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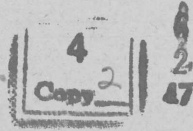
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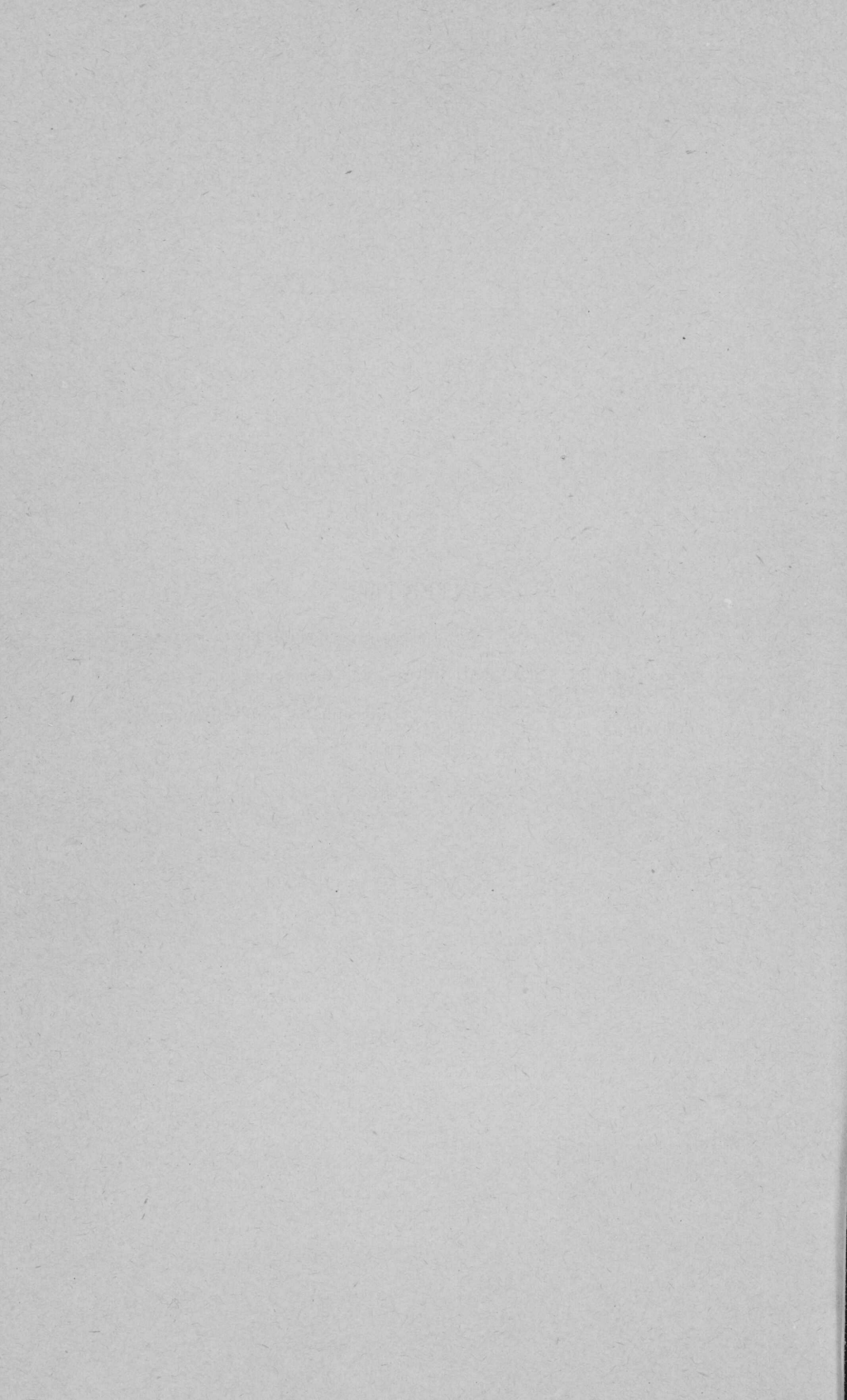
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THE TOXICITY AND POTENTIAL DANGERS OF METHYL  
BROMIDE WITH SPECIAL REFERENCE TO ITS USE IN  
THE CHEMICAL INDUSTRY, IN FIRE EXTINGUISHERS,  
AND IN FUMIGATION

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# THE TOXICITY AND POTENTIAL DANGERS OF METHYL BROMIDE WITH SPECIAL REFERENCE TO ITS USE IN THE CHEMICAL INDUSTRY, IN FIRE EXTINGUISHERS, AND IN FUMIGATION

## INTRODUCTION

Methyl bromide has been used extensively as an intermediate in the chemical industry, and more recently it has gained importance as a fire extinguishing agent and as a fumigant for insecticidal purposes. The literature on methyl bromide poisoning shows that it is a highly toxic material of insidious action and that certain precautions are necessary in handling it to prevent untoward effects. The chemistry of methyl bromide, its determination, its effects on animals and men, and the potential dangers of its use under different conditions, as well as preventive measures are here discussed in order to caution against untoward effects and to promote the safe use of this important chemical.

## CHEMISTRY

Methyl bromide, monobromomethane,  $\text{CH}_3\text{Br}$ , has a molecular weight of 94.95. It is a colorless gas of specific gravity  $1.732 \frac{0^\circ}{0^\circ} \text{ C.}$  which solidifies at  $-93^\circ \text{ C.}$  and boils at  $4.5^\circ \text{ C.}$  (758 mm. Hg.). It is very slightly soluble in water, but is soluble in alcohol, ether, chloroform, and carbon disulfide (Lange, 1944). Heilbron (1936) gives its heat of combustion as  $\text{Cp } 184.71 \text{ Cal.}$  and its latent heat at  $20^\circ \text{ C.}$  as  $61.52 \text{ kg.-cal./kg.}$  According to Coward and Jones (1939) all mixtures of methyl bromide in air appear to be incapable of ignition and propagation of flame in a tube 2 inches in diameter, but mixtures containing 13.5 to 14.5 percent methyl bromide were ignited by an induction coil spark in a  $2\frac{1}{2}$ -liter jar.

Methyl bromide can be prepared: (1) by interaction of hydrogen bromide with methanol,  $\text{CH}_3\text{OH} + \text{HBr} = \text{CH}_3\text{Br} + \text{H}_2\text{O}$ ; (2) by reacting bromine with methanol in the presence of red phosphorus,  $\text{P} + 5\text{CH}_3\text{OH} + 5\text{Br} = 5\text{CH}_3\text{Br} + \text{H}_2\text{O} + \text{H}_3\text{PO}_4$ ; and (3) by the action of a boiling solution of potassium bromide on dimethyl sulfate (Duyoir, Fabre, and Layani, 1939),  $\text{SO}_4(\text{CH}_3)_2 + 2\text{KBr} = 2\text{CH}_3\text{Br} + \text{K}_2\text{SO}_4$ . On a large scale it is manufactured by heating sodium bromide, methanol, and sulfuric acid to  $130^\circ \text{ C.}$  in a closed process.

Although methyl bromide-air mixtures are incapable of ignition or of propagation of flame, such mixtures undergo decomposition at elevated temperatures. Nuckolls (1933) showed that methyl bromide-air mixtures are decomposed in the presence of a burning gas flame, a mixture of 0.7 volume percent containing, after 5 minutes, 0.003 percent hydrogen bromide and less than 0.001 percent bromine, and, after 30 minutes, 0.003 percent hydrogen bromide, less than 0.001 percent bromine, 1.4 percent carbon dioxide, and less than 0.1 percent carbon monoxide. Air containing 2.2 volume percent methyl bromide contained, after 5 minutes, 0.011 percent hydrogen bromide and 0.004 percent bromine, and, after 30 minutes, 0.010 percent hydrogen bromide, 0.002 percent bromine, 1.6 percent carbon dioxide, and 0.4 percent carbon monoxide. Air containing 5.4 percent methyl bromide contained, in the presence of an oil fire, 0.15 percent hydrogen bromide but no carbonoxybromide or bromine, and, in the presence of a wood fire, air originally containing 2.2 percent methyl bromide contained 0.34 percent hydrogen bromide and 0.023 percent carbonoxybromide, but no bromine. It is, therefore, apparent that with thermal decomposition several irritant gases—hydrogen bromide, bromine, and carbonoxybromide—may be formed in addition to carbon monoxide and carbon dioxide. Similarly, Beyne and Goett (1934) and Duvoir, Fabre, and Layani (1939), referring to analytical data of Kohn-Abrest, stated that air originally containing 12 percent methyl bromide (500–600 mg./l.), when heated to 1000–1100° C. contained 8.90 percent carbon dioxide, 2.65 percent carbon monoxide, 10.90 percent hydrogen bromide, 10.90 percent water vapor, 2.45 percent oxygen, and 64.70 percent nitrogen.

It is obvious that such a concentration of hydrogen bromide will not only cause more or less severe irritation of the respiratory organs but it will also aggravate the toxic action of carbon monoxide in a similar way as reported with nitrous fumes (Rubino 1912, and Wirth 1930) and thus increase the hazard from carbon monoxide exposure in fires.

#### DETERMINATION OF METHYL BROMIDE IN AIR

For the detection and estimation of methyl bromide in air the halide lamp may be used. Dudley, Miller, Neal, and Sayers (1940) found that under ideal conditions of light and in the absence of air currents the lower limit of this type of lamp is about 50 p. p. m. of methyl bromide. At 50 p. p. m. a moderately strong reaction is seen, whereas at 35 p. p. m. an unreliable test is seen, and lower concentrations give a negative reaction. It is, therefore, apparent that this method is not sufficiently sensitive to detect concentrations of methyl bromide in air which may cause toxic effects with continued exposure. Schwarz and Zangger (1926) suggested absorbing methyl bromide in alcohol and

adding this solution to a current of hydrogen which is then burned, the flame burning bluish green when copper screen is held into the flame in case alkyl halides are present.

Busbey and Drake (1938) determined methyl bromide in air by collecting it in 2-liter vacuum flasks and hydrolyzing it with alcoholic potassium hydroxide; this was followed by oxidation of the bromide formed to bromate by means of sodium hypochlorite and iodometric determination of the bromate. Stenger, Shrader, and Beshgetoor (1939) hydrolyzed methyl bromide by means of ethanolamine and determined the resulting bromide by the Kolthoff-Yutzy procedure, or, in the presence of larger quantities, by Volhard's method. This method was slightly modified by Chisholm and Koblitsky (1944). Lubatti (1945) determined methyl bromide in air by passing it over a glowing platinum wire and estimating the amount of bromine liberated by means of test papers saturated with a pale yellow solution of fluorescein, more or less of which is changed to eosin according to the amount of bromine liberated. The resulting colors can be matched against standards prepared from known amounts of methyl bromide in the same way. Williams (1945) passed air containing methyl bromide through a quartz tube heated to 950–1,000° C. and absorbed the hydrogen bromide formed in a diluted solution of sodium hydroxide in which he determined the bromine content by the Kolthoff-Yutzy procedure. Williams, Haines, and Heindel (1945) devised an apparatus for the continuous determination of methyl bromide at different points of the atmosphere which is based on the same principle except that the hydrogen bromide formed is absorbed in a stream of water and the electrical conductivity of the aqueous solution is determined by means of a recording conductivity meter.

For the determination of bromide in food the method of Stenger, Shrader, and Beshgetoor (1939) may be used. Dudley (1939) published a method for the determination of the bromide content of food-stuffs fumigated with methyl bromide; Shrader, Beshgetoor, and Stenger (1942) described a method which allows the determination of volatile and inorganic bromine compounds in such products.

#### TECHNICAL USES OF METHYL BROMIDE

Methyl bromide is used extensively as methylating agent in the chemical industry, as for instance in the manufacture of antipyrine. Because of its low boiling point it has been used as a refrigerant.

It is used in fire extinguishers, and it is used extensively in fumigation as an insecticidal agent. The insecticidal action of methyl bromide was discovered by Le Goupil (1932) and subsequently studied by Lindgren (1936), Lepesne (1938), Mackie (1938), Fisk and Shepard (1938), Jones (1938), Piper and Davidson (1938), Phillips,

Monro, and Allen (1938), Hawkins (1938 and 1939), Monro and Delisle (1943) and others, using a great variety of insects. Jones (1938) showed that the insecticidal action on adult red flour beetles may be markedly increased by the admixture of 10 percent carbon dioxide. Since it does not affect the germination of seeds or cause injury of plants when used properly (as shown by Le Goupil (1932) and later by Mackie and Carter (1937), Fisk and Shepard (1938) and others) methyl bromide has gained great importance as an insecticide in past years.

#### EFFECT OF METHYL BROMIDE ON THE ANIMAL ORGANISM

*The absorption of methyl bromide* usually takes place through the lungs; if ingested it may be absorbed from the gastro-intestinal tract (Irish, Adams, Spencer, and Rowe, 1941, and Miller and Haggard, 1943); but the question as to whether it is absorbed through the intact skin in sufficient quantities to cause systemic effects does not appear to be definitely answered. Schifferli (1942) mentions two cases of systemic poisoning in fumigators who had been working with close-circuit respirators for 1½ hours in a fumigated area, and Bruhin (1943) reported three similar cases of workers who developed systemic poisoning after an exposure of 55 and 33 minutes (with an exposure-free interval of 10 minutes between) to a concentration of presumably 30.9 mg. per liter (?) (7941 p. p. m.). But in view of the reports of Butler, Perry, and Williams (1945) and Billet and Abel (1940) who observed no symptoms of systemic poisoning in soldiers within a tank after the use of methyl bromide fire extinguishers (or only minor symptoms), although all three suffered severe injury of the skin, it appears doubtful whether the cases reported by Schifferli (1942) and Bruhin (1943) actually absorbed the toxic material through the skin.

With regard to the *fate of methyl bromide in the organism*, Irish, Adams, Spencer, and Rowe (1941) assumed that after its absorption it is partly broken down in the body because during exposure of rabbits to methyl bromide they noted a definite increase of the non-volatile bromide in the blood which rose from a normal value of 1 mg. percent to 11 mg. percent after exposure for 8 hours to 60 p. p. m. of methyl bromide in air. This was confirmed by Miller and Haggard (1943).

With regard to the *distribution of methyl bromide in the organism*, Löffler and Rüttimeyer (1920) claimed to have demonstrated the presence of volatile bromide in various organs of guinea pigs exposed to high concentrations of methyl bromide as illustrated in table 1, from which it would appear that in acute methyl bromide poisoning volatile bromides may be detected even after 24 hours. However, the same authors were unable to detect volatile bromide in the organs

of a victim of methyl bromide poisoning who died within approximately one-half this period of time. Duvoir, Fabre, and Layani (1939) determined the bromine content in the organs of one rabbit and one dog and found the amounts, calculated as methyl bromide, as given in table 2. From these data it appears that methyl bromide is stored in those tissues which are rich in lipid material and that even after 40 minutes appreciable amounts of bromine compounds may be present whereas in the case of methanol the distribution corresponds very closely to the water content of various tissues as shown by Yant and Schrenk (1937). As pointed out above, Löffler and Rüttemeyer were unable to detect volatile bromide in tissues of a victim of methyl bromide poisoning. Similar negative results were reported by Rohrer (1920) who also was unable to find measurable amounts of nonvolatile bromine compounds, whereas Glaser (1928), although unable to detect volatile bromine compounds, found bromide in various tissues and in urine. Heimann (1944) determined the bromine content of the tissues of a victim of acute methyl bromide poisoning and found in the lungs 38 mg., in the liver 56 mg., in the brain 62 mg., and in a

**Table 1. DISTRIBUTION OF VOLATILE BROMIDE IN GUINEA PIGS AFTER EXPOSURE TO METHYL BROMIDE VAPORS**

(Löffler and Rüttemeyer, 1920)

| Time of analysis                               | Blood | Liver | Brain | Lungs | Kidneys | Heart | Muscle |
|------------------------------------------------|-------|-------|-------|-------|---------|-------|--------|
| Immediately after death.....                   | +     | +     | ±     | 0     | -----   | ----- | -----  |
| ½ hour after death.....                        | +     | +     | ±     | 0     | -----   | ----- | -----  |
| 24 hours after death (on ice).....             | ----- | +     | ±     | ±     | +       | ----- | -----  |
| Animals allowed to live 45' after exposure.... | ±     | ±     | ±     | ±     | ±       | +     | ±      |
| Animals exposed for 2'; killed 70' later.....  | 9     | 0     | ----- | ----- | -----   | ----- | -----  |

**Table 2. DISTRIBUTION OF BROMINE IN ORGANS OF ANIMALS FOLLOWING EXPOSURE TO METHYL BROMIDE AND CALCULATED AS METHYL BROMIDE**

(Duvoir, Fabre, and Layani, 1939)

| Organ              | Rabbit exposed for 180' to 31.7 mg./l., which died 8-10 hours later | Dog exposed for 180' to 48 mg./l., which died 40' later | Organ            | Rabbit exposed for 180' to 31.7 mg./l., which died 8-10 hours later | Dog exposed for 180' to 48 mg./l., which died 40' later |
|--------------------|---------------------------------------------------------------------|---------------------------------------------------------|------------------|---------------------------------------------------------------------|---------------------------------------------------------|
| Blood.....         | 3.2                                                                 | 0.8                                                     | Bone marrow..... | Trace                                                               | 2                                                       |
| Brain.....         | 3.8                                                                 | 3.2                                                     | Pancreas.....    | None                                                                | 1.3                                                     |
| Liver.....         | 5.2                                                                 | 6.1                                                     | Spleen.....      | None                                                                | -----                                                   |
| Kidney.....        | 6.5                                                                 | 3.9                                                     | Testes.....      | Trace                                                               | -----                                                   |
| Lungs.....         | 3.0                                                                 | 0.6                                                     | Adrenals.....    | Trace                                                               | Trace                                                   |
| Abdominal fat..... | 6.4                                                                 | 7.3                                                     | Thyroid.....     | Trace                                                               | -----                                                   |



composite sample of heart, spleen, and kidney 32 mg. per 300 gm. of tissue in addition to traces of methanol and formaldehyde.

*The excretion of methyl bromide* takes place partly through the lungs, as demonstrated in rats by Miller and Haggard (1943) who also showed that this takes place rather promptly, in that after the intraperitoneal injection of 60 mg./kg. body weight in olive oil 90 percent of the total amount exhaled was excreted during the first 30 minutes. Part of the methyl bromide absorbed is decomposed in the organism and is excreted as bromide with the urine, as demonstrated by Clarke, Roworth, and Holling (1945). However, it may take a considerable period of time for the bromine to be completely eliminated as illustrated by the slow disappearance of bromine from the blood demonstrated by these authors in 4 victims of acute methyl bromide poisoning. This is shown in table 3.

**Table 3. CONCENTRATION OF BROMIDE IN MG. PER 100 CC. OF BLOOD IN PATIENTS SUFFERING FROM ACUTE METHYL BROMIDE POISONING**

(Clarke, Roworth, and Holling, 1945)

| Time after exposure  | No. 1 fatal poisoning | No. 2 fatal poisoning | No. 3 severe poisoning | No. 4 light poisoning |
|----------------------|-----------------------|-----------------------|------------------------|-----------------------|
| Day of accident..... | 9.2                   | 8.3                   | 6.9                    | 6.9                   |
| 12 days later.....   |                       |                       | 6.9                    | 6.9                   |
| 31 days later.....   |                       |                       |                        | 0.8                   |
| 39 days later.....   |                       |                       | 0.0                    |                       |
| 41 days later.....   |                       |                       |                        | 0.0                   |

*The toxicity of methyl bromide for laboratory animals* has been studied by Roth and von Frey (quoted from Schuler, 1899), Bachem (1927), Merzbach (1928), Sayers, Yant, Thomas, and Berger (1929), Glaser and Frisch (1929), Nuckolls (1933), Beyne and Goett (1934), Winter (1936), Duvoir, Fabre, and Layani (1939), and Irish, Adams, Spencer, and Rowe (1940) whose data are summarized in tables 4 and 5. An exact evaluation of these data is difficult because the experimental conditions used in the various studies show considerable variation. In addition, in many instances the calculated concentration of methyl bromide in the air has not been checked by analysis and for this reason the actual concentration may have been different from that stated. It appears, however, that the toxicological picture of methyl bromide poisoning varies with the concentration. With certain concentrations there is some evidence of a narcotic action, characteristic of all aliphatic chlorinated hydrocarbons. With lower concentrations the irritant action on mucous membranes of the eyes and nose is at first quite distinct, as indicated by restlessness of the animals, lacrimation, and salivation. The animals become quiet, stagger when trying to

walk, and finally tolerate side position. With the beginning of this stage, the respiration is slowed; later it becomes labored and dyspneic, and foamy froth may appear around the mouth. During the dyspneic stage, cardiac irregularities, tachycardia, and arrhythmias may be observed, and dilatation of the right heart is seen not infrequently. The depression of the central nervous system may be preceded by, associated with, or followed by tremors or twitchings which may pass into epileptiform (clonic-tonic) convulsions; these symptoms may also develop after discontinuance of the exposure. Even if these symptoms are not severe, or even if they are absent and the animals survive the exposure proper, death may ensue within a few hours or days. This delayed and insidious action is one of the characteristics of methyl bromide poisoning.

With regard to quantitation of the acute toxicity of methyl bromide for laboratory animals the data collected in table 4 (with the exception of those for guinea pigs) cover too small a number of animals in each group to establish a minimal fatal or maximal tolerated concentration. The data of Sayers, Yant, Thomas, and Berger (1929) for guinea pigs as illustrated in figure 1, and those of Irish, Adams, Spencer, and Rowe (1940) for rats and rabbits as illustrated in table 5, give a summary of these experiments. These data show that the toxicity is in part a function of the duration of the exposure. Rabbits survive an exposure to 514 p. p. m. for 6 hours and exposure to 237 p. p. m. for 15 hours, whereas rats tolerate the former for only 2 hours and the latter for only 8 hours. Guinea pigs are about as sensitive as rats in that they tolerate exposure to concentrations up to 200 p. p. m. for 6 hours and up to concentration 100 p. p. m. for 10 hours of exposure. Rabbits suffered 100 percent mortality with exposure to 514 p. p. m. for 11 hours or to 217 p. p. m. for 24 hours. The death rate of rats was 100 percent if exposed for 6 hours to 514 p. p. m. or for 22 hours to 257 p. p. m., and that of guinea pigs was 100 percent if exposed for 6 hours to 450 p. p. m. or more or for 10 hours to about 280 p. p. m. As pointed out by Irish, Adams, Spencer, and Rowe (1940) the irritation of the lungs is somewhat less pronounced in rabbits than in rats; on the other hand, it appears that the former have a greater tendency to respond with motor paralyses.

With regard to the *toxic effects of methyl bromide with continued exposure*, Glaser and Frisch (1929) exposed two guinea pigs for 15 minutes daily to relatively high concentrations. One animal became nervous and died on the fifth day of the experiment; the other, which survived for 11 days, developed a paresis of the hind legs from which it recovered prior to its death. Irish, Adams, Spencer, and Rowe (1940) published a similar experiment with a larger number of animals

**Table 4. ACUTE TOXICITY OF METHYL BROMIDE FOR VARIOUS LABORATORY ANIMALS**

DOGS

| Concentration        |          | Duration of exposure in minutes | Symptoms                                                                                                       | Author                            |  |
|----------------------|----------|---------------------------------|----------------------------------------------------------------------------------------------------------------|-----------------------------------|--|
| As stated            | P. p. m. |                                 |                                                                                                                |                                   |  |
| 0.00054 mol./l.....  | 13, 107  | 45                              | 5' salivation, slowed respiration; 30' marked dyspnea; after exposure respiration very poor, ✚ after 1½ hours. | Merzbach (1928).                  |  |
| 0.00036 mol./l.....  | 8, 738   | 60                              | 15' salivation labored respiration, staggering, light narcosis; after exposure coma, ✚ after 80'.              |                                   |  |
| 0.00018 mol./l.....  | 4, 369   | }                               | Similar, but no details given.....                                                                             |                                   |  |
| 0.00009 mol./l.....  | 2, 185   |                                 |                                                                                                                |                                   |  |
| 0.000036 mol./l..... | 874      |                                 | Not fatal, no details given.....                                                                               |                                   |  |
| 21 mg./l.....        | 5, 397   | 38                              | Somnolence, salivation, deep respiration; after exposure ✚ in 30'.....                                         | Beyne and Goett (1934).           |  |
| 19.3 mg./l.....      | 4, 960   | 30                              | Somnolence, staggering; after exposure rapid respiration, tachycardia, arrhythmia, ✚ after 3 hours 17'.        |                                   |  |
| 10 mg./l.....        | 2, 570   | 35                              | Sight trembling; 15' after exposure vomiting; 2½ hours impaired respiration; ✚ 4½ to 5½ hours after exposure.  |                                   |  |
| 6.2 mg./l.....       | 1, 530   | 35                              | No effects during exposure; sneezing, diarrhea after exposure; lack of appetite; recovery                      |                                   |  |
| 48 mg./l.....        | 12, 336  | 90                              | Irritation, restlessness; after exposure temporary recovery, convulsions; ✚ after 40'.                         | Duvoir, Fabre, and Layani (1939). |  |

CATS

|                 |         |    |                                                                                      |                           |
|-----------------|---------|----|--------------------------------------------------------------------------------------|---------------------------|
| 120 mg./l.....  | 30, 840 | 10 | } No symptoms during exposure, apparently no toxic effects.....                      | Glaser and Frisch (1929). |
| 340 mg./l.....  | 87, 380 | 12 |                                                                                      |                           |
| 31.7 mg./l..... | 8, 146  | 30 | Temporary excitement, no other symptoms.....                                         |                           |
| 20 mg./l.....   | 5, 140  | 22 | After 15' narcosis, later restlessness, recovery.....                                |                           |
| 70 mg./l.....   | 17, 990 | 25 | After 23' narcosis; after exposure salivation, incoordination, dyspnea; ✚ after 80'. |                           |

# RABBITS

|                                    |        |    |                                                                                                                  |                                                |
|------------------------------------|--------|----|------------------------------------------------------------------------------------------------------------------|------------------------------------------------|
| 4000 cc./50 l.....                 | 80,000 | 30 | Irritation, slowing of respiration, narcosis; after exposure cyanosis, progressive depression, ✚ after 2½ hours. | Roth and von Frey (quoted from Schuler, 1899). |
| 1000 cc./50 l.....                 | 20,000 | 25 | Some irritation, slowing of respiration; after exposure lack of appetite, ✚ 26 hours later.                      |                                                |
| 98 mg./l.....                      | 25,186 | 78 | No symptoms during exposure, no appetite, ✚ 20 hours later.....                                                  | Glaser and Frisch (1929).                      |
| 36 mg./l.....                      | 9,252  | 25 | Slowing of respiration, foamy froth, depression; after exposure dyspnea, ✚ after 2½ hours.                       |                                                |
| 23 mg./l.....                      | 6,425  | 30 | Slowing of respiration; after exposure foamy froth, progressive depression, sick for several days.               | Beyne and Goett (1934).                        |
| 19 mg./l.....                      | 4,883  | 25 | Slowing of respiration; after exposure rapid recovery.....                                                       |                                                |
| 20 Gm./0.315 m. <sup>3</sup> ..... | 16,316 | ?  | Immediate severe convulsions; when immediately removed complete recovery.                                        | Duvour, Fabre, and Layani (1939).              |
| 10 Gm./0.315 m. <sup>3</sup> ..... | 8,158  | 5  | Irritation, restlessness, after 2-5' convulsions; after exposure somnolence in 90', ✚ 8-10 hours later.          |                                                |

# MICE

|                      |        |            |                                                                                            |                                                |
|----------------------|--------|------------|--------------------------------------------------------------------------------------------|------------------------------------------------|
| 500 cc./50 l.....    | 10,000 | 45         | Moderate depression; ✚ next day.....                                                       | Roth and von Frey (quoted from Schuler, 1899). |
| 0.001 mol./l.....    | 24,415 | 25         | Fatal.....                                                                                 |                                                |
| 0.001 mol./l.....    | 24,415 | 75         | Fatal.....                                                                                 | Bachem (1927).                                 |
| 0.000017 mol./l..... | 488    | 150        | ✚ 6 hours                                                                                  |                                                |
| 0.000008 mol./l..... | 195    | Many hours | Only light narcosis.....                                                                   | Glaser and Frisch (1929).                      |
| 28 mg./l.....        | 7,196  | 75         | Moderate irritation, narcosis in 35', died during night.....                               |                                                |
| 204 mg./l.....       | 52,428 | 17         | After 3' narcosis, paralysis, ✚ after 1 hour.....                                          |                                                |
| 5.2 mg./l.....       | 1,336  | 45         | No narcosis, staggering at end of exposure, temporary recovery, ✚ 10 hours after exposure. |                                                |
| 49.5 mg./l.....      | 12,721 | 11         | No narcosis, temporary excitation, recovery.....                                           |                                                |

# RATS

|                   |     |   |                                                                         |                |
|-------------------|-----|---|-------------------------------------------------------------------------|----------------|
| 0.05 percent..... | 500 | ? | All affected within 2 hours, 2 ✚ after 5 hours, all ✚ next morning..... | Winter (1936). |
|-------------------|-----|---|-------------------------------------------------------------------------|----------------|

Table 4. ACUTE TOXICITY OF METHYL BROMIDE FOR VARIOUS LABORATORY ANIMALS—Continued

## GUINEA PIGS

| Concentration          |                 | Duration of exposure in minutes | Symptoms                                                                                                                           | Author                                         |
|------------------------|-----------------|---------------------------------|------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------|
| As stated              | P. p. m.        |                                 |                                                                                                                                    |                                                |
| 2000 cc./50 l.....     | 40, 000         | 15                              | Progressive depression; ✕ ½ hour after exposure.....                                                                               | Roth and von Frey (quoted from Schuler, 1899). |
| 1000 cc./50 l.....     | 20, 000         | 40                              | Some irritation, slowed respiration, progressive depression, ✕.....                                                                |                                                |
| 5 and 9.6 percent..... | 50, 000-96, 000 | 7-15                            | Immediate uneasiness, after 1-2' side position, struggling, convulsive respiration, ✕ in 7-15'.                                    |                                                |
| 2.9 percent.....       | 29, 000         | 5                               | All animals died.....                                                                                                              |                                                |
| 2.2 percent.....       | 22, 000         |                                 | After 7' coughing, retching; after 8-15' unsteadiness; after 30' marked weakness; ✕ 10' after exposure.                            | Sayers, Yant, Thomas, and Berger (1929).       |
| 1.3 percent.....       | 13, 000         |                                 | After 13' increased respiration, lacrimation, discharge from nose, unsteadiness in 23-28', convulsive respiration, ✕ after 43-68'. |                                                |
| 0.7 percent.....       | 7, 000          |                                 | Similar effects as with 1.3 percent but delayed; 30' exposure ✕ 1-2 hours, 90' exposure ✕ immediately.                             |                                                |
| 0.54 percent.....      | 5, 400          | 10 and 20                       | With exposure for 10' no apparent effects or death, with exposure for 20' no acute symptoms but ✕ after 6 days.                    |                                                |
| 0.2-0.23 percent.....  | 2, 000-2, 300   | 30                              | No acute symptoms; 1/6 ✕ 9 hours after exposure.....                                                                               |                                                |
| 0.2-0.23 percent.....  | 2, 000-2, 300   | 90                              | Slight weakness, lacrimation, secretion about nose and mouth, after 160' 4/6 side position, majority ✕ in 2½ hours or less.        |                                                |
| 0.2-0.23 percent.....  | 2, 000-2, 300   | 170                             | All animals ✕ at end of exposure.....                                                                                              |                                                |
| 0.05-0.06 percent..... | 500-600         | 90                              | No fatalities.....                                                                                                                 |                                                |
| 0.05-0.06 percent..... | 500-600         | 270                             | Slight salivation and nasal discharge, all ✕ during following 2 days.....                                                          |                                                |
| 0.05-0.06 percent..... | 500-600         | 440                             | 3/6 ✕ at end of exposure.....                                                                                                      |                                                |
| 0.05-0.06 percent..... | 500-600         | 480                             | 3/3 ✕ within 3½ hours following exposure.....                                                                                      |                                                |
| 0.03 percent.....      | 300             | 270                             | No symptoms, no ✕.....                                                                                                             |                                                |
| 0.03 percent.....      | 300             | 300                             | No symptoms, 1/6 ✕.....                                                                                                            |                                                |
| 0.03 percent.....      | 300             | 540                             | After 540' rales, all animals ✕ during subsequent 3 days.....                                                                      |                                                |
| 0.03 percent.....      | 300             | 810                             |                                                                                                                                    |                                                |
| 0.015 percent.....     | 150             | 540                             | No symptoms during exposure, majority of animals ✕ in 1-3 days.....                                                                |                                                |
| 0.015 percent.....     | 150             | 810                             | No symptoms, no fatalities.....                                                                                                    |                                                |
| 0.010 percent.....     | 100             | 300                             | No symptoms, no fatalities.....                                                                                                    |                                                |
| 0.010 percent.....     | 100             | 600                             |                                                                                                                                    |                                                |



|                      |                 |             |                                                                                                                                 |                                                    |
|----------------------|-----------------|-------------|---------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------|
| 0.7-1.0 percent..... | 7, 000-10, 000  | 5           | Within 1' uneasiness, 3' slight tremors and irregular respiration, 30' after exposure weakness, tremors, retching, recovery.    | } Nuckolls (1933).                                 |
| 0.7-1.0 percent..... | 7, 000-10, 000  | 30          | After 5' lacrimation, cough, retching; after 15' weakness; after exposure stupor, irritation, ✱ 18 hours after exposure.        |                                                    |
| 0.7-1.0 percent..... | 7, 000-10, 000  | 60          | Progressive depression, retching, respiration slow; after exposure convulsive movements, ✱ in 20'.                              |                                                    |
| 0.7-1.0 percent..... | 7, 000-10, 000  | Until death | One animal each died after 95', 110'; 1 survived exposure for 180' but ✱ 10' after exposure.                                    |                                                    |
| 2.0-2.5 percent..... | 20, 000-25, 000 | 5           | After 1' lacrimation, irritation; after 4' incoordination; after exposure marked weakness, irregular respiration, recovery.     | } Kohn-Abrest (quoted from Beyne and Goett, 1934). |
| 2.0-2.5 percent..... | 20, 000-25, 000 | 30          | After 5' stupor; after 20' unable to stand, slow labored respiration; after exposure semiconsciousness, tremors, ✱ within 150'. |                                                    |
| 2.0-2.5 percent..... | 20, 000-25, 000 | 60          | Rapidly unconscious, 1 ✱ after 30', 1 ✱ after 50', 2 within 60', and 1 after 65'.                                               |                                                    |
| 80 mg./l.....        | 20, 560         | 15          | Excitement, irritation, narcosis; ✱ 15' after exposure.....                                                                     |                                                    |
| 25 mg./l.....        | 6, 425          | 60          | Lassitude, excitement, narcosis, ✱ 150' after exposure.....                                                                     |                                                    |
| 10-12 mg./l.....     | 2, 570-3, 084   | 68          | No symptoms during exposure, ✱ several hours after exposure.....                                                                |                                                    |

**Table 5. ACUTE TOXICITY OF METHYL BROMIDE FOR RATS AND RABBITS**

(Irish, Adams, Spencer, and Rowe, 1940)

| Concentration |          | 520 Rats                     |                                 | 154 Rabbits                  |                                 |
|---------------|----------|------------------------------|---------------------------------|------------------------------|---------------------------------|
| mg./liter     | p. p. m. | 100% fatal exposure in hours | 100% survival exposure in hours | 100% fatal exposure in hours | 100% survival exposure in hours |
| 50            | 12,850   | 0.1 (6 min.)                 | 0.05 (3 min.)                   | 0.5 (30 min.)                | 0.2 (12 min.)                   |
| 20            | 5,140    | 0.4 (24 min.)                | 0.1 (6 min.)                    | 1.4 (84 min.)                | 0.6 (36 min.)                   |
| 10            | 2,570    | 0.7 (42 min.)                | 0.4 (24 min.)                   | 2.2 (132 min.)               | 1.0 (60 min.)                   |
| 2.0           | 514      | 6                            | 2                               | 11                           | 6                               |
| 1.0           | 257      | 22                           | 8                               | 24                           | 15                              |
| 0.85          | 218      | 26                           | 12                              | 32                           | 20                              |
| 0.42          | 108      | -----                        | 22                              | -----                        | -----                           |

**Table 6. EFFECT OF REPEATED EXPOSURE OF LABORATORY ANIMALS TO METHYL BROMIDE**

(Irish, Adams, Spencer, and Rowe, 1940)

| Concentration |          | Symptoms                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             |
|---------------|----------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| mg./liter     | p. p. m. |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      |
| 0.85          | 218      | 20/20 <i>rats</i> died after 3-4 exposures for 7-8 hours; they appeared normal until shortly before death and showed no significant histopathological changes.<br>14/16 <i>guinea pigs</i> died after 1-3 exposures; 2 were killed when showing marked symptoms of poisoning. All showed respiratory difficulties, few apparently paralyzed. Autopsy: congestion, edema, leucocytic infiltration of the lungs with frequent hemorrhages into the alveoli; liver, spleen, and kidney showing no significant changes.                                                                                                                                                                                                                                                                                                                                                                                  |
| 0.42          | 108      | 9/30 <i>rats</i> showed almost immediately loss of weight. These 9 were sacrificed after 6-14 exposures when moribund; 2 developed convulsions. 16/30 appeared to tolerate fairly well 16-58 exposures; 5 of these developed convulsions. 5/30 female rats survived 83-100 exposures; they showed generally poor appearance and retarded growth. Sixty percent showed some histological changes varying from congestion to marked pneumonic consolidation with multiple abscesses, 6 being essentially normal. Animals showing marked pulmonary changes; also gave evidence of liver and kidney injury.<br>4/11 <i>guinea pigs</i> died after 64-91 exposures and showed at the end no significant changes of liver, kidney, spleen, pancreas, or adrenals, the pulmonary damage being insignificant.<br>One <i>monkey</i> developed severe convulsions after 11 exposures over a period of 14 days. |
| 0.25          | 64       | <i>Rats</i> and <i>guinea pigs</i> showed no demonstrable toxic effects over an exposure period of 6 months.<br><i>Rabbits</i> developed weakness passing into paralysis, usually lung irritation, and loss of weight.<br><i>Monkeys</i> developed paralyses and became excitable. If exposure was discontinued after the first sign of paralysis all animals recovered promptly. Monkeys showed no blood changes except a moderate increase of neutrophils, but these decreased again after discontinuation of the exposure.                                                                                                                                                                                                                                                                                                                                                                        |
| 0.13          | 33       | No noticeable toxic effects were observed in <i>rats</i> , <i>guinea pigs</i> , or <i>monkeys</i> .<br><i>Rabbits</i> showed irritation of lungs and paralyses as observed with 64 p. p. m.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |
| 0.065         | 16       | This concentration was tolerated by all species including rabbits.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   |

of different species. Their results are summarized in table 6 which shows that rats, rabbits, guinea pigs, and monkeys tolerated daily exposures to 33 p. p. m. for 7 to 8 hours for many months without toxic effects, whereas rabbits showed signs of pulmonary irritation but no paralysis, and tolerated continued exposure to 16 p. p. m.

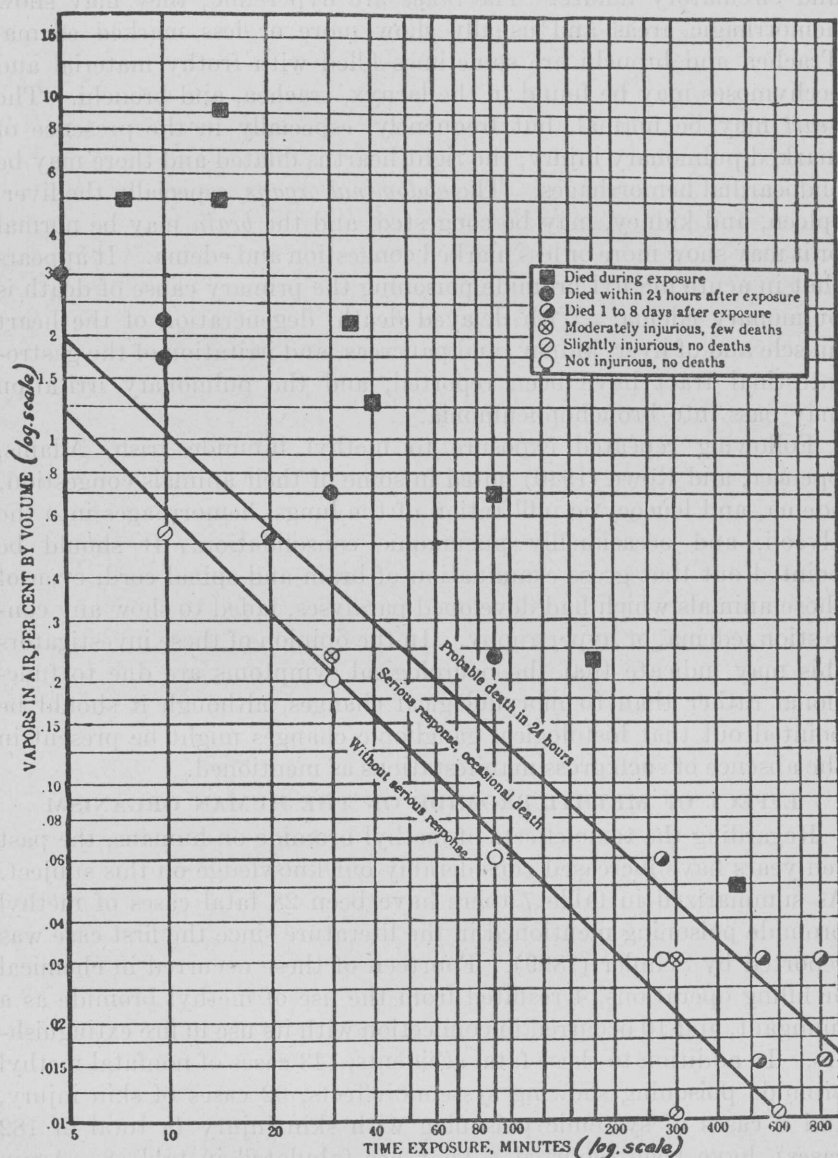


FIGURE 1. The acute toxicity of methyl bromide for guinea pigs.

(Sayers, Yant, Thomas, and Berger, 1929)

without any untoward effects. It appears, therefore, that for rats, guinea pigs, and monkeys, approximately 30 p. p. m. represents the maximal safe concentration for continued exposure.

*The pathological changes observed in animals* that died from acute methyl bromide poisoning are characteristic for pulmonary irritation and circulatory failure. The *lungs* are hyperemic; they may show hemorrhagic areas and usually show more or less marked edema. Trachea and bronchi are sometimes filled with frothy material and ecchymoses may be found in the larynx, trachea, and bronchi. The *heart* may be normal, but frequently, especially in the presence of marked pulmonary injury, the right heart is dilated and there may be endocardial hemorrhages. The *abdominal organs*, especially the liver, spleen, and kidney, may be congested, and the *brain* may be normal or it may show more or less marked congestion and edema. It appears that in acute methyl bromide poisoning the primary cause of death is pulmonary edema. With delayed death, degeneration of the heart muscle and of liver, kidney, and pancreas, and irritation of the gastrointestinal tract have been reported, and the pulmonary irritation may pass into bronchopneumonia.

Following repeated exposure to methyl bromide Irish, Adams, Spencer, and Rowe (1940) noted in some of their animals congestion, edema, and leucocytic infiltration of the lungs, hemorrhages into the alveoli, and occasionally pneumonic consolidation. It should be pointed out that gross examination of brain and spinal cord, even of those animals which had developed paralyses, failed to show any congestion, edema, or hypertrophy. In the opinion of these investigators this may indicate that the neurological symptoms are due to functional rather than to morphological changes, although it should be pointed out that histological ganglionic changes might be present in the absence of such gross manifestations as mentioned.

#### EFFECT OF METHYL BROMIDE ON THE HUMAN ORGANISM

Regarding the toxic effects of methyl bromide on humans, the past ten years have increased considerably our knowledge on this subject. As summarized in table 7 there have been 28 fatal cases of methyl bromide poisoning mentioned in the literature since the first case was reported by Schuler (1899). Fourteen of these occurred in chemical or filling operations, 4 resulted from the use of methyl bromide as a fumigant, and 10 occurred in connection with its use in fire extinguishers. In addition to these fatal accidents, 143 cases of nonfatal methyl bromide poisoning showing systemic effects, 32 cases of skin injury, and 6 cases of systemic poisoning with skin injury (a total of 182 cases), have been reported. They are tabulated in table 8. Again the majority of these (122) resulted from exposure in chemical and filling operations (6 of these in the laboratory); 39 were observed when

it was used as a fumigant; 5 resulted from leaking fire extinguishers; 6 from the use of methyl bromide fire extinguishers; 10 from accidental discharge of fire extinguishers.

The *fatal accidents* following exposure to methyl bromide, as given in table 7, *resulted from exposures to relatively high concentrations*. Wyers (1945) reported that his patients had a potential exposure to up to 60,000 p. p. m.; those of Schifferli (1942) and Bruhin (1943) had been exposed to approximately 8,000 p. p. m. for a few hours, whereas Miller's (1943) patient was presumably exposed for several hours to concentrations of less than 8,000 p. p. m.

**Table 7.—INCIDENCE OF FATAL METHYL BROMIDE POISONINGS**

| Author                                          | Type of exposure                              | No. of cases |
|-------------------------------------------------|-----------------------------------------------|--------------|
| Schuler (1899).....                             | Chemical operation.....                       | 1            |
| Rohrer (1920).....                              | Chemical operation.....                       | 1            |
| Löffler and Rüttimeyer (1920).....              | Chemical operation.....                       | 1            |
| Goldschmid and Kuhn (1920).....                 | Chemical operation.....                       | 3            |
| Adler-Herzmark (1927).....                      | } Filling operation.....                      | 1            |
| Meixner (1928).....                             |                                               |              |
| Friemann (1937).....                            | Chemical operation.....                       | 1            |
| Duvoir, Fabre, and Layani (1939).....           | Chemical and filling operations.....          | 2            |
| Heimann (1944).....                             | Filling operation.....                        | 1            |
| Michaux, Clercy, and Lechevalier (1944-45)..... | Filling operation.....                        | 2            |
| Viner (1945).....                               | Filling operation.....                        | 1            |
| Total.....                                      | .....                                         | 14           |
| Mackie (1938).....                              | Fumigation.....                               | 1            |
| Schifferli (1942).....                          | Fumigation.....                               | 1            |
| Miller (1943).....                              | Fumigation.....                               | 1            |
| Bruhin (1943).....                              | Fumigation.....                               | 1            |
|                                                 |                                               | 4            |
| Wyers (1945).....                               | Fire extinguisher (use).....                  | 1            |
| Clarke, Roworth, and Holling (1945).....        | Fire extinguisher (leak).....                 | 2            |
| Nixon (quoted from Gray, 1944).....             | Fire extinguisher (leak).....                 | 3            |
| Holling and Clarke (1944).....                  | Fire extinguisher (accidental discharge)..... | 4            |
|                                                 |                                               | 10           |
| Total.....                                      | .....                                         | 28           |

The onset of toxic symptoms is usually delayed, and *this latent period* may vary considerably. It may be as short as 2 hours (Wyers, 1945); usually it is from 4 to 6 hours (Schuler, 1899, Löffler and Rüttimeyer, 1920, Friemann, 1937, Schifferli, 1942, and Clarke, Roworth, and Holling, 1945); but occasionally it may be longer (12, 24, or even 48 hours) as reported by Duvoir, Fabre, and Layani (1937), Rohrer (1920), and Goldschmid and Kuhn (1920).



Early *symptoms* of fatal methyl bromide poisoning are malaise, headache, visual disturbances, nausea, and vomiting. Sometimes the first symptoms are irritation of the skin (Bruhin, 1943) or smarting of the eyes (Clarke, Roworth, and Holling, 1945), listlessness, somnolence, and vertigo, and there are tremors of the hands. It seems to be only exceptionally that the poisoning starts very abruptly with severe symptoms such as sudden unconsciousness as reported by Goldschmid and Kuhn (1920) and Michaux, Clercy, and Lechevalier (1944-45). Later the tremors pass into twitchings and finally into convulsions which become more and more violent, and the intervals shorter and shorter. Between the onset of the convulsive stage and the very first symptoms there may be a period when the patient is delirious (Bruhin, 1943), disorientated and excited (manic) (Bruhin, 1943). The convulsions are of the epileptiform type and in the beginning are restricted to the extremities, but later, they affect the entire body. The respiration may be deep and labored, and as pulmonary edema develops there may be froth in and around the mouth. Less frequently the respiration may be rapid and shallow. In the early stages of the poisoning the pulse may be normal but toward the terminal stage it becomes rapid and shallow. In the beginning the skin is frequently flushed; this is followed later by cyanosis due to poor oxygenation of the blood in the edematous lungs. Frequently the body temperature rises to high levels as reported by Schuler (1899), Meixner (1928), Friemann (1937), and Heimann (1944). Whether this is the outcome of the convulsions or whether it is due to central stimulation is open to discussion. Death occurs either during the convulsive stage or while the patient is in coma following convulsions, most frequently within 4 to 6 hours although it may occasionally occur within 24 to 48 hours. It appears, therefore, that if the patient survives the first 2 days after the onset of toxic symptoms the prognosis as to his survival is relatively good. If death occurs early the immediate cause is frequently pulmonary edema (Wyers, 1945, Duvoir and Dérobert, 1944-45, and Clarke, Roworth, and Holling, 1945); if delayed, it is due to circulatory failure (Meixner, 1928) or to both (Clarke, Roworth, and Holling, 1945).

With regard to neurological findings in fatal poisonings, the picture is usually dominated by the clonic-tonic convulsions. The pupils are dilated, with poor or negative response to light, especially during the convulsive stage. The reflex excitability is either unchanged as reported by Wyers (1945) and Meixner (1928) or exaggerated as reported by Löffler and Rüttimeyer (1920) and Michaux, Clercy, and Lechevalier (1944-45). The same inconsistency exists with regard to Oppenheim and Babinski reflexes. In view of the rapid course and the seriousness of the clinical picture of fatal methyl bromide poisoning,

detailed clinical studies are exceptional. Löffler and Rüttimeyer (1920) noted in their patient a spinal pressure of 40 cc. of water; the spinal fluid contained blood. With regard to changes of the blood picture, Bruhin (1943) reported polyglobulia and hyperchromasia, Heimann (1944) noted an increase of hemoglobin above normal, and leucocytosis was reported by Löffler and Rüttimeyer (1920) and Heimann (1944). As to changes of the blood chemistry in methyl bromide poisoning, Löffler and Rüttimeyer (1920) noted a considerable increase in the blood urea (85 mg. percent) whereas Heimann (1944) found the non-protein nitrogen and creatinine values within normal limits (24.4 mg. percent and 1.8 mg. percent) and the uric acid content only slightly increased (5 mg. percent). Similarly, Viner (1945) found a serum urea nitrogen content of 14 mg. percent. The same author found, in a fatal case of methyl bromide poisoning, 211.6 mg. percent of serum bromide. With regard to urinary findings in fatal methyl bromide poisoning it appears that albuminuria of varying degree is the rule and occasionally casts and a few cells may be found, but other pathological constituents such as sugar, urobilin, urobilinogen, and acetone have not been reported.

With regard to *pathological changes in methyl bromide poisoning*, some reports refer to the marked lividity of the skin, to hyperemia of the conjunctiva, and to periorbital ecchymoses (Clarke, Roworth, and Holling, 1945). The tongue may be furred, perhaps as the outcome of dehydration by edema in other organs.

The most significant and characteristic changes are found in the *respiratory tract*. The trachea may contain blood-stained fluid (Clarke, Roworth, and Holling, 1945). The bronchi are hyperemic as reported by Schuler (1899) and Heimann (1944), inflamed (Goldschmid and Kuhn, 1920, Miller, 1943, and Wyers, 1945), or show the picture of purulent bronchitis (Friemann, 1937). The lungs are hyperemic and edematous as reported by Schuler (1899), Goldschmid and Kuhn (1920), Meixner (1928), Duvoir, Fabre, and Layani (1937 and 1939), Miller (1943), Duvoir and Dérobert (1944-45), Wyers (1945), and Clarke, Roworth, and Holling (1945), and they may show consolidation, especially in the lower lobes (Heimann, 1944), emphysematous areas (Duvoir and Dérobert, 1944-45, and Clarke, Roworth, and Holling, 1945), foci of bronchopneumonia (Friemann, 1937, Miller, 1943, and Viner, 1945), and hemorrhagic areas and hemorrhages into the alveoli (Heimann, 1944, Duvoir and Dérobert, 1944-45, Clarke, Roworth, and Holling, 1945, and Viner, 1945). The latter also noted considerable quantities of blood-stained fluid in the pleural cavity. It should be pointed out, however, that in one case of methyl bromide poisoning no pulmonary changes were reported (Bruhin, 1943).

The *heart* occasionally exhibits characteristic changes. Clarke, Roworth, and Holling (1945) noted dilatation of the right heart, and Heimann (1944) and Viner (1945) saw scattered petechial hemorrhages on the epicardial surface.

The *abdominal organs* offer no persistent pathological changes. The liver may be normal, pale yellow, congested, or show early fatty

**Table 8.—Incidence of nonfatal methyl bromide poisonings**

A. SYSTEMIC POISONINGS

| Author                                          | Type of exposure                              | Number of cases |
|-------------------------------------------------|-----------------------------------------------|-----------------|
| Schuler (1899).....                             | Chemical operation.....                       | 2               |
| Jaquet (1901).....                              | Chemical operation.....                       | 3               |
| Bing (1910).....                                | Chemical operation.....                       | 2               |
| Floret (1915).....                              | Chemical operation.....                       | 3               |
| Steiger (1918).....                             | Chemical operation.....                       | 1               |
| Löffler and Rüttimeyer (1920).....              | Chemical operation.....                       | 1               |
| Goldschmid and Kuhn (1920).....                 | Chemical operation.....                       | 6               |
| Cadé and Mazel (1923).....                      | Chemical operation.....                       | 2               |
| Tietze (1933).....                              | Filling operation.....                        | 2               |
| Oppermann (1933).....                           | Chemical operation.....                       | 2               |
| DeMorsier and Steinmann (1936).....             | Chemical operation.....                       | 2               |
| Mackie (1938).....                              | Laboratory.....                               | 4               |
| Duvor, Fabre, and Layani (1939).....            | Chemical operation.....                       | 3               |
| Watrous (1942).....                             | Filling operation.....                        | 31              |
| DeJong (1944).....                              | Chemical and filling operations.....          | 3               |
| Heimann (1944).....                             | Filling operation.....                        | 3               |
| Wyers (1945).....                               | Filling operation.....                        | 3               |
| Viner (1945).....                               | Filling operation.....                        | 1               |
| Ramond and Lacorne (1942).....                  | Filling operation.....                        | 1               |
| Pagniez and Plichet (1942).....                 | Filling operation.....                        | 1               |
| Michaux, Courchet, and Lechevalier (1944).....  | Filling operation.....                        | 7               |
| Michaux, Clercy, and Lechevalier (1944-45)..... | Filling operation.....                        | 11              |
|                                                 |                                               | 94              |
| Schifferli (1942).....                          | Fumigation.....                               | 1               |
| Bruhü (1943).....                               | Fumigation.....                               | 2               |
| Johnstone (1945).....                           | Fumigation.....                               | 34              |
|                                                 |                                               | 37              |
| Clarke, Roworth, and Holling (1945).....        | Fire extinguisher (leak).....                 | 2               |
| Holling and Clarke (1944).....                  | Fire extinguisher (accidental discharge)..... | 7               |
| Holling and Clarke (1944).....                  | Fire extinguisher (leak).....                 | 3               |
|                                                 |                                               | 13              |
| Total.....                                      |                                               | 144             |

B. SYSTEMIC POISONINGS ASSOCIATED WITH SKIN INJURIES

|                        |                                               |   |
|------------------------|-----------------------------------------------|---|
| Carter (1945).....     | Chemical and filling operations.....          | 2 |
| Schifferli (1942)..... | Fumigation.....                               | 2 |
| Gray (1944).....       | Fire extinguisher (accidental discharge)..... | 2 |
|                        |                                               | 6 |

Table 8.—*Incidence of nonfatal methyl bromide poisonings—Continued* ]

## C. SKIN INJURIES ONLY

| Author                                  | Type of exposure                              | Number of cases |
|-----------------------------------------|-----------------------------------------------|-----------------|
| Mackie (1938).....                      | Laboratory.....                               | 2               |
| Watrous (1942).....                     | Filling operation.....                        | 22              |
| Wyers (1945).....                       | Filling operation.....                        | 2               |
|                                         |                                               | 26              |
| Billet and Abel (1940).....             | Fire extinguisher (tank).....                 | 1               |
| Schifferli (1942).....                  | Fire extinguisher (use).....                  | 2               |
| Gray (1944).....                        | Fire extinguisher (accidental discharge)..... | 1               |
| Butler, Perry, and Williams (1945)..... | Fire extinguisher (tank).....                 | 2               |
|                                         |                                               | 6               |
| Total.....                              |                                               | 32              |

degeneration; the spleen may be hyperemic and enlarged; and the kidneys may be hyperemic and show cloudy swelling and degenerative changes in the tubular apparatus. The lining of the stomach may be hyperemic. Heimann (1944) saw hemorrhagic congestion of the adrenals.

Next to the lungs the most important pathological changes are seen in the *brain*. The meninges and the brain are hyperemic (Schuler, 1899, Duvoir, Fabre, and Layani, 1937 and 1939, Heimann, 1944, Duvoir and Dérobert, 1944-45, Clarke, Roworth, and Holling, 1945, Meixner, 1928, and Wyers, 1945) and the latter may show petechial hemorrhages in various areas. Frequently the brain tissue is edematous as reported by Friemann (1937), Duvoir, Fabre, and Layani (1937 and 1939), Bruhin (1943), and Heimann (1944), and ganglionic changes characterized by fatty degeneration, shrinkage, and pyknosis may be found in different sections (Goldschmid and Kuhn, 1920, Friemann, 1937, and Bruhin, 1943). According to Meixner (1928) the large nuclei in the base of the brain seem to be the first to show edematous changes.

The pathological findings in fatal methyl bromide poisoning are, therefore, in accordance with the clinical picture. The immediate cause of death is evidently the pulmonary injury associated with circulatory failure, whereas the cerebral changes, although significant for the clinical picture, are as a rule less extensive.

Table 8 gives a summary of 150 *nonfatal methyl bromide poisonings* as reported in the literature, 6 of which are associated with more severe cutaneous lesions; in addition it lists 32 cases of skin injury only.

*The clinical picture of nonfatal methyl bromide poisoning* shows great variations. Although many of the symptoms discussed below are



quite common, their sequence is not always the same. The onset of toxic symptoms usually occurs after a latent period of several hours. The most common early symptoms are the feeling of fatigue and complaints about blurred and double vision mentioned by Jaquet (1901), Floret (1915), Steiger (1918), Cadé and Mazel (1923), DeMorsier and Steinmann (1936), Mackie (1938), Heimann (1944), DeJong (1944), Carter (1945), Viner (1945), and Wyers (1945). They may also occur in a later stage of the poisoning as reported by Carter (1945), and pass into temporary blindness (Jaquet, 1901, Duvoir, Fabre, and Layani, 1937, and Wyers, 1945). They may be associated with conjunctivitis as reported by Jaquet (1901), Heimann (1944), Clarke, Roworth, and Holling (1945) or with edema of the eyelids as observed by Schifferli (1942). On the other hand, no serious eye injuries have been reported even in cases of direct spills into the eyes (Mackie, 1938, and Wyers, 1945). Many patients suffer from headache (Cadé and Mazel, 1923, Oppermann, 1933, DeMorsier and Steinmann, 1936, Duvoir, Fabre, and Layani, 1937, Mackie, 1938, Michaux, Clercy, and Lechevalier, 1944-45, Viner, 1945, and others). This may be associated with tinnitus (Tietze, 1933, DeJong, 1944, and Wyers, 1945), acoustic hyperesthesia (Michaux, Clercy, and Lechevalier, 1944-45), dizziness (Jaquet, 1901, Mackie, 1938, Schifferli, 1942, and DeJong, 1944), or vertigo (Steiger, 1918, Oppermann, 1933, Duvoir, Fabre, and Layani, 1937, and DeJong, 1944). Occasionally there are complaints about pain in joints (Watrous, 1942, and Michaux, Clercy, and Lechevalier, 1944-45). Many patients experience at an early stage of the poisoning (sometimes later) nausea and vomiting, as reported by Schuler (1899), Jaquet (1901), Floret (1915), Tietze (1933), DeMorsier and Steinmann (1936), Duvoir, Fabre, and Layani (1937), Mackie (1938), Bruhin (1943), and Wyers (1945). Many victims suffer from confusion (Jaquet, 1901, Tietze, 1933, DeMorsier and Steinmann, 1936, Heimann, 1944, and Clarke, Roworth, and Holling, 1945) and loss of memory (Tietze, 1933, and Wyers, 1945). They may become somnolent, shortly after exposure, as observed by Steiger (1918), Ramond and Lacorne (1942), and Bruhin (1943), or occasionally later, as seen by DeJong (1944). Patients may suffer from fainting spells (Bruhin, 1943) and fall into unconsciousness (Schuler, 1899, Tietze, 1933, Viner, 1945, and Wyers, 1945) and later into collapse (Schuler, 1899). At an early stage the speech may become impaired, blurred, and hesitant (Cadé and Mazel, 1923, Tietze, 1933, Duvoir, Fabre, and Layani, 1937, Ramond and Lacorne, 1942, Michaux, Clercy, and Lechevalier, 1944-45, Wyers, 1945, Viner, 1945, and Carter, 1945) and this may pass into aphasia as seen by Duvoir, Fabre, and Layani (1937). Many patients complain of muscular weakness (Oppermann,



1933, DeJong, 1944, Carter, 1945, Viner, 1945, and Wyers, 1945) and this may lead to incoordination and pareses of some or all limbs as reported by Jaquet (1901), Steiger (1918), Cadé and Mazel (1923), Duvoir, Fabre, and Layani (1937), Ramond and Lacorne (1942), DeJong (1944), Michaux, Clercy, and Lechevalier (1944-45), Carter (1945), Viner (1945), and Wyers (1945). This incoordination may be associated with tremors as seen by Schuler (1899), Steiger (1918), Tietze (1933), Ramond and Lacorne (1942), DeJong (1944), Michaux, Clercy, and Lechevalier (1944-45), and Wyers (1945), and these tremors may pass into twitchings (Schuler, 1899, and Viner, 1945) and finally into convulsions as reported by Schuler (1899), DeMorsier and Steinmann (1936), Duvoir, Fabre, and Layani (1937), Mackie (1938), Ramond and Lacorne (1942), Michaux, Clercy, and Lechevalier (1944-45), Wyers (1945), Clarke, Roworth, and Holling (1945), Viner (1945), and others. These convulsions are frequently restricted at first to one extremity but gradually spread to others and finally affect the musculature of the entire body. Occasionally these attacks may be preceded by prodromal symptoms and an aura as reported by Tietze (1933). Some patients experience at an early stage, others later, paresthesias and analgesias (Cadé and Mazel, 1923, Duvoir, Fabre, and Layani, 1937, DeJong, 1944, Michaux, Clercy, and Lechevalier, 1944-45, Carter, 1945, Viner, 1945, and others). At times the body temperature may be elevated as seen by Schuler (1899) and Tietze (1933) and the patient may suffer from chills as reported by Mackie (1938). The symptoms described above may be associated with or followed by psychic disturbances such as euphoria (Wyers, 1945), delirium (Floret, 1915, and Wyers, 1945), or psychoses (Jaquet, 1901, Bing, 1910, Floret, 1915, Steiger, 1918, and others). It should be pointed out that in contrast to the diversity and severity of the neurological symptoms other organ functions are affected comparatively little. In most cases the blood pressure is normal and the heart action unimpaired, although occasionally cyanosis (Wyers, 1945) and tachycardia (Floret, 1915, and DeJong, 1944) have been reported. In contrast to fatal poisonings, which as shown above are frequently associated with severe pulmonary injury, the respiratory organs seem to be affected comparatively little in nonfatal poisonings. Sometimes the patient loses his appetite and body weight as reported by DeJong (1944) and Viner (1945). The incidence of these various signs and symptoms is illustrated in table 9 which is taken from the publication of Johnstone (1945) who reported on 34 cases of methyl bromide poisoning in a date-packing operation where concentrations of 100-500 p. p. m. of methyl bromide in air were found. In these cases the high incidence of visual, speech, and sensory disorders and of mental confusion is most conspicuous. In most instances fatigue,

impaired speech, and, especially, impaired vision were among the first serious symptoms. Watrous (1942) reported on 31 cases of light methyl bromide poisoning in an ampoule-filling operation resulting from an exposure to about 35 p. p. m. of methyl bromide in air, the signs and symptoms showed the following distribution: 23 of 31 cases developed anorexia, nausea, and in severe cases, vomiting; 16, headache (not severe and only during exposure); 4, vertigo; 4, mild visual disturbances; 3, sleepiness; 3, severe epigastric and substernal pain; 2, faintness; 2, doyness; 2, dimmed vision; 2, severe muscular pain (late); 1, amaurosis lasting 12 hours.

Michaux, Courchet, and Lechevalier (1944) noted such prodromal symptoms as headache, vertigo, somnolence, and mental depression in 6/7 of their patients; auditory hyperesthesia in 4/7; pain in joints in 3/7; psychoses in 2/7; adiadokokinesis and intention tremor in 5/7; epileptic convulsions in 4/7; and symptoms of injury of the pyramidal tract in 2/7.

**Table 9.—INCIDENCE OF VARIOUS SIGNS AND SYMPTOMS OF METHYL BROMIDE POISONING**

(Johnstone, 1945)

| Symptoms                     | Total | Intensity |          |       | Duration |           |               |                    |
|------------------------------|-------|-----------|----------|-------|----------|-----------|---------------|--------------------|
|                              |       | Severe    | Moderate | Mild  | Few days | Few weeks | 2 to 5 months | Possibly permanent |
| Visual disturbances.....     | 34    | 2         | 7        | 25    | 27       | 5         | 2             | 0                  |
| Speech disturbances.....     | 23    | 5         | 8        | 10    | 23       | 0         | 0             | 0                  |
| Mild mental confusion.....   | 15    | -----     | -----    | 15    | 15       | 0         | 0             | 0                  |
| Hallucinations.....          | 7     | 7         | -----    | ----- | 7        | 0         | 0             | 0                  |
| Melancholia.....             | 2     | -----     | -----    | 2     | -----    | -----     | 2             | 0                  |
| Coma.....                    | 4     | 4         | -----    | ----- | 4        | 0         | 0             | 0                  |
| Convulsions.....             | 1     | 1         | -----    | ----- | 1        | 0         | 0             | 0                  |
| Apathy.....                  | 1     | 1         | -----    | ----- | -----    | -----     | -----         | 1                  |
| Tremor.....                  | 4     | -----     | -----    | 4     | -----    | 3         | 1             | -----              |
| Neurosis.....                | 2     | -----     | 2        | ----- | -----    | -----     | -----         | 2                  |
| Fainting attacks.....        | 3     | -----     | -----    | 3     | -----    | 2         | 1             | -----              |
| Numbness of extremities..... | 18    | -----     | -----    | 18    | 4        | 11        | 3             | -----              |

Comparison of these data with those given in table 9 illustrates the dependence of the clinical picture upon the intensity of the exposure.

As indicated in table 9, severe and even moderate poisonings may lead to prolonged illness and possibly to permanent injury. This is also illustrated by other case histories reported in the literature. After two months had elapsed Steiger's (1918) patient still suffered from sensory disturbances, Cadé and Mazel's (1923) was still nervous and easily fatigued, and one of DeJong's (1944) patients suffered

from weakness, paresthesias, and blurred vision. The other of DeJong's patients showed neurological abnormalities even after 3 months. One of the patients reported by Bruhin (1943) showed neurological abnormalities after one year, two of Michaux, Clercy, and Lechevalier (1944-45) after 6 and 7 months; Wyers (1945) and Viner (1945) after 8 months; Jaquet (1901) after 9 months. It appears, therefore, that systemic effects resulting from methyl bromide poisoning may persist and incapacitate the individual for a long time.

Aside from the systemic effects discussed above, *methyl bromide causes marked local reactions*. These may precede the systemic effects as reported by Schifferli (1942), Bruhin (1943), and others; they may follow a moderate systemic reaction as seen by Billet and Abel (1940); or they may be the sole symptoms in case the toxic agent comes in contact with the skin only. Exposure to high concentrations (about 8000 p. p. m.) of the vapors of methyl bromide in air may cause itching and prickling of the skin; later erythema and formation of vesicles, which have a tendency to form blisters, may occur. As pointed out by these authors, these effects are most marked in parts of the body which are irritated by close fitting garments. Quite frequent are those skin injuries which result from splashes of methyl bromide on the skin, even if it is protected by garments, boots, and gloves, as illustrated by the reports of Mackie (1938), Wyers (1945), Butler, Perry, and Williams (1945), and Watrous (1942). Watrous published a series of 22 cases of skin injury from methyl bromide observed in an ampoule-filling operation. When liquid methyl bromide is spilled on the skin it evaporates immediately, producing a cool (but not cold) burning sensation. The repeated application of small quantities or single applications of large quantities give rise to a characteristic train of symptoms. Immediately following the contact there is a tingling sensation and burning; with more severe exposure this is followed by numbness and later by aching pain. In the early stages the skin appears red and slightly swollen. After several hours, with sufficiently severe exposure, vesicles or blebs appear on the exposed surface. Resembling second degree burns, they are markedly distended and contain straw-colored fluid, but show little tendency to refill after being punctured. In case the exposure is not sufficiently severe to produce blisters, frequently a fine papular, vesicular, itching dermatitis develops after a latent period of 7 or more days. In most cases healing is followed by considerable desquamation of the skin similar to that seen after sunburn. Carter (1945) considered this to be the outcome of topical disturbances. It has been shown by Watrous (1942) that these skin injuries are not thermal effects, and it appears that they are the result of a toxic effect of methyl bromide

on the peripheral nerve endings, causing degenerative changes of a similar nature as described by Hueper (1936) in blister formation following prolonged contact with carbon disulfide.

With regard to *the clinical detection of methyl bromide poisoning*, as a rule the blood pressure shows no abnormalities, and, as pointed out above, the heart action is not impaired except in rare cases. In contrast to the findings in fatal methyl bromide poisonings resulting from massive exposure, less severe poisonings cause only occasionally clinical changes of the lungs. Similarly, the abdominal organs show only occasional abnormalities such as enlargement of the liver as reported by Heimann (1944). More frequently there is some injury of the kidneys indicated by the presence of albumin in the urine as reported by Jaquet (1901), Steiger (1918), Tietze (1933), and Viner (1945); of casts and red blood cells as reported by the latter two authors; and of polyuria as observed by Ramond and Lacorne (1942). As illustrated in table 10, the hematological studies reported on non-fatal cases of methyl bromide poisoning offer very little that is characteristic. In most instances there is a moderate increase of the white blood cells due to a moderate increase of the polymorphonuclears and a reduction of the lymphocytes, and there may be an increase of the eosinophiles, stabforms, basophiles, and juvenile cells, but reports on such findings are too limited to allow an evaluation of their importance for the clinical picture of methyl bromide intoxication. Similarly, the blood chemistry offers very little evidence in making the diagnosis of methyl bromide poisoning. Tietze (1933) and Wyers (1945) found normal values for the blood sugar, and only in severe poisoning did the former note a temporary increase of the blood urea (65 mg. percent) and of the bilirubin (1.4 mg. percent). As shown by DeJong (1944), Viner (1945), and Clarke, Roworth, and Holling (1945), the bromide level in the blood may be increased for a considerable period of time, and this may help in establishing the diagnosis of methyl bromide poisoning. The neurological examination alone will aid in the diagnosis of methyl bromide poisoning. As illustrated in table 11, the patellar reflexes are most often exaggerated and in more severe cases this is associated with patellar clonus; the same holds true for the achilles reflexes, whereas abdominal and cremaster reflexes are evidently less frequently increased. Babinski's and Oppenheim's reflexes are often positive. Romberg's syndrome is also often positive and the same holds true for nystagmus and intention tremor. The coordination is often impaired as indicated by the reports of Jaquet (1901), Steiger (1918), Cadé and Mazel (1923), Duvoir, Fabre, and Layani (1937), DeJong (1944), Carter (1945), Wyers (1945), and others. There may be pareses as observed by Steiger (1918), and analgesias and paresthesias. These may be among the early symp-



Table 10.—CHANGES OF THE BLOOD PICTURE IN METHYL BROMIDE POISONING

| Red blood cells millions | Hemo-<br>globin percent | White blood cells thousands | Differential counts (percent) |             |       |           |                   |           |       |              |                            | Author                                  |           |
|--------------------------|-------------------------|-----------------------------|-------------------------------|-------------|-------|-----------|-------------------|-----------|-------|--------------|----------------------------|-----------------------------------------|-----------|
|                          |                         |                             | Poly-<br>morpho-<br>nuclears  | Lymphocytes |       | Monocytes | Eosino-<br>philes | Juveniles | Stabs | Basophililes | Myelocytes and myeloblasts |                                         | Platelets |
|                          |                         |                             |                               | Large       | Small |           |                   |           |       |              |                            |                                         |           |
| 5.4                      | 115                     | 7.4                         | 54                            | 38          | 4.0   | 1.9       | -----             | 0.8       | 0.5   | -----        | -----                      | Normal. <sup>1</sup><br>Steiger (1918). |           |
| 5.3                      | 84                      | 6.5                         | 48                            | 38          | 7     | 5         | -----             | -----     | 1.8   | 0.2          | -----                      |                                         |           |
| 4.82                     | 94                      | 9.3                         | 77                            | 10          | 4     | -----     | 0.5               | 8.5       | ----- | -----        | -----                      | Tietze (1933).                          |           |
| 3.6                      | 86                      | 13.3                        | -----                         | ---         | ----- | -----     | -----             | -----     | ----- | -----        | -----                      | Heimann (1944).                         |           |
| 3.8                      | 72                      | -----                       | -----                         | ---         | ----- | -----     | -----             | -----     | ----- | -----        | -----                      | Heimann (1944).                         |           |
| 3.47                     | 78                      | 7.7                         | -----                         | ---         | ----- | -----     | -----             | -----     | ----- | -----        | -----                      | Heimann (1944).                         |           |
| 4.8                      | 97                      | 17.25                       | 84                            | 9           | 7     | -----     | -----             | -----     | ----- | -----        | Normal                     | DeJong (1944).                          |           |
| 4.7                      | 90                      | 9.0                         | 63                            | 30          | 5     | 2         | -----             | -----     | ----- | -----        | -----                      | DeJong (1944).                          |           |
| 4.85                     | 90                      | 9.0                         | 70                            | 26          | ----- | -----     | -----             | -----     | ----- | -----        | -----                      | Carter (1945).                          |           |
| 4.4                      | -----                   | 9.8                         | 90                            | 8           | 2     | 0         | -----             | -----     | ----- | -----        | -----                      | Wyers (1945).                           |           |
| 5.6                      | 115                     | 10.0                        | -----                         | ---         | ---   | -----     | -----             | -----     | ----- | -----        | -----                      | Viner (1945).                           |           |

<sup>1</sup> Normal values for males of 14-30 years of age or 19-30 years, respectively, according to Osgood, 1935.

Table 11.—BEHAVIOR OF VARIOUS REFLEXES IN METHYL BROMIDE POISONING

| Patellar | Patellar clonus | Achillea | Achillea clonus | Abdominal | Cremaster | Babinski | Oppenheim | Romberg | Nystagmus | Intention tremor | Author                 |
|----------|-----------------|----------|-----------------|-----------|-----------|----------|-----------|---------|-----------|------------------|------------------------|
| ±        | -----           | 0        | -----           | +++       | Normal    | +        | -----     | -----   | -----     | -----            | Carter (1945).         |
| +        | -----           | +        | -----           | -----     | -----     | -----    | -----     | -----   | -----     | -----            | Cadé and Mazel (1923). |
| ++       | -----           | -----    | -----           | -----     | -----     | -----    | -----     | -----   | -----     | -----            | Schuler (1899).        |
| ++       | +               | Normal   | -----           | Normal    | -----     | -----    | -----     | ++      | +         | -----            | Tietze (1933).         |
| ++       | -----           | ++       | -----           | +         | 0         | ±        | ±         | +       | -----     | +                | Steiger (1918).        |
| ±        | 0               | -----    | -----           | -----     | -----     | -----    | -----     | +       | +         | -----            | Floret (1915).         |
| +        | -----           | ++       | ±               | -----     | -----     | ±        | -----     | -----   | +         | -----            | Floret (1915).         |
| ++       | -----           | ++       | ±               | -----     | -----     | ++       | -----     | +       | +         | +                | DeJong (1944).         |
| ++       | -----           | Reduced  | -----           | -----     | -----     | -----    | -----     | +       | +         | -----            | DeJong (1944).         |
| -----    | -----           | -----    | -----           | -----     | -----     | -----    | -----     | -----   | +         | -----            | Carter (1945).         |



**TABLE 12.—CHANGES OF PRESSURE AND CHEMISTRY OF THE CEREBROSPINAL FLUID IN METHYL BROMIDE POISONING**

| Author                                          | Initial pressure | Appearance      | Cells per (cubic millimeter) mm. <sup>3</sup> | Glob-ulin   | Protein mg. percent | Chlo-rides as NaCl mg. percent | Bro-mides as NaBr mg. percent | Non-protein nitro-gen mg. percent | Sugar mg. percent | Urea mg. percent | Nonne test glob-ulin | Pandy test glob-ulin | Mei-necke   |
|-------------------------------------------------|------------------|-----------------|-----------------------------------------------|-------------|---------------------|--------------------------------|-------------------------------|-----------------------------------|-------------------|------------------|----------------------|----------------------|-------------|
| Normal <sup>1</sup> .....                       | 70-190.....      | Clear.....      | 0-5.....                                      | 0.....      | 15-45.....          | 720-750.....                   | <sup>2</sup> 1.....           | 12-18.....                        | 50-75.....        | 12-15.....       | .....                | .....                | .....       |
| DeMorsier and Steinmann (1936).....             | Normal.....      | .....           | .....                                         | .....       | .....               | .....                          | .....                         | .....                             | .....             | .....            | .....                | .....                | .....       |
| Tietze (1933).....                              | Normal.....      | .....           | .....                                         | .....       | .....               | .....                          | .....                         | .....                             | .....             | .....            | .....                | .....                | .....       |
| DeJong (1944).....                              | Normal.....      | Clear.....      | 3.....                                        | .....       | 48.....             | .....                          | .....                         | .....                             | .....             | .....            | .....                | .....                | .....       |
| Cadé and Mazel (1923).....                      | Normal.....      | .....           | 0.....                                        | .....       | 25.....             | .....                          | .....                         | .....                             | .....             | .....            | .....                | .....                | .....       |
| Wyers (1945).....                               | Normal.....      | Opalescent..... | .....                                         | .....       | 45.....             | .....                          | 16.....                       | .....                             | Normal.....       | .....            | .....                | .....                | .....       |
| Wyers (1945).....                               | Increased.....   | .....           | { WBC 2<br>RBC 20 }                           | 0.....      | 40.....             | 740.....                       | .....                         | .....                             | 91.....           | 32.....          | .....                | .....                | .....       |
| Oppermann (1933).....                           | 200.....         | .....           | 2-3.....                                      | +           | .....               | .....                          | .....                         | .....                             | .....             | .....            | +                    | 0.....               | 0.....      |
| DeJong (1944).....                              | 150.....         | .....           | 0.....                                        | .....       | 23.....             | .....                          | .....                         | .....                             | .....             | .....            | .....                | .....                | .....       |
| Michaux, Clercy, and Lechevalier (1944-45)..... | Normal.....      | Normal.....     | Normal.....                                   | Normal..... | Normal.....         | Normal.....                    | Normal.....                   | Normal.....                       | Normal.....       | Normal.....      | Normal.....          | Normal.....          | Normal..... |
| Víner (1945).....                               | Normal.....      | Normal.....     | Normal.....                                   | Normal..... | Normal.....         | Normal.....                    | Normal.....                   | Normal.....                       | Normal.....       | Normal.....      | Normal.....          | Normal.....          | Normal..... |

<sup>1</sup> Osgood (1935).

<sup>2</sup> Wyers (1945).

toms as seen by Floret (1915), Steiger (1918), and Cadé and Mazel (1923), or they may become manifest later as reported by DeJong (1944) and others. Tietze (1933) considered chronaxy measurements as a sensitive indicator in methyl bromide poisoning, and the same opinion was expressed by Schwarz (1937). As illustrated in table 12, examination of the spinal pressure is less liable to be of diagnostic value than the neurological examination. With one exception (Wyers, 1945) this has been found to be within normal limits; the spinal fluid has usually been found to be clear and of normal composition. Only in severe cases can abnormalities be expected. This contrast between positive neurological findings and the negative behavior of spinal pressure and spinal fluid indicates that the injurious effect of methyl bromide on the central nervous system is due more to injury of the ganglionic cells and less to circulatory disturbances in the brain. The visual disturbances, such as blurred and double vision, are probably due to pareses of the oculomotor nerves. According to Steiger (1918), although occasionally stasis in the small vessels of the optic disc and of the papillary zone and small retinal hemorrhages have been reported, the different structures of the eye (cornea, lens, anterior and posterior chamber, and eyeground) offered in his case no pathological findings.

With regard to *the treatment of methyl bromide poisoning* it appears that moderate symptoms will disappear upon discontinuation of the exposure and transfer of the patient to fresh air. All soiled garments should be removed as soon as possible. The patient should be kept under observation and at rest for at least 48 hours. In case he becomes cyanotic, straight oxygen should be given intermittently as used by Heimann (1944) and Wyers (1945). If signs and symptoms of incipient pulmonary edema should develop, venesection may be indicated. Moderate convulsions may be controlled by phenobarbital, but in the experience of Clarke, Roworth, and Holling (1945) even the intravenous administration of barbiturates may not be sufficient to control severe convulsions and in such cases the inhalation of chloroform and oxygen may be the treatment of choice. In case of enlargement of the liver (Heimann, 1944) or epigastric pain (Gray, 1944) glucose may be administered intravenously, but such injections should be given only if there is no danger of pulmonary edema. With regard to the treatment of skin lesions, Wyers (1945) suggested bathing the affected parts of the body with sodium carbonate solution in order to destroy any methyl bromide present. Watrous (1942) advocated aspirating the fluid from the blisters and covering these with a cod liver oil ointment. Butler, Perry, and Williams (1945) removed the dead epithelium and covered the area with 2 percent tannic acid in triple dye solution. In view of the recent unfavorable experience with the use of tannic acid for burns, this procedure appears to be inadvisable,

especially since these authors also got good results with the application of a sulfathiazole powder containing 5 percent light magnesium oxide and 2000 units of penicillin per gram and subsequent dressing, given on three occasions at 48-hour intervals. They obtained, however, optimal results by leaving the blisters intact, cleaning the affected area with saline, and covering the whole area with a propamidine-isothionate cream and renewing this every 48 hours.

#### THE MECHANISM OF ACTION OF METHYL BROMIDE

In severe acute methyl bromide poisoning of man and animals, the toxicological picture is characterized by pulmonary edema, circulatory failure, and nervous disturbances. It appears at present open to discussion whether the pulmonary injury is primary or secondary to the circulatory changes and whether methyl bromide acts fundamentally on the vascular system as assumed by Duvoir, Fabre, and Layani (1937). But in less acute poisonings signs or symptoms referring to the circulatory or respiratory apparatus may be insignificant or even absent, and the picture may be entirely controlled by neurological symptoms so that it appears that methyl bromide does not produce, like other aliphatic narcotics, a functional depression of certain nervous structures which is retrogressive as soon as the narcotic agent is eliminated, but it does cause severe and possibly irreparable damage of the central nervous system. This is in accordance with the observation of histopathological changes in the ganglionic cells of victims of methyl bromide poisoning as reported by Goldschmid and Kuhn (1920), Friemann (1937), and Bruhin (1943). On the other hand, Irish, Adams, Spencer, and Rowe (1940), and others were unable to find any histopathological changes in animals which had shown definite but retrogressive neurological symptoms; from this it appears that methyl bromide also causes functional changes. These may be of a different character and produced by a different mechanism from those produced by other aliphatic narcotics.

It is generally accepted that the narcotic action of aliphatic narcotics such as chloroform is due to the entire molecule and is closely connected with their lipid solubility, and that the intensity of their action is determined at least in part by their partition coefficient oil/water or their solubility coefficient in oils. That this holds true for the toxic manifestations of methyl bromide is questionable. As compared with chloroform and its higher homologue, ethyl bromide, the narcotic action of methyl bromide (that is, the depressing effect on the cerebral cortex) is comparatively slight as is shown by the findings of Meyer and Gottlieb-Billroth (1921). These investigators determined the minimal narcotic concentration in volume percent with mice as  $>2.3$  for methyl bromide,  $1.9 \pm 0.3$  for ethyl bromide, and  $0.44 \pm 0.04$  for

chloroform, and the solubility coefficient of these compounds in vegetable oils as  $32 \pm 1.5$  for methyl bromide,  $95 \pm 4$  for ethyl bromide, and  $265 \pm 7$  for chloroform. It is, therefore, apparent that the narcotic action of methyl bromide is in accordance with its relatively low lipoid solubility, and that the toxic manifestations characteristic for methyl bromide which are not shown either by ethyl bromide or by chloroform must be due to a different mechanism. Lewin and Guilery (1913) left unanswered the question as to whether the toxic manifestations of methyl bromide were due to the intact molecule, the bromine content, or the methyl radical. Irish, Adams, Spencer, and Rowe (1941) demonstrated that in rabbits showing functional changes after repeated exposures for 8 hours to a concentration of 60 p. p. m. of methyl bromide in air the inorganic bromide level in the blood increased from a normal of 1 mg. percent to 11 mg. percent. However, when rabbits were fed sodium bromide in sufficient doses to produce this and even higher levels of bromide in the blood (62 percent) they failed to show any functional response similar to that observed in animals fed methyl bromide. Similarly, rabbits exposed to methanol vapors in concentrations equivalent to or many times higher than those which would result from such concentrations of methyl bromide as used in the exposure experiments to methyl bromide failed to show a functional response similar to that produced by methyl bromide. Such negative results were also seen in rabbits exposed to 5,000 p. p. m. methanol in air and receiving at the same time daily doses of 0.1 gm./kg. of sodium bromide, in spite of the fact that the bromide level in their blood rose as high as 90 mg. percent. It is, therefore, evident that neither a high bromide level nor a high methanol level, or a combination of both in the blood results in the typical picture of methyl bromide poisoning. Irish, Adams, Spencer, and Rowe (1941) found that feeding rabbits 22 daily doses of 0.03 gm./kg. of methyl bromide dissolved in olive oil produced a bromide level of 20 mg. percent in the blood and the same type of response (paralysis) as seen after the inhalation of methyl bromide vapors, but that it required 50 daily doses of 0.30 gm./kg. of ethyl bromide with a bromide level of 42 mg. percent in the blood to elicit the same effect. From this they concluded that the typical response to methyl bromide is a function of the methyl bromide molecule proper. It should, however, be pointed out that according to Miller and Haggard (1943) olive oil alone, given in doses up to 15 cc. daily over a period of 3 to 8 weeks, produces in rabbits loss of weight, illness, sometimes paralysis of the hind legs, and in most animals death. They also showed that rats, which tolerate large doses of olive oil better than rabbits, died within 5 to 7 hours after the oral administration of 100 mg./kg. of methyl bromide, whereas animals given as much as 1200 mg./kg. of ethyl bromide in

the same way neither died nor showed any serious ill effects. It should also be pointed out that ethyl bromide has been used as a narcotic agent without causing any signs or symptoms similar to those resulting from even less severe exposure to methyl bromide. The few cases of human ethyl bromide poisoning reported by Jendritza (1892) and Scherbatscheff (1902) have nothing in common with the clinical picture of methyl bromide poisoning except a delayed action. It appears, therefore, that the toxic effects of methyl bromide are fundamentally different from those produced by ethyl bromide, and that one has to look for a different explanation for the peculiar effects of methyl bromide. Miller and Haggard (1943) refer to Harrison, Darrow, and Yannet (1936), Manery and Hastings (1939), and Wallace and Brodie (1939) as having demonstrated that the bromide ions present in the blood stream do not penetrate the cellular membranes in appreciable quantity (except red blood cells) but that they are limited to the extracellular fluid. They pointed out that alkyl halides, such as methyl bromide, penetrate the cells and dissolve in the intracellular fluid. In case hydrolysis takes place within the cells, bromide ions are liberated within the cell where they are trapped because they pass only with difficulty through the cellular membrane and therefore their pharmacodynamic action is prolonged. They assumed further that under these conditions even small quantities of bromide may produce an atypical and prolonged reaction and that for this reason the bromide level in the blood would be no indication of the extent of the bromide action. They presented experimental data to show that in rats there is a different distribution of bromide between intracellular and extracellular fluid after the oral administration of methyl bromide in oil than after the oral administration of sodium bromide in water, whereas the distribution of bromide after oral administration of ethyl bromide in oil corresponds closely to that found after sodium bromide, indicating that more bromide is retained within the cells after administration of methyl bromide than after administration of ethyl bromide or sodium bromide. As pointed out by Miller and Haggard (1943) there is at present no evidence that the intracellular bromide has certain pharmacological peculiarities not yet demonstrated or that within the cell it may cause direct organic injury to the cell. They also pointed out that in case the intracellular bromide is formed from intracellular hydrolysis of methyl bromide, the other product of hydrolysis should be methanol, that for each mg. of sodium bromide 0.311 mg. of methanol should be formed, and that with a retention of a total average amount of 109.8 mg. per kg. of sodium bromide at death the corresponding quantity of methanol would be 33.1 mg. per kg., an amount which would cause no symptoms of poisoning. It appears, however, that with some justification one



can also assume that the same quantity of methanol formed within the cells may have more marked effects than when distributed throughout the organism. As pointed out by Jaquet (1901) the similarity between the toxic action of methyl bromide and methyl iodide on the one hand and the difference in the toxicological picture between methyl bromide and ethyl bromide on the other, point to the importance of the methyl group for the characteristic action of methyl bromide. Although the possibility of intracellular hydrolysis with the formation of bromide and methanol and its oxidation products formaldehyde and formic acid may offer one explanation of the specific toxicity of methyl bromide, it may also be possible that methyl bromide as well as methyl iodide forms a quaternary ammonium compound with certain nitrogen groups of enzymes and other biologically important amines in the organism.

It has been shown that methyl bromide differs from other aliphatic chlorinated hydrocarbons in that it has a very weak narcotic action, but that after a latent period it produces functional and perhaps also pathological changes in the lower sections of the brain, the basal ganglia, the cerebellum, and the pyramidal tract, as indicated by the changes of the reflex excitability and visual and speech disturbances. In this action it resembles manganese and carbon monoxide, both of which may affect the oxygen metabolism in certain sections of the brain. It has been suggested (von Oettingen, 1945) that some toxic phenomena observed in carbon monoxide poisoning may be caused by the effect of carbon monoxide on the iron of certain iron-containing pigments of the brain, and Egg (1927) showed that bivalent iron may form a complex with methyl alcohol and thus interfere with the catalytic action of hemoglobin. It is, therefore, possible that a similar reaction may take place with methyl bromide and other monohalogenated methyl derivatives. On the other hand, the possibility that methyl bromide may form quaternary ammonium bases with biologically important amines, as in the case of alkaloids, may also be considered. The existence of such quaternary amines has been recently demonstrated by the discovery by Najjar and Wood (1940) of a fluorescent compound "F<sub>2</sub>" which was later identified as N methyl-chloro-nicotinamide by Huff and Perlzweig (1943), Coulson and Ellinger (1943), and Ellinger and Coulson (1943).

#### THE HAZARDS CONNECTED WITH THE USE OF METHYL BROMIDE, AND THEIR PREVENTION

*Methyl bromide poisonings in chemical operations* result most frequently from the escape of methyl bromide from leaking pipe lines and containers as reported by Schuler (1899), Jaquet (1901), Löffler and Rüttimeyer (1920), Cadé and Mazel (1923), Meixner (1928), and De

Morsier and Steinmann (1936). Others result from the displacement and inhalation of methyl bromide vapor laden air from collecting vessels and in filling operations as reported by Jaquet (1901), Watrous (1942), Ramond and Lacorne (1942), Pagniez and Plichet (1942), DeJong (1944), Heimann (1944), Michaux, Courchet, and Lechevalier (1944), Michaux, Clercy, and Lechevalier (1944-45), and Wyers (1945). Occasionally accidents are caused by the bursting of pipe lines and containers due to sudden temperature changes and other causes as observed by Rohrer (1920) and Goldschmid and Kuhn (1920), and finally the exposure may take place during repair work as in the case of Friemann (1937). In addition, poisonings may result from carelessness, spilling of methyl bromide, and disregard of preventive measures. In order to prevent such accidents it appears necessary to prevent leakage by periodic examination of pipe lines and containers for corrosion and other defects while the operation is shut down. If possible, pipe lines should be of copper, heavy rubber, or neoprene tubing as suggested by Wyers (1945). Pipe lines and cylinders should preferably be located outdoors or, if this is not possible, the adjoining area should be provided with adequate local downward exhaust ventilation. From the report of Meixner (1928) it appears that concentrations of about 500 p. p. m. may still be injurious, and animal experiments reported by Irish, Adams, Spencer, and Rowe (1941) seem to indicate that the concentration of methyl bromide in air for continued exposure should be kept below 30 p. p. m. However, Watrous (1942) reported cases of methyl bromide poisoning from exposure to 35 p. p. m. which may indicate that the maximal permissible concentration should be well below this level and that, in addition to the contamination of the atmosphere, much attention should be paid to the contamination of the close surroundings of the worker. All operations in which methyl bromide is handled should be equipped with an adequate number of gas masks, preferably of the open air type, because canister masks may not offer adequate protection against high concentrations of methyl bromide. As pointed out by Wyers (1945) joints should be lubricated with soap because methyl bromide is soluble in oils and fats, and gaskets should be made from soft copper, leather, sheet lead, or hard felt. Workers must be instructed as to the hazards of methyl bromide, and meticulous personal hygiene must be taught and enforced. This holds true especially for the prompt removal of soiled garments and shoes and any other material contaminated with methyl bromide. It should also be emphasized that gloves of rubber or leather not only offer no protection but, on the contrary, may prolong and intensify the exposure by preventing the rapid evaporation and dissemination of methyl bromide.

With regard to *the hazards of methyl bromide as a fire extinguishing agent* it appears that these deserve serious consideration. Von Gronow (1927), Henning (1933), and May and Baker (1936) advocated the use of methyl bromide in fire extinguishers because of its prompt action and because in contrast to carbon tetrachloride no phosgene but less dangerous hydrogen bromide is formed by thermal decomposition, the irritant action of which would act as warning signal to prevent further exposure. It appears that at that time the toxicity and potential dangers of methyl bromide were not properly appreciated although Joachimoglu (1930) pointed out that it is more toxic than carbon tetrachloride when used for the same purpose. In this connection the hazards connected with the filling of fire extinguishers with methyl bromide deserve less attention because these have been discussed above. Such accidents were reported by Meixner (1928), Tietze (1933), Duvoir, Fabre, and Layani (1937), and Carter (1945). Methyl bromide poisoning may result from the escape of methyl bromide into the air from leaking fire extinguishers as reported by Nixon (quoted from Gray, 1944), Holling and Clarke (1944), and Clarke, Roworth, and Holling (1945) and also from its use as an extinguishing agent in poorly ventilated rooms as reported by Holling and Clarke (1944) and Wyers (1945) or in small enclosures such as tanks as described by Billet and Abel (1940) and Butler, Perry, and Williams (1945). Toxic effects may also result from the inhalation of volatilized methyl bromide and from contamination of garments, including shoes, with this material during the use of the extinguisher resulting in contamination of the skin as reported by Dérobert (1941). It is, therefore, apparent that the safe use of methyl bromide in fire extinguishers is bound to certain precautions. In view of the high incidence of methyl bromide poisonings in filling operations, the filling of fire extinguishers with methyl bromide should be done only by trained personnel, and as suggested by de Gaulejac and Dervillé (1939) (quoted from Fischer, 1940) the filling should take place under a hood with downward exhaust ventilation and the container should first be evacuated and then filled by gravity in order to reduce to a minimum the overflow of methyl bromide contaminated air. Since, as pointed out by Beyne and Goett (1934), the danger from leakage is much greater with methyl bromide than with carbon tetrachloride extinguishers, and because of the high specific gravity of the vapors of the former, methyl bromide extinguishers should be stored at low levels. Their weight should be checked periodically in order to detect any loss by evaporation of methyl bromide, and the equipment should be examined for signs of corrosion and other defects. In addition, a proper gas mask should be kept near the extinguisher so that it is easily available in case of emergency or repair. With regard to the

safe use of methyl bromide extinguishers the directions given by de Gaulejac and Dervillé (1939) (quoted from Fischer, 1940) and of Beyne and Goett (1934) may be accepted and summarized as follows: In using methyl bromide fire extinguishers the operator should stand near the exit, preferably at an elevated level or under the wind, and any contamination of garments or shoes should be avoided. In using methyl bromide extinguishers for small fires excessive amounts of methyl bromide should not be used and special care should be exercised when using it on overheated motors because of its very rapid volatilization.

After the use of the methyl bromide extinguisher the area should be adequately ventilated, and it should be emphasized that entering such areas without this precaution, even with adequate protection by a gas mask, may cause severe injury of the skin although no systemic effects may result. Personnel handling methyl bromide fire extinguishers should receive special training, and persons suffering from respiratory disturbances should be excluded. It has been suggested that some odorant be added to methyl bromide as a warning agent, but it appears to be questionable to what extent this would be effective in view of the high volatility of methyl bromide. It is essential to construct the release mechanism in such a fashion that methyl bromide cannot be released inadvertently. In view of the fact that most cases of methyl bromide poisoning resulting from the use of this material in fire extinguishers have been caused by accidents beyond human control, it appears that the use of methyl bromide fire extinguishers should be restricted to very few occasions and that such extinguishers should be operated only by adequately trained personnel. The use of permanent methyl bromide fire extinguishing equipment, as on board ship, offers dangers by possible leakage or inadvertent discharge as illustrated by the reports of Gray (1944), Holling and Clarke (1944), and Clarke, Roworth, and Holling (1945). The toxicity of methyl bromide and its inherent dangers are too great to advocate the use of methyl bromide fire extinguishers by the general public as for use in automobiles and garages.

With regard to *the use of methyl bromide as an insecticidal agent for the fumigation of foodstuffs* both the dangers connected with the fumigation and the possible contamination of the fumigated material have to be considered. The latter was studied by Dudley (1939) and Dudley, Sayers, and Neal (1939) who found that after fumigation with methyl bromide fresh fruits and vegetables, dried fruits, and whole grains absorb only minor amounts of the fumigant but that milled grain and fatty or oily foods (nutmeats and cheese) absorb greater amounts and in general retain considerable quantities of bromide after aeration for 48 hours. Phillips, Monro, and Allen (1938)

found that after fumigation of apples the bromide residue is below the danger point for human consumption, being of the order of 3 to 5 parts per million; Dudley, Miller, Neal, and Sayers (1940) showed in animal experiments that it seems unlikely that the small amounts of methyl bromide or bromide residues on commercially fumigated fresh vegetables and fruits or dried fruits is harmful to the consumer, and Dudley and Neal (1942) believed it unlikely that the small amounts of methyl bromide or bromide residues on commercially fumigated fresh vegetables and fruits is harmful to the consumer and that milled cereal products fumigated with one pound of methyl bromide per 1,000 cubic feet will probably offer no risk when such food is consumed. Laug (1941) found that the concentration of volatile bromides in various foodstuffs decreases rapidly within one hour after fumigation, in walnut meats to 18.4 percent and in figs to 5 percent of the amount present immediately after fumigation. The values decreased in the course of 24 hours to such small amounts as to be of no practical significance. On the other hand, the amounts of residual inorganic bromide remained constant and in some instances were rather high. Shrader, Beshgetoor, and Stenger (1942) showed that retention of bromide is greater with fumigation on a laboratory scale than under commercial conditions and that most of the methyl bromide disappears within one day and is completely gone within one week after fumigation, mainly due to volatilization, only a small fraction being decomposed to inorganic bromides after removal from the fumigation chamber. Roehm, Shrader, and Stenger (1942) published similar findings, as illustrated in figure 2, and they found that at higher humidities water displaces part of the methyl bromide from the surface and enhances the conversion of methyl bromide to bromide. Roehm, Stenger, and Shrader (1943) showed that dairy products could be safely fumigated with methyl bromide and that they retained only insignificant amounts of bromide. Spencer, Rowe, Adams, and Irish (1944) found that rats kept for one year on diets fumigated with 2 and 6 pounds of methyl bromide per 1,000 cubic feet with an average bromine content of 26.2 and 63.7 mg. per 100 gm., respectively, made normal weight gains and showed no evidence of intoxication or nutritional deficiency as evidenced by hematological, histopathological, analytical, and organ weight studies. It appears, therefore, that fumigation of food products with 1 to 2 pounds of methyl bromide per 1,000 cubic feet with adequate subsequent aeration will leave no residues which may have deleterious effects when such food products are included as a large part of the human diet. With regard to the potential dangers to humans resulting from the use of methyl bromide as a fumigant, a number of accidents are on record. Mackie (1938) mentioned two cases of methyl bromide poi-



soning with one fatality in two fumigators who had been working with M. S. A. Comfo respirators with small canisters for 75 minutes in a storage room of 300,000 cubic feet capacity which was half filled with rice and which was fumigated with 147 pounds of methyl bromide. Schifferli (1942) mentioned two similar accidents with one fatality, and Bruhin (1943) reported three cases with one fatality in persons who had been working in an atmosphere containing probably 8,000 p. p. m. with closed circuit respirators for 55 and 33 minutes

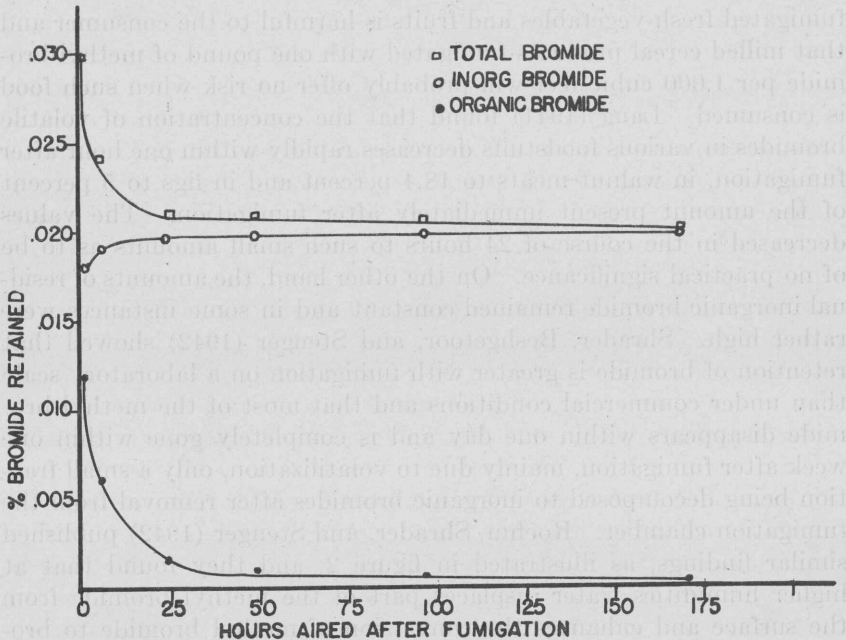


FIGURE 2.—Retention of total, organic, and inorganic bromide in whole wheat flour after fumigation with methyl bromide.

with a 10-minute interval between. Miller (1943) discussed one fatal accident resulting from the unauthorized entering of a freight car under fumigation, and Johnstone (1945) reported on 34 cases of severe, moderate, or mild methyl bromide poisoning from fumigation vapors escaping to an adjoining room. From the histories of these accidents it is apparent that methyl bromide poisoning in fumigators resulted from the wearing of inadequate gas masks which were not suitable for high concentrations or which were probably defective; one accident was due to the access of an unauthorized person to a gassed area; the others were caused by diffusion of methyl bromide to adjoining localities (three common causes of accidents from the use of fumigants which were also pointed out by Schwarz (1937)). These accidents emphasize the importance of the selection of adequate gas masks

and their regular inspection, the need for marking gassed areas to caution against unauthorized entering, and the necessity for checking the localities to be gassed for communication to adjoining rooms or buildings. In view of the tremendous quantities of methyl bromide used in past years for fumigation purposes, the number of accidents from such use is small indeed, indicating that methyl bromide may be used safely if certain precautions are observed. The Division of Industrial Hygiene of the National Institute of Health, U. S. Public Health Service in 1938 made the following preliminary recommendations:

1. Avoid breathing air containing methyl bromide.
2. On completion of fumigation, provide thorough ventilation for cars, rooms, or buildings before entering.
3. When necessary to enter space containing methyl bromide, use a gas mask provided with a canister giving protection against organic vapors, or a positive pressure hose mask (masks and canisters to be approved under the U. S. Bureau of Mines Schedules 14D or 19A. Canister black, type B).
4. Avoid spilling of methyl bromide. Get to fresh air immediately in case of spillage. Remove any clothing in contact with the skin which has become impregnated with the liquid.
5. Post warning signs, notifying that methyl bromide is being used and that the gas is toxic.
6. Containers of methyl bromide should be stored in a cool, well-ventilated place, outside inhabited buildings. Avoid leakage by seeing that valves of cylinders are tightly closed.

Further directions and technical details regarding the safe handling of methyl bromide, the types of fumigation chambers, the dosage schedule, the methods of applying methyl bromide, and also the methods of venting fumigated enclosures were given by the Division of Control Investigations, Bureau of Entomology and Plant Quarantine, Agricultural Research Administration, U. S. Department of Agriculture, in Publication E-601 (Sept. 1943).

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THE EFFECTS OF ALIPHATIC NITROUS AND NITRIC ACID  
ESTERS ON THE PHYSIOLOGICAL FUNCTIONS WITH  
SPECIAL REFERENCE TO THEIR CHEMICAL CONSTITUTION

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## TABLES

# THE EFFECTS OF ALIPHATIC NITROUS AND NITRIC ACID ESTERS ON THE PHYSIOLOGICAL FUNCTIONS WITH SPECIAL REFERENCE TO THEIR CHEMICAL CONSTITUTION

## INTRODUCTION

For nearly one century the aliphatic esters of nitrous and nitric acids have aroused the interest of pharmacologists and toxicologists. Whereas the aliphatic nitrous acid esters or alkyl nitrites are mainly used as therapeutic agents, the aliphatic nitric acid esters are also used very extensively as explosives. Because the physiological changes produced by nitric acid esters are to a certain extent similar to those produced by nitrous acid esters and by inorganic nitrites, these types of compounds may be discussed together. It is hoped that a detailed knowledge of the effects of these chemicals on the physiological functions of the normal, and also, to a certain extent, of the diseased organism, will facilitate the understanding of the potential dangers which may result from the handling of these materials during their production, their use in the manufacture of explosives, and from the loading of ammunition.

## I. THE ALIPHATIC NITROUS ACID ESTERS

The physico-chemical properties of some of the aliphatic esters of nitrous acid, the alkyl nitrites, are summarized in table 1. This shows that with the exception of methyl nitrite, which is a gas, all members of this group are liquids, the boiling points of which increase with the molecular weight, and that the iso, secondary, and tertiary compounds have a lower boiling point than the corresponding normal derivatives. According to Dunstan (1888-89) and Cash and Dunstan (1894) the higher homologues are more readily decomposed than the lower ones, and the secondary and tertiary alkyl nitrites suffer decomposition more easily than the corresponding normal compounds.

The aliphatic esters of nitrous acid are characterized by the following reactions (Rosenthaler, 1923): (1) They are hydrolyzed with the formation of nitrous acid and alcohol. (2) If reduced with tin and hydrochloric acid, the nitrogen is split off as ammonia or hydroxylamine. (Difference from the isomeric nitroalkyl compounds.)

Table 1.—*PHYSICO-CHEMICAL PROPERTIES OF ALIPHATIC NITROUS ACID ESTERS*

| Name                                  | Formula                                                                                  | Molecular weight | Form                | Specific gravity                   | Melting point °C. | Boiling point °C.      | Solubility in—         |               |           |
|---------------------------------------|------------------------------------------------------------------------------------------|------------------|---------------------|------------------------------------|-------------------|------------------------|------------------------|---------------|-----------|
|                                       |                                                                                          |                  |                     |                                    |                   |                        | Water                  | Alcohol       | Ether     |
| Methyl nitrite <sup>1</sup> .....     | CH <sub>3</sub> ONO.....                                                                 | 61.04            | Gas.....            | 0.991 <sup>15°</sup> .....         | .....             | —12.....               | Insoluble.....         | Soluble.....  | Soluble.  |
| Ethyl nitrite <sup>1</sup> .....      | CH <sub>3</sub> CH <sub>2</sub> ONO.....                                                 | 75.07            | Liquid.....         | 0.990 <sup>15, 50°</sup> .....     | .....             | 17.....                | Very slightly soluble. | Miscible..... | Miscible. |
| n-Propyl nitrite <sup>1</sup> .....   | CH <sub>3</sub> CH <sub>2</sub> CH <sub>2</sub> ONO.....                                 | 89.09            | do.....             | 0.935 <sup>20°<br/>4°</sup> .....  | .....             | 57.....                | Insoluble.....         | Soluble.....  | Soluble.  |
| i-Propyl nitrite <sup>2</sup> .....   | (CH <sub>3</sub> ) <sub>2</sub> CHONO.....                                               | 89.09            | do.....             | .....                              | .....             | 39.5.....              | do.....                | do.....       | Do.       |
| n-Butyl nitrite <sup>1</sup> .....    | CH <sub>3</sub> CH <sub>2</sub> CH <sub>2</sub> CH <sub>2</sub> ONO.....                 | 103.12           | do.....             | 0.911 <sup>6°</sup> .....          | .....             | 77-79.....             | .....                  | Miscible..... | Miscible. |
| i-Butyl nitrite <sup>1</sup> .....    | (CH <sub>3</sub> ) <sub>2</sub> CH-CH <sub>2</sub> ONO.....                              | 103.12           | do.....             | 0.870 <sup>20°<br/>20°</sup> ..... | .....             | 67-68.....             | Slightly soluble.....  | do.....       | Do.       |
| sec-Butyl nitrite <sup>2</sup> .....  | CH <sub>3</sub> CH <sub>2</sub> CH(CH <sub>3</sub> )ONO.....                             | 103.12           | do.....             | .....                              | .....             | 68.5.....              | do.....                | do.....       |           |
| tert-Butyl nitrite <sup>2</sup> ..... | CH <sub>3</sub> -C(CH <sub>3</sub> ) <sub>2</sub> ONO.....                               | 103.12           | do.....             | .....                              | .....             | 63.....                | do.....                | do.....       |           |
| n-Amyl nitrite <sup>1</sup> .....     | CH <sub>3</sub> CH <sub>2</sub> CH <sub>2</sub> CH <sub>2</sub> CH <sub>2</sub> ONO..... | 117.15           | Pale yellow liquid. | 0.853 <sup>20°<br/>4°</sup> .....  | .....             | 104 (761 mm. Hg.)..... | do.....                | do.....       |           |
| α-i-Amyl nitrite <sup>1+3</sup> ..... | (CH <sub>3</sub> ) <sub>2</sub> CHCH <sub>2</sub> CH <sub>2</sub> ONO.....               | 117.15           | Liquid.....         | 0.872 <sup>20°<br/>4°</sup> .....  | .....             | 99.....                | do.....                | do.....       |           |
| β-i-Amyl nitrite <sup>2+4</sup> ..... | CH <sub>3</sub> CH <sub>2</sub> CH(CH <sub>3</sub> )CH <sub>2</sub> ONO.....             | 117.15           | do.....             | .....                              | .....             | 95.96.....             | Insoluble.....         | Soluble.....  | Do.       |
| tert-Amyl nitrite <sup>2</sup> .....  | CH <sub>3</sub> CHC(CH <sub>3</sub> ) <sub>2</sub> ONO.....                              | 117.15           | do.....             | .....                              | .....             | 92.....                | do.....                | do.....       |           |

<sup>1</sup> Quoted from Lange's Handbook of Chemistry, Fifth edition, 1944.<sup>2</sup> Quoted from Cash and Dunstan, 1894.<sup>3</sup> 1=amyl nitrite.<sup>4</sup> 2=methylbutyl nitrite.

It is generally assumed that the pharmacological and toxicological actions of the aliphatic nitrites are due, at least to a certain extent, to the splitting off of the nitrous group, resulting in the formation of nitrite. According to Cash and Dunstan (1894), this holds true for the higher homologues only, whereas with the lower members of this series the pharmacological action rests with the molecule itself. For this reason several methods have been developed to determine nitrites in biological specimens.

These *determinations* are based on the diazotization of an aromatic amine by the nitrite and coupling of the resulting azo compound with some chromogenic agent. Goroncy (1928) used a mixture of a solution of 0.5 gm. sulfanilic acid in 150 cc. acetic acid and a solution of 0.1 gm. alpha-naphthylamine hydrochloride in 150 cc. of 30 percent acetic acid. This yields a red color upon the addition of nitrite which can be matched with standards made up with known concentrations of nitrite. Essentially the same procedure was used by Crandall, Leake, Loevenhart, and Muehlberger (1929) and Rath and Krantz (1942a). Stieglitz and Palmer (1934) determined the nitrite content in blood by precipitating first the proteins by zinc sulfate and sodium hydroxide, and, after separation of the precipitate, producing a violet-blue color by adding a solution of alpha-naphthylamine and of G acid (disodium salt of beta-naphthylamine-6,8-disulfonic acid) which can be matched against standards of known strength. Wallace and Neave (1927) and Germuth (1929) suggested replacing the alpha-naphthylamine with dimethyl-alpha-naphthylamine because of the greater stability of the color produced by the latter, and Giblin and Chapman (1936) used dimethylaniline and sulfanilic acid which results in the formation of methyl orange. Yagoda and von Oettingen (1944) worked out a procedure for the determination of nitrite in biological materials which is a modification of the American Public Health Association's method (1936) for the determination of nitrite in water by substituting dimethyl-alpha-naphthylamine for alpha-naphthylamine as recommended by Wallace and Neave (1927) and Germuth (1929). With this modification it is possible to differentiate 0.05 microgram of  $\text{NO}_2$  at a dilution of one part per billion from a tube containing distilled water. In blood, the proteins were removed by the procedure of Somogyi (1930) prior to the determination of the nitrite.

For the *determination of nitrite in blood* 1 cc. of whole blood is transferred to a 50-cc. centrifuge tube containing 5 cc. of 0.3 percent potassium oxalate and 20 cc. of water, and 2 cc. of 3 percent zinc sulfate is added and mixed by gently shaking the tube. During mixing, 0.4 cc. of normal sodium hydroxide solution is added, drop by drop, from a calibrated medicine dropper, whereupon the mixture is centrifuged



until the precipitate is reduced to approximately 3 cc. At this stage an additional 0.1 cc. of normal sodium hydroxide is added, drop by drop, in order to form a secondary precipitate of zinc hydroxide; and after standing for 1 minute the supernatant liquid including the suspended zinc hydroxide is filtered into a Nessler tube.

A standard containing 1 microgram of  $\text{NO}_2$  is prepared by diluting 1 cc. of the primary standard containing 0.15 percent sodium nitrite in aqueous solution to one liter. Measured volumes of the diluted standard are transferred to Nessler tubes and filled with distilled water to the 25-cc. calibration mark.

In the unknown and in the standards the color is then developed by adding 0.5 cc. each of the sulfanilic acid and the dimethyl- $\alpha$ -naphthylamine solutions. In case nitrite is present in excess of 0.1 microgram, a characteristic pink color develops within 15 minutes at room temperature. With smaller quantities this develops after 30 minutes.

Since an average of 25 percent of the nitrite in blood is partly decomposed and partly absorbed during the preparation of the blood sample for the final color reaction, the results obtained with the determination outlined above have to be multiplied by a correction factor 1.34. This was obtained by adding known amounts of sodium nitrite to blood and comparing the color developed with that obtained with aqueous standards of the same concentration. In order to secure reliable results it is essential that the preparation of the blood sample for the final color reaction be performed as rapidly as possible and that the blood sample be analyzed as soon as possible after collection in order to avoid decomposition of the nitrite. By the use of "internal" standards prepared by adding known concentrations of nitrite to normal blood and treating this in the same way, it is possible to differentiate the color produced by the addition of 0.05 microgram of nitrite from the blank reaction of normal blood.

The same procedure may be applied to the *determination of nitrite in urine*. A measured volume of urine (1 to 20 cc.) is pipetted into a 50-cc. glass-stoppered cylinder and diluted with water to 50 cc. One gram of activated charcoal (Norite A) is added and mixed slowly by inverting the cylinder several times. On standing for several minutes, the charcoal flocculates and when this point is reached the mixture is filtered into a 100-cc. flask through an 11 cm. paper similar to C. S. & S. No. 598. Then 2 cc. of 5-percent zinc sulfate are added and the liquid is filtered off from the zinc phosphate together with its coprecipitated colloidal carbon through a second 11-cm. paper, catching the filtrate in a 50-cc., tall-form, Nessler tube. Finally, 1 cc. each of sulfanilic acid and dimethyl- $\alpha$ -naphthylamine reagents are added,



mixed, and the pink color developed is compared, after standing for 30 minutes, with internal standards.

These standards are prepared by adding known amounts of nitrite to a volume of urine equal to that used in the analysis of the unknown. In samples of normal urine it is possible to differentiate the color produced by 0.1 microgram of nitrite ( $\text{NO}_2$ ) added to the urine from the faint pink color of the nitrite normally present in the urine. The absolute value of the normal nitrite content can be estimated by removing part of the solution from the standard tube and measuring the length of the residual column which just matches in intensity the color produced by the unknown. If  $h_x$  and  $h_s$  are the lengths of the balancing columns for the unknown and the internal standard, respectively, and  $k$  is the amount of nitrite added as internal standard, then the amount of nitrite in the unknown,  $c$ , can be obtained from the equation:

$$c = \frac{k \cdot h_s}{h_x - h_s}.$$

A similar procedure can be used for the *determination of nitrite in gastric juice and saliva*. One cc. of the viscous sample is measured by means of a syringe into a 100-cc. flask containing 50 cc. of water and shaken until the slimy matter is dispersed. The solution is clarified by adding 5 cc. of 5-percent zinc sulfate and precipitating zinc hydroxide by the dropwise addition of 1 cc. of 1 *M* sodium hydroxide. If the gastric juice is deeply colored it should be decolorized prior to the addition of zinc sulfate by adsorption on about 0.2 gm. of activated charcoal. The mixture is filtered through an 11-cm. paper (C. S. & S. No. 598) and 50 cc. of the filtrate is collected in a Nessler tube. The color is developed by the addition of 1-cc. portions of the two reagent solutions and compared with aqueous standards of known nitrite content. In calculating the results the volume of the sample is multiplied by the ratio of the volume of the filtrate to the volume of the initial system, i. e.  $50/57=0.88$ .

The *pharmacological and toxicological actions of aliphatic nitrites* are characterized by a fall of the blood pressure due to peripheral vasodilatation, acceleration of the pulse rate, and, with sufficient concentrations, by the formation of methemoglobin. As will be discussed below, the fall of the blood pressure is due to peripheral vasodilatation on account of a depression of the smooth muscles in the vascular walls.

Cash and Dunstan (1894) studied the effect of various aliphatic nitrites on the pulse rate, blood pressure, and muscular function, as illustrated in table 2. This shows that the *acceleration of the pulse rate* is moderate with the lower homologues, with which it may be absent or even reversed to slowing, perhaps because of reflex stimula-

tion of the medullary centers by irritation of the nasal mucous membranes. The accelerative action increases with the molecular weight of these compounds, and the isomeric, secondary, and tertiary derivatives are more potent in this respect than the normal compounds. Although this effect is qualitatively the same and follows the same order when the compounds are given intravenously as when they are inhaled, with the latter form of administration the effect is invariably more marked.

**Table 2.—EFFECT OF ALIPHATIC NITRITES ON PULSE RATE, BLOOD PRESSURE, AND MUSCULAR CONTRACTION**

(Cash and Dunstan, 1894)

| Name                                        | Formula                                                                   | Acceleration of pulse rate | Reduction of blood pressure | Duration of fall of blood pressure | Degree of passive muscular contraction | Rapidity of muscular contraction |
|---------------------------------------------|---------------------------------------------------------------------------|----------------------------|-----------------------------|------------------------------------|----------------------------------------|----------------------------------|
| Methyl nitrite.....                         | $\text{CH}_3\text{ONO}$ .....                                             | 1+                         | 4+                          | 10+                                | 1+                                     | 10+                              |
| Ethyl nitrite.....                          | $\text{CH}_3\text{CH}_2\text{ONO}$ .....                                  | 2+                         | 2+                          | 9+                                 | 2+                                     | 9+                               |
| n-Propyl nitrite.....                       | $\text{CH}_3\text{CH}_2\text{CH}_2\text{ONO}$ .....                       | 3+                         | 1+                          | 1+                                 | 6+                                     | 6+                               |
| i-Propyl nitrite.....                       | $(\text{CH}_3)_2\text{CHONO}$ .....                                       | 4+                         | 10+                         | 2+                                 | 7+                                     | 8+                               |
| n-Butyl nitrite.....                        | $\text{CH}_3\text{CH}_2\text{CH}_2\text{CH}_2\text{ONO}$ .....            | 5+                         | 3+                          | 5+                                 | 4+                                     | 2+                               |
| i-Butyl nitrite.....                        | $(\text{CH}_3)_2\text{CHCH}_2\text{ONO}$ .....                            | 7+                         | 7+                          | 3+                                 | 10+                                    | 1+                               |
| sec-Butyl nitrite.....                      | $\text{CH}_3\text{CH}_2\text{CH}(\text{CH}_3)\text{ONO}$ .....            | 6+                         | 8+                          | 6+                                 | 8+                                     | 4+                               |
| tert-Butyl nitrite.....                     | $\text{CH}_3\text{C}(\text{CH}_3)_2\text{ONO}$ .....                      | 8+                         | 9+                          | 7+                                 | 5+                                     | 5+                               |
| $\alpha$ -i-Amyl nitrite <sup>1</sup> ..... | $(\text{CH}_3)_2\text{CHCH}_2\text{CH}_2\text{ONO}$ .....                 | 9+                         | 5+                          | 4+                                 | 3+                                     | 3+                               |
| $\beta$ -i-Amyl nitrite <sup>2</sup> .....  | $\text{CH}_3\text{CH}_2\text{CH}(\text{CH}_3)\text{CH}_2\text{ONO}$ ..... |                            |                             |                                    |                                        |                                  |
| tert-Amyl nitrite.....                      | $\text{CH}_3\text{CHC}(\text{CH}_3)_2\text{ONO}$ .....                    | 10+                        | 6+                          | 8+                                 | 9+                                     | 7+                               |

<sup>1</sup> 1-amyl nitrite.

<sup>2</sup> 2-methylbutyl nitrite.

With regard to the *lowering of the blood pressure*, there is a decrease of the effectiveness from methyl to n-propyl nitrite but an increase of the depressor action with n-butyl nitrite as compared with n-propyl nitrite. As illustrated by the response to the butyl and amyl nitrites, the secondary and tertiary derivatives are more potent in this respect than the iso derivatives, and, as indicated by a comparison of i-propyl and i-butyl nitrite with the normal nitrites, the former are more potent than the latter. With regard to the duration of the fall of the blood pressure, methyl and ethyl nitrite are the most effective, n-propyl nitrite is the least effective, and the iso derivatives of propyl and butyl nitrite are more effective than the normal compounds. It is difficult to give an adequate interpretation of these findings in view of the paucity of physico-chemical data on the compounds. The same holds true with regard to their effect on the passive muscular contraction and on the rapidity of the muscular contraction. As pointed out by Bradbury (1895), the effectiveness of these compounds appears to be determined partly by their solubility in water and partly by

their liability to decompose with the formation of nitrites, the latter probably being the more important factor because methyl nitrite, which is the most soluble of these alkyl nitrites, has comparatively little effect on the vasodilator action, whereas the most stable nitrites are the least effective in this respect.

Krantz, Carr, and Forman (1938a; 1939) studied the depressant effect of n-hexyl nitrite, n-heptyl nitrite, n-octyl nitrite, 2-ethyl-n-hexyl nitrite, n-nonyl nitrite, n-decyl nitrite, 5-ethyl-n-nonyl-2-nitrite, n-duodecyl nitrite, 7-ethyl-2-methyl-undecyl nitrite, n-tetra-decyl

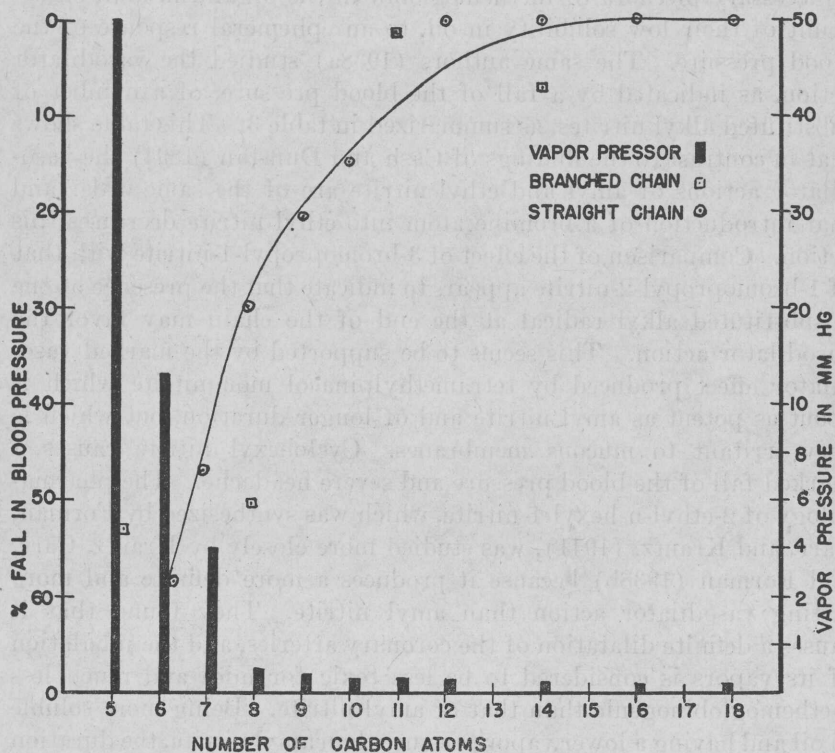


FIGURE 1.—Relation between blood pressure, vapor pressure, and molecular weight of alkyl nitrites.

nitrite, n-hexadecyl nitrite, and n-octadecyl nitrite on the blood pressure and correlated this with their molecular weight (as indicated by the number of carbon atoms) and their vapor pressure. As illustrated in figure 1, the reduction of the blood pressure resulting from the inhalation of these compounds is a function of their vapor pressure. In addition, it shows that with this form of administration the alkyl nitrites with more than 12 carbons are devoid of such depressor action whereas with intravenous injection they elicit a distinct effect. It appears further, in accordance with the findings of Cash and Dunstan

(1894) made with the lower homologues, that compounds with branched carbon chains are more active than those with straight chains of equimolecular weight because the latter have a lower vapor tension. This correlation has also been observed with aliphatic alcohols. With regard to the duration of the pressor action, Krantz, Carr, and Forman (1938a; 1939) are of the opinion that with the higher alkyl nitrites this is determined by the greater partition coefficient in oil over water. In their opinion the optimal vapor pressure for therapeutic results lies between 3 and 4 mm. They believe that higher pressures lead to unnecessary plethora of methemoglobin in the organism, and, on account of their low solubility in oil, to an ephemeral response of the blood pressure. The same authors (1938a) studied the vasodilator action, as indicated by a fall of the blood pressure, of a number of substituted alkyl nitrites, as summarized in table 3. This table shows that in contrast to the findings of Cash and Dunstan (1894) the vasodilator actions of amyl and ethyl nitrite are of the same order and that introduction of a bromine atom into ethyl nitrite decreases this action. Comparison of the effect of 3-bromopropyl-1-nitrite with that of 1-bromopropyl-2-nitrite appears to indicate that the presence of one unsubstituted alkyl radical at the end of the chain may favor the vasodilator action. This seems to be supported by the marked vasodilator effect produced by tetramethylpinacol mononitrite which is about as potent as amyl nitrite and of longer duration, but which is very irritant to mucous membranes. Cyclohexyl nitrite causes a marked fall of the blood pressure and severe headache. The pharmacology of 2-ethyl-n-hexyl-1-nitrite, which was synthesized by Forman, Carr, and Krantz, (1941), was studied more closely by Krantz, Carr, and Forman (1938b) because it produces a more definite and more lasting vasodilator action than amyl nitrite. They found that it causes a definite dilatation of the coronary arteries, and the inhalation of its vapors is considered to be less toxic for mice and much less methemoglobinogenic than that of amyl nitrite. Being more soluble in oil and having a lower vapor pressure than amyl nitrite, the duration of its vasodilator action is about six times longer than that of amyl nitrite. Field (1944) reported on four cases of achalasia of the cardia in which the inhalation of 2-ethylhexyl nitrite gave prompt relief without causing the nausea and vomiting observed after the administration of amyl nitrite and acted more promptly than trinitroglycerol which is also less effective in alleviating vomiting. Freedberg, Spiegel, and Riseman (1941) found it definitely effective in the treatment of angina pectoris, having all the advantages and disadvantages of trinitroglycerol and amyl nitrite, differing however from the former in that it can be inhaled, is more rapidly absorbed, and hence acts more



promptly, which they consider a definite advantage in the treatment of this disease.

**Table 3.—THE VASODILATOR ACTION OF A NUMBER OF SUBSTITUTED ALKYL NITRITES AS INDICATED BY THEIR EFFECT ON THE BLOOD PRESSURE**

(Krantz, Carr, and Forman, 1938 (a))

| Compound                         | Formula                                                                                                                                                                                                       | Original blood pressure in mm. Hg. | Maximal fall of blood pressure in mm. Hg. | Duration of fall of blood pressure in minutes |
|----------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------|-------------------------------------------|-----------------------------------------------|
| Amyl nitrite.....                | $C_5H_{11}ONO$ .....                                                                                                                                                                                          | 154                                | 53                                        | 2.0                                           |
| Ethyl nitrite.....               | $C_2H_5ONO$ .....                                                                                                                                                                                             | 140                                | 64                                        | 1.8                                           |
| 2-Bromo-ethyl-nitrite.....       | $CH_2BrCH_2ONO$ .....                                                                                                                                                                                         | 94                                 | 15                                        | 4.0                                           |
| 3-Bromopropyl-1-nitrite.....     | $CH_2BrCH_2CH_2ONO$ .....                                                                                                                                                                                     | 140                                | 20                                        | 5.0                                           |
| 1-Bromopropyl-2-nitrite.....     | $CH_2BrCH(ONO)CH_3$ .....                                                                                                                                                                                     | 112                                | 40                                        | 2.0                                           |
| Tetramethylpinacol mono-nitrite. | $  \begin{array}{c}  CH_3 \qquad \qquad CH_3 \\  \diagdown \quad \diagup \\  C \quad \quad C \\  \diagup \quad \diagdown \quad \diagup \quad \diagdown \\  CH_3 \quad OH \quad ONO \quad CH_3  \end{array}  $ | 146                                | 62                                        | 5.0                                           |
| Cyclohexyl nitrite               | $  \begin{array}{c}  H_2 \quad H_2 \\  \diagdown \quad \diagup \\  H_2 \quad \quad \quad ONO \\  \diagup \quad \diagdown \\  H_2 \quad H_2  \end{array}  $                                                    | 142                                | 63                                        | 14.0                                          |
| 2-Ethyl-n-hexyl-1-nitrite....    | $CH_3CH_2CH_2CH_2CH(C_2H_5)CH_2ONO$ ....                                                                                                                                                                      | 100                                | 50                                        | 15.0                                          |

In contrast to alkyl nitrites, characterized by the radical  $O-NO$ , nitro derivatives of aliphatic hydrocarbons with the group  $-N \begin{smallmatrix} O \\ // \\ O \end{smallmatrix}$  have no vasodilator properties, as demonstrated by Schadow (1876) for nitroethane and nitropentane. Similarly, nitramine with the group  $-NH-N=O_2$  has no nitrate action, as shown by Stokvis and Spruyt (1899).

It appears, therefore, that the vasodilator action resulting in lowering of the blood pressure following the inhalation of alkyl nitrites is bound to the grouping  $R-ONO$  and that its intensity depends upon the vapor pressure of the aliphatic nitrites and their partition coefficient oil over water. Earlier investigators assumed that the vaso-depressor action of alkyl nitrites depends entirely upon the speed of liberation of free nitrite in the organism, but Krantz, Carr, and Forman (1938 b) showed that this is not the only factor because they found that amyl nitrite and 2-ethyl-n-hexyl-1-nitrite have the same rate of hydrolysis although the higher homologue has a much more lasting effect.



## ETHYL NITRITE

Of the nitrous acid esters listed in table 1, ethyl nitrite, the nitrous acid ester of ethyl alcohol, has some pharmacological importance in that it is used therapeutically as a vasodilator agent in the form of the spiritus aethylis nitritis (sweet spirit of niter) an alcoholic solution of ethyl nitrite of about 4 percent strength (U. S. P.) or 2 percent (B. P.). As indicated in table 2, Cash and Dunstan (1894) found it more effective than methyl nitrite in increasing the pulse rate but less effective than propyl nitrite, and with regard to the reduction of the blood pressure it also ranks in between these two compounds. Like other nitrous acid esters, it changes oxyhemoglobin into methemoglobin, but, in contrast to the nitric acid ester, doses of 20 to 60 mg. per kg., given in oil, intraperitoneally to cats, do not give rise to the formation of Heinz bodies (Pulina, 1942).

Lewin (1929) stated that workers handling ethyl nitrite suffered from headache, increased pulse rate, and cyanosis. According to the same author, the ingestion of 4 gm. of the spirit of ethyl nitrite caused, in a boy, severe vomiting and colic lasting several hours. The continued ingestion of 30 gm. of the spirit daily, for 3 weeks, caused, in an adult, delirium and mental disturbances with tendency to suicide, dryness of the skin, slowing of the pulse, and albuminuria. Hill (1878) reported one fatal case of a child approximately 3 years old who had ingested 90 to 120 gm. of sweet spirit of niter. The child vomited freely and had diarrhea; after some time it collapsed completely, became cold and almost pulseless, the respiration was hardly perceptible, and the pupils were fixed and widely dilated. Upon application of hot-water bottles there was temporary improvement, but after several hours the vomiting and diarrhea were repeated, the respiration became stertorous, and the child died 12 hours after the ingestion of the spirit. Upon autopsy the gastric contents had a strong odor of alcohol, the mucosa of the stomach and of the duodenum were inflamed, and the kidneys were slightly congested, the other abdominal organs being grossly normal. The meninges were highly congested and contained large quantities of dark colored blood. Seggel (1936) reported four cases of industrial poisoning from ethyl nitrite. All patients developed methemoglobinemia which lasted 24 hours, and three out of four showed, on the day of the accident, a distinct enlargement of the spleen which was thought to be caused by the destruction of methemoglobin. All patients had completely recovered after 10 days had elapsed.

## AMYL NITRITE

At present the most important aliphatic nitrous acid ester is iso-amyl nitrite, usually referred to as amyl nitrite. It is used as a vasodilator agent especially in angina pectoris, in the perfume industry, and in the manufacture of diazo and nitroso compounds.

Chemical characteristics: Amyl nitrite,  $(\text{CH}_3)_2\text{CHCH}_2\text{CH}_2\text{ONO}$ , has the molecular weight 117.15. It is a light yellow, transparent liquid of the specific gravity  $0.872 \frac{20^\circ}{4^\circ}$  C. which boils at  $99^\circ$  C. and

which is very slightly soluble in water but miscible with alcohol and ether. It has a penetrating, fragrant odor and decomposes upon exposure to air and sunlight (Horswell and Silverman, 1941).

Chemical identification: When amyl nitrite is poured upon a freshly prepared mixture of equal parts of a 10-percent solution of ferrous sulfate and concentrated sulfuric acid a brown layer is formed (Rosenthaler, 1923).

When 2 cc. of sulfuric acid are added to a mixture of 2 drops of amyl nitrite and 2 drops of distilled water, amyl valerate is produced which may be identified by its odor upon dilution with water (Pharmacopeia of the United States, 1942).

*Determination.*—For the determination of amyl nitrite, 20 cc. of aldehyde-free alcohol are placed in a 100-cc. glass-stoppered volumetric flask and weighed accurately, 3 to 4 cc. of amyl nitrite are added, and the mixture is stoppered and weighed again, thus determining the weight of the amount of amyl nitrite used for assay. The volume is then made up to 100 cc. at  $25^\circ$  C. with aldehyde-free alcohol, mixed thoroughly, and 10-cc. samples are used immediately for further determination. For this purpose 10 gm. of potassium iodide are dissolved in 40 cc. of boiling, deaerated, distilled water in a 300-cc. Erlenmeyer flask which is closed with a two-hole stopper, and carbon dioxide is passed through the mixture at the rate of five bubbles per second until the solution is cooled, then 10 cc. of hydrochloric acid (1:2) are added and carbon dioxide is passed through for another 5 minutes. (In case any iodine is liberated, as indicated by a yellow color, this is decolorized by careful addition of N/10 sodium thiosulfate.) Ten cc. of the alcoholic solution of amyl nitrite are added, and the liberated iodine is titrated with N/10 sodium thiosulfate, 1 cc. of which corresponds to 0.01172 gm. of amyl nitrite (Pharmacopeia of the United States, 1942).

For the *determination of amyl nitrite in air*, Horswell and Silverman (1941) recommended the following procedure which is based on

the color change from faint blue to deep red when a minute amount of amyl nitrite is added to a mixture of phenol solution, copper sulfate solution, and glacial acetic acid. For the determination, air is passed at a rate of 40 cc. per minute through 30 cc. of a mixture of 4.8 cc. of a 5-percent phenol solution, 0.2 cc. of a 2-percent hydrated copper sulfate solution, and 35 cc. of glacial acetic acid. The maximal color is produced after 15 minutes at 25° C. and in 5 minutes at 80° C. It remains stable for 24 hours and its intensity can be measured spectrophotometrically at a wave length of 515 m $\mu$ . It is stated that 5 to 100 p. p. m. may be determined in this way.

*Absorption, fate, and excretion of amyl nitrite.*—Amyl nitrite is readily absorbed through the lungs and from the digestive tract but it does not appear to be established whether or not it is absorbed through the skin. Very little is known with regard to its fate in the organism. Leech (1893) believed that with oral administration it is slowly decomposed by the gastric juice with the formation of nitrite, so that it appears questionable whether undecomposed amyl nitrite enters the circulation with this form of administration. After inhalation a similar decomposition takes place but with this form of administration both the amyl nitrite molecule and the nitrite derived from it may be effective.

*Effects of amyl nitrite on the organism.*—Evidently the first reference to the effect of amyl nitrite on the human organism is the statement by Guthrie (1859) who, following the description of the chemical properties of amyl nitrite, stated that the inhalation of its vapors caused throbbing in the arteries of the neck followed immediately by flushing of the neck, the temples, and the forehead, and acceleration of the pulse rate; these symptoms disappeared shortly after discontinuation of the exposure.

With regard to the *effect of amyl nitrite on the central nervous system*, Wood (1871) showed that in pigeons, cats, and dogs the continued inhalation of amyl nitrite causes a progressive depression of the central nervous system and, in pigeons, also, frequently, convulsions. In the opinion of Mayer and Friedrich (1875) these are due to stimulation of certain sections of the brain. Filehne (1879) believed that they are probably caused by asphyxia. Fischer and Fischer (1914) reviewed the different studies of the effect of amyl nitrite on the central nervous system and studied the mechanism of the convulsions in rabbits. They found that reduction of the increased intracranial pressure by trepanation (the mechanism of which will be discussed below) does not modify the convulsant action, furthermore, they found no evidence that it arises from the spinal cord or in the cortex of the brain, and they assumed that it is due to a direct stimulation of the hypothalamic region.

Pilcher and Sollmann (1915) showed that amyl nitrite usually produces a moderate stimulation of the *vasomotor center*, probably due to anemia from the fall of the blood pressure, but they saw no evidence of a depressant effect.

Similarly, Mayer and Friedrich (1875) assumed that the increase in rate and depth of the *respiration* following the inhalation of amyl nitrite was due to a primary stimulation of the respiratory center which later on may be followed by a depression. In the opinion of Filehne (1879) this stimulation results from hypoxemia caused by methemoglobin formation. This central effect may be obscured or overshadowed by an inhibition caused by reflexory reactions in the upper respiratory tract, as shown by Dautrebande (1932 a, b; 1933). Rosenblüth and Wassermann (1929) believed that the deepening of the respiration is probably due to a disturbance of the respiratory center. It appears, therefore, that following inhalation of amyl nitrite the respiration may be increased by stimulation of the medullary center because of anoxia caused by deficient blood supply or by methemoglobinemia, and that when the anoxia increases this may result later in a depression. On the other hand, the inhalation of high concentrations of amyl nitrite may cause slowing of the respiration by a reflex mechanism arising in the upper respiratory tract.

Ladendorf (1874) showed that inhalation of amyl nitrite results in an increase of the *temperature* of the skin of the head and axilla which outlasts the inhalation. He attributed this to the vasodilatation produced by amyl nitrite, whereas Fischer and Fischer (1914) believed it to be due to stimulation of the central heat-regulating mechanism. In contrast to this increase of the temperature of the skin, Krauss (1913) found that amyl nitrite decreases the rectal temperature of normal rabbits, and, to a greater extent, that of rabbits in which the body temperature has been raised by heat puncture. Jacobj (1913) pointed out that in such animals even small doses which do not cause any changes in the temperature of normal animals are effective; that with intermediate doses this effect becomes less marked; but that large doses again cause a definite decrease. He believed that this twofold action can be explained by variations in the peripheral circulation and, with large doses, by interference with the oxygen metabolism. It appears, therefore, that following inhalation of amyl nitrite the skin temperature may be increased because of greater heat dissipation caused by vasodilatation, and that if this persists over a sufficient period of time the body temperature is reduced because of heat loss, especially if the heat-regulating center is depressed by an inadequate blood supply, lack of oxygen, or a reduction of the oxygen metabolism by methemoglobinemia.



Biedl and Reiner (1900) showed that immediately following the injection of amyl nitrite into a side branch of the carotid artery there is a rise of the *spinal pressure* which is parallel to the fall of the blood pressure. Dixon and Halliburton (1914) showed that in animals the inhalation of 3 to 4 drops of amyl nitrite increases the secretion of cerebrospinal fluid, which parallels the fall of the blood pressure, both returning to normal values at the same time. They also showed that this effect is often associated with a rise of the venous pressure and of the torcula pressure. They believed that the latter is the outcome of lower peripheral resistance, resulting in increased cardiac output, and

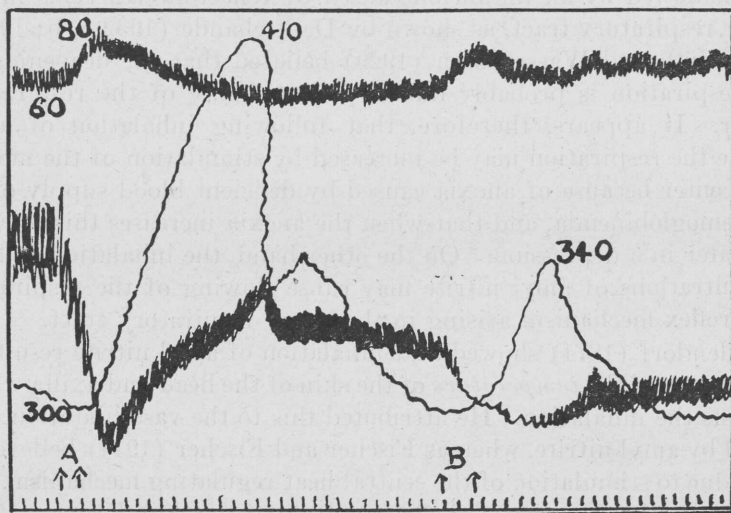


FIGURE 2.—Effect of amyl nitrite on cerebrospinal pressure, arterial pressure, and torcula pressure.

of a small increase of pressure in the right auricle so that the torcula pressure parallels the cardiac output. They showed further that the increase of the spinal pressure following inhalation of amyl nitrite is not due to an increase in the venous pressure because it occurs before the venous pressure is changed. On the other hand, there seems to be little relation between spinal pressure and torcula pressure because in some instances both the cardiac output and the torcula pressure show their maximum increase after the spinal pressure is already at its maximum. Figure 2 shows the relation between spinal pressure, arterial pressure, and torcula pressure following the inhalation of amyl nitrite. Stefan (1934) believed that the increase of the spinal pressure resulting from the inhalation of amyl nitrite is the result of vasodilatation in the skull and increased secretion of spinal fluid. As



illustrated in figure 3, he found that in chronic inflammatory processes such as postencephalitic Parkinsonism, Wilson's pseudosclerosis, Alzheimer's disease, and in genuine epilepsy, this rise is much less pronounced and may be absent, whereas in patients with brain tumors the rise is greater than normal. This seems to indicate that the rise of the spinal pressure is, at least in part, the result of an increase of the intracranial pressure due to vasodilatation.

The effect of amyl nitrite on the vision is, in part, closely affiliated with its vasodilator action. During the flushing of the face there may be distinct injection of conjunctival vessels, sometimes associated with

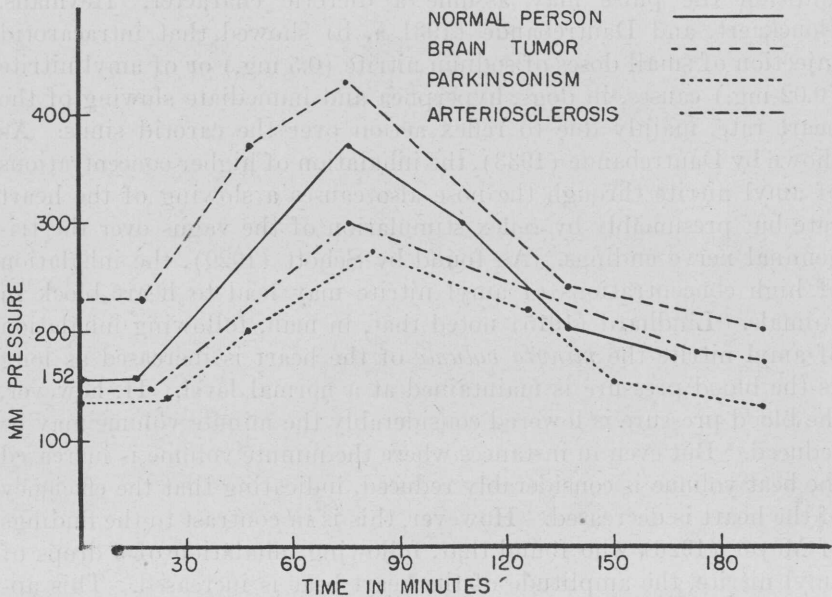


FIGURE 3.—Changes of the spinal pressure of normal individuals under different pathological conditions following the inhalation of amyl nitrite.

more or less marked lacrimation and heaviness of the upper eyelids. The pupils are usually dilated, only in severe poisoning are they constricted, and their reaction to light may be exaggerated or abolished. Some persons experience subjective color sensations of varying character (Lewin and Guillery, 1913) and the acuity of the vision may be temporarily reduced.

Wessely (1915) showed that in animals the administration of amyl nitrite is followed by an increase of the *intraocular pressure* resulting from vasodilatation within the bulbus.

Effect of amyl nitrite on the circulation: As shown by Bock (1898), amyl nitrite has no direct effect on the frog's *heart* or the isolated

mammalian heart, and, as found by Plumier (1905), only in large doses does it cause a depression of the heart action, diminishing the force of the cardiac contraction without affecting the rate of the heart beat. In contrast to these negative findings with the isolated heart, the inhalation of amyl nitrite through a tracheal cannula causes an increase of the *heart rate* as described by Guthrie (1859), Cash and Dunstan (1894), Hewlett (1906 a, b), and many others. In the opinion of Mayer and Friedrich (1875) this is due to decreased vagus tone, which, according to Meyer, Gottlieb, and Pick (1933), is the result of decreased blood pressure. As pointed out by Cushny (1923), in addition the pulse may assume a dicrotic character. Heymans, Bouckaert, and Dautrebande (1931 a, b) showed that intracarotid injection of small doses of sodium nitrite (0.5 mg.) or of amyl nitrite (0.02 mg.) causes, in dogs, hyperpnea and immediate slowing of the heart rate, mainly due to reflex action over the carotid sinus. As shown by Dautrebande (1933), the inhalation of higher concentrations of amyl nitrite through the nose also causes a slowing of the heart rate but presumably by reflex stimulation of the vagus over the trigeminal nerve endings. As found by Schott (1920), the inhalation of high concentrations of amyl nitrite may lead to heart block in animals. Lindhard (1915) noted that, in man, following inhalation of amyl nitrite the *minute volume* of the heart is increased as long as the blood pressure is maintained at a normal level. If, however, the blood pressure is lowered considerably the minute volume may be reduced. But even in instances where the minute volume is increased the beat volume is considerably reduced, indicating that the efficiency of the heart is decreased. However, this is in contrast to the findings of Meyer (1926) who found that, following inhalation of 5 drops of amyl nitrite, the amplitude of the heart beat is increased. This apparent contradiction may be explained by the observations of Winkler (1898) who found that, following administration of amyl nitrite, both blood pressure and cardiac efficiency are reduced but that with repeated administration the latter may be entirely overcome. He believed that small doses of amyl nitrite have a favorable effect on the heart energy but that with large doses it is decreased, as indicated by an increase of the left auricular pressure.

Amyl nitrite has a marked *vasodilator action*. Lauder-Brunton (quoted from Bernheim, 1874) believed that this was of peripheral origin, whereas Bernheim (1874) and Filehne (1879) were of the opinion that it was due to a depression of the vasomotor center. But the conclusions of the latter were proved to be based on an inadequate technic by Lichtwitz and Hirsch (1910) and other students of the subject such as Mayer and Friedrich (1875), Kobert (1887), Atkinson (1887-88), Marshall (1897-98), and others. Pilcher and Sollman

(1915) showed that amyl nitrite causes no depression of the vasomotor center, and according to Jacobj (1913) the peripheral vasodilator action is more marked if the vascular tone is increased by central stimulation. Leech (1893) showed that in concentrations of 1:10,000 amyl nitrite usually dilates but sometimes contracts the blood vessels, but higher concentrations always cause vasodilation in the perfused turtle. Danielopolu and Aslan (1925) noted that inhalation of amyl nitrite causes a primary vasoconstriction and subsequent dilatation, and Johnson (1931) observed that, following inhalation of amyl nitrite, the finger volume was decreased by 0.2 to 0.25 cc. which sometimes was associated with a decrease of the pulse rate and later followed by an increase in volume. He believes that the decrease in volume was due to an overwhelming splanchnic dilation, causing drainage of the blood from the peripheral area, the blood pressure being too low to distend the vessels. Cow (1911) found that rings of isolated arteries (coronary, pulmonary, and others) are dilated by amyl nitrite. Therefore, it appears to be established that amyl nitrite dilates the blood vessels by a peripheral mechanism which is produced (as will be shown below) by a direct depression of the muscular structures. In the intact organism this effect may be complicated by other factors, so that with systemic action of amyl nitrite the vascular response may be modified. In the opinion of Cotton, Slade, and Lewis (1916) the rise of the pulse rate and the fall of the blood pressure (vasodilation) are generally greater in persons with "irritable heart" than in normal individuals.

Much attention has been paid to the response of the cerebral vessels to the inhalation of amyl nitrite. Schüller (1874) showed that amyl nitrite causes dilatation of the pial vessels, and found that this effect is less marked after dissection of the superior cervical ganglion. Jacobj (1913) and later Danielopolu and Aslan (1925) pointed out that the vasodilator action of amyl nitrite is more marked if the blood vessels are high in tone. It appears, therefore, that the observation of Schüller (1874) is not indicative of a nervous mechanism of the amyl nitrite action. Similarly, Pick (1876) and Schadow (1876) found that, in rabbits, inhalation of amyl nitrite causes dilation of the pial vessels, and this was confirmed by Mosso (1881) and Hürthle (1889) (both quoted from Wolff, 1929) and by Hirschfelder (1915), Raphael and Stanton (1919), and others. Wolf (1929) found by direct measurements that this vasodilatation is paralleled by the fall of the blood pressure and the rise of the cerebrospinal pressure. Biedl and Reiner (1900) showed that *injection* of amyl nitrite into a side branch of the carotid artery causes a considerable reduction in the blood pressure in the vessels at the base of the brain (*circulus arteriosus Willemsii*), resulting in an increased blood flow through the veins.

Dixon and Halliburton (1913-14), on the other hand, found that *inhalation* of amyl nitrite diminishes the vascular volume of the brain, and they assumed that in the intact animal and with systemic administration of amyl nitrite the peripheral vasodilator action on the cerebral blood vessels is overshadowed by the fall of the blood pressure from splanchnic dilatation, resulting in a shift of the blood to the splanchnic area. It appears, therefore, that with systemic administration of amyl nitrite the blood vessels of the brain may be dilated but the flow of blood reduced on account of inadequate blood supply which may possibly result in anoxia of the brain.

In view of the therapeutic use of amyl nitrite in angina pectoris, the effect of this chemical on the *coronary blood vessels* has aroused

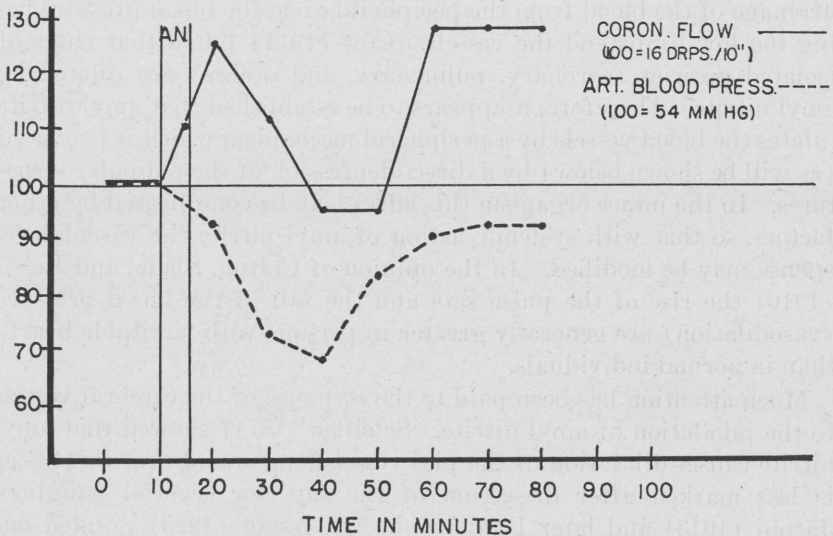


FIGURE 4.—Effect of inhalation of amyl nitrite on the coronary circulation and the arterial blood pressure of cats.

much interest. Loeb (1903) showed that concentrations of amyl nitrite which do not markedly affect the heart action cause dilatation of the coronary arteries, and similar results were reported by Meyer (1912) and Voegtlin and Macht (1913) who pointed out that this effect is less lasting than observed with nitroglycerine. Schloss (1913) studied the relation between the dilatation of the coronary blood vessels and the changes of the systemic blood pressure. He found (as illustrated in fig. 4) that, following inhalation of amyl nitrite, the coronary circulation is first increased, that it decreases parallel to the fall of the blood pressure, but that it increases again more rapidly as the blood pressure returns to normal, and finally reaches values of 20 percent above normal. He believed that the primary



coronary dilatation precedes the peripheral vasodilatation and hence also the fall of the blood pressure, and that only with toxic doses is the fall of the systemic blood pressure so marked that it overshadows the effect of the coronary dilatation and decreases the coronary flow. Nagl (1935) showed that coronary dilatation may be produced by very small doses of amyl nitrite which do not affect the peripheral circulation by stimulation of the carotid sinus. It appears, therefore, that in small doses amyl nitrite causes dilatation of the coronary arteries and an increase of the coronary flow, but that with larger doses this effect may be overshadowed and antagonized by its effect on the blood pressure.

With regard to the effect of amyl nitrite on the *splanchnic blood vessels*, Pick (1876) noted in rabbits that it causes dilatation of the abdominal blood vessels. Amsler and Pick (1920) studied the effect of sodium nitrite and amyl nitrite on the splanchnic blood vessels in perfusion experiments with the isolated splanchnic vascular system of frogs. With concentrations of 1:100 to 1:1000 of sodium nitrite and with concentrations of 10 drops of amyl nitrite per 200 cc. Ringer's solution, they noted always constriction of the splanchnic vessels which was sometimes followed by vasodilatation. They tried to explain the paradoxical behavior by a stimulation of peripheral vasoconstrictors by the nitrites which overshadows the vasodilator action, and they refer in this to experiments by Fröhlich and Loewi (1908 a, b) who believed they had shown that the nitrites paralyze the vasodilator fibers of the chorda tympani of the cat so that the effect of vasoconstrictor fibers becomes predominant. However, the presence of such vasoconstrictor fibers in the chorda tympani was not confirmed by Bayliss (1908).

With regard to the vascular response of the abdominal organs to amyl nitrite, Edmunds (1921) showed that under the influence of amyl nitrite the portal pressure and the *liver* volume are decreased, these changes being secondary to the fall of the systemic blood pressure, indicating that the liver does not participate in the dilatation of the splanchnic vessels. Tournade, Malméjac, and Rocchisani (1934) showed by cross perfusion of normal and denervated *kidneys* in dogs that in addition to its peripheral vasodilator effect amyl nitrite may have some vasoconstrictor properties of central origin. Herrick, Mann, and Sheehan (1939) showed that under the influence of amyl nitrite there is a rapid increase of the renal blood flow.

With regard to the effect of amyl nitrite on the *capillaries*, Carrier (1922) found that its intradermal injection and its inhalation cause slight dilatation of the capillaries and an increase of the capillary flow, indicating a dilator effect on the arterioles. Similarly, Schul-



mann and Marek (1926), who studied the effect of inhalation on wheal formation, found that this favors the disappearance of the wheal by increasing the capillary diameter and the capillary flow and possibly also by changing the permeability of the capillary walls.

The effect of amyl nitrite on the *blood pressure* has aroused much comment. Berger (1874), Mayer and Friedrich (1875), Bradford and Dean (1894), Pilcher and Sollmann (1915), and others noted that the inhalation of amyl nitrite causes a fall of the blood pressure by peripheral vasodilatation, and Berger (1874) pointed out that this form of administration may give rise to confusing results, in that the irritation of the upper respiratory tract produced by the vapors may result in a number of reflexes which overshadow the fundamental reaction. Hewlett (1906 a, b) studied the effect of its inhalation in man and found that within 30 to 60 seconds this causes a rapid fall of the *systolic blood pressure* of an average of 13 mm. Hg., which is rapidly followed by a secondary rise to 15 mm. Hg. above the original level, the maximum being reached in 1 to 3 minutes after the inhalation, after which it gradually falls again to normal or slightly subnormal levels. Similarly, Meyer (1926) stated that inhalation of 5 drops of amyl nitrite causes tachycardia, arterial hypotension, and sometimes secondary slowing of the pulse rate and hypertension. Hewlett (1906, a) found that in most instances the *diastolic pressure* followed the changes of the systolic pressure with certain variations, so that the pulse pressure is reduced during the fall of the blood pressure and increased during its rise. Since during the first 30 seconds after inhalation of amyl nitrite the pulse rate is rapidly increased, this may, in part, compensate for the fall of the blood pressure from peripheral vasodilatation. Von Rzentkowski (1909) pointed out that the fall of the blood pressure is much more marked in certain arteriosclerotic patients than in normal persons, that it is of longer duration and that it is associated with a very marked decrease of the diastolic blood pressure, presumably because of a lower peripheral vascular tone, especially in the splanchnic region. Wessely (1915) confirmed the statement of Berger (1874) that in animal experiments inhalation of amyl nitrite through the nose may complicate the fundamental reaction of superimposed reflexes; he believed that with small doses the central stimulant, and with large doses the peripheral depressant effect was predominant. Dautrebande (1932 a, b) pointed out that in unanesthetized dogs inhalation of amyl nitrite causes an invariable rise of the blood pressure which can be prevented by cocaineization of the upper respiratory tract, after which it causes a fall of the blood pressure. Dautrebande (1933) pointed out that in different persons inhalation of amyl nitrite may cause a fall, a rise, or no effect on the blood pressure. Maréchal and Dautrebande (1933) and Maréchal (1935)

showed that the response to inhalation of amyl nitrite may vary with the dose of amyl nitrite and the duration and depth of the inhalation. In the opinion of Gaucher, Lequime, and van Bogaert (1933), amyl nitrite, as a rule, causes a fall of the blood pressure which sometimes is preceded by a moderate temporary rise, the fall persisting usually for some time after discontinuation of the exposure. It appears, therefore, that inhalation of amyl nitrite causes fundamentally a fall of the blood pressure caused by peripheral dilatation and associated with an increase of the heart rate. If inhaled in high concentrations it may cause reflex irritation of the medullary centers, resulting in a temporary rise of the blood pressure and bradycardia.

With regard to the effect of amyl nitrite on the *venous pressure*. Burton-Opitz and Wolf (1910) found that when administered to a restricted area (femoral artery of the dog) small doses of amyl nitrite cause a dilatation of the arterioles and a rise of the venous pressure in this area, whereas with systemic administration (inhalation) the marked fall of the arterial pressure was associated with a slight decrease of the venous pressure. Similarly, Payan and Giraud (1925) noted, as a rule, a reduction of the venous pressure which in only one instance was preceded by a short primary rise. This was confirmed by McDowall (1922-23) who noted also a rise of the venous pressure, but more frequently a primary rise and subsequent fall. He believed that because of the peripheral vasodilatation the venous return was greater and the pressure in the right auricle and in the venous system increased. He explained the secondary fall of the venous pressure by an accumulation of blood in the dilated capillaries which acted as a sponge. It appears, therefore, that as a rule the inhalation of amyl nitrite does not increase the venous pressure in spite of the peripheral dilatation, and this is presumably due to an inadequate blood supply to the periphery because of the shift of the blood to the splanchnic blood vessels.

The effect of amyl nitrite on the *pressure in the pulmonary arteries* has aroused much interest. Von Openchowski (1889) found that following administration of amyl nitrite to dogs the pressure in the pulmonary artery was either unchanged or increased as the systemic blood pressure was reduced, and in a few instances it was slightly below the systemic blood pressure. In his opinion the increase of the pulmonary arterial pressure is due to a stasis in the left auricle because the heart is unable to remove all the blood from the smaller circulation. In addition, it appears possible that as the peripheral vasodilatation wears off and the vessels contract they may deliver an abnormally large quantity of blood into the right heart, and hence to the lungs. On the other hand, the lowering of the pulmonary pressure below the level of the systemic blood pressure may be explained by a reduction

of the venous return because of excessive vasodilatation in the splanchnic area. Similarly, Bradford and Dean (1894), Plumier (1905 and 1906), and Frey (1909) noted that following administration of amyl nitrite there is a marked increase of the pulmonary pressure during the fall of the systemic blood pressure, followed by a more gradual return to normal. Winkler (1898) who made similar observations, pointed out that the increase of the pulmonary arterial pressure combined with the increased pressure in the left auricle, interfering with the return of the blood to the larger circulation, may easily lead to capillary stasis in the lungs and pulmonary edema. Other investigators tried to explain the rise in pulmonary arterial pressure by an abnormal response of the pulmonary arteries resulting in vasoconstriction instead of dilatation. Macht (1914) stated that amyl nitrite, like sodium nitrite and nitroglycerin, causes constriction and subsequent dilatation of isolated pulmonary arteries, whereas Cow (1911) had observed only relaxation of pulmonary arterial rings, and Dixon (quoted from McDowall, 1922-23) showed that perfusion of the isolated lung with nitrites causes an increased flow through this organ and therefore vasodilatation. Pic and Petitjean (1906) reported that in dogs the intravenous administration of amyl nitrite causes a fall of the systemic blood pressure and a rise of the pulmonary arterial pressure due to vasoconstriction of the pulmonary vessels as indicated by the blanching of the lungs, and Meyer (1926) was also inclined to associate the increase of the pulmonary arterial pressure with a vasoconstriction in this area. It appears, however, that there is no evidence for such an abnormal response of the pulmonary arteries, as also stated by McDowall (1922-23). He noted that in cats under light ether anesthesia the inhalation of amyl nitrite resulted in a fall of the pulmonary arterial and systemic blood pressure, the former frequently preceding the latter. He observed, however, that in deep anesthesia the fall of the pulmonary arterial pressure was frequently replaced by a rise, which may indicate that this phenomenon may only be observed if the peripheral resistance is lowered and the venous return increased. It appears, therefore, that the response of the pulmonary vessels to amyl nitrite does not differ from that of other blood vessels and that under the influence of increased venous return the pulmonary arterial pressure may be increased, which, if associated with a rise of the pressure in the left auricle, may lead to pulmonary edema.

With regard to the effect of amyl nitrite on the *blood*, Gamgee (1866-69) noted in animals that after inhalation of amyl nitrite the blood assumed a brown color and that in such blood the two absorption bands of oxyhemoglobin had more or less disappeared. He recognized this new pigment as some type of hemoglobin but did not identify it as methemoglobin. Wood (1871) showed that this pigment cannot

be oxygenated to oxyhemoglobin. Jolyet and Regnard (1876) found that in the presence of such dark colored blood the oxygen metabolism of the organism is reduced, because of a reduction of the oxygen-carrying power of the blood. Similarly, Filehne (1879) found that this pigment is unable to sustain the oxygen metabolism, and Giacosa (1879) established its identity with methemoglobin. There is no information available as to whether or not this methemoglobinemia is associated with the formation of Heinz bodies in blood cells, but experiments reported by Pulina (1942) with ethyl nitrite suggest that since this compound does not give rise to their formation, amyl nitrite will not. This is in contrast to the effect of ethyl nitrate which gives rise to the formation of both methemoglobin and Heinz bodies. Although it has been definitely established that following inhalation of amyl nitrite the methemoglobin formed interferes with the oxygen metabolism, it was shown by Slavu (1908) that after administration of amyl nitrite considerable amounts of oxygen are still present in the blood, so that it appears that death from amyl nitrite cannot be due exclusively to lack of oxygen. A similar assumption was made by Haldane, Makgill, and Mavrogordato (1897).

With regard to the *effect of amyl nitrite on muscular structures*, Wood (1871) showed that it decreases the response of the striated muscle to electric stimuli, Leech (1893) found that it decreases the contraction of the striated muscle, and Cash and Dunstan (1894) noted that it causes a passive muscular contraction.

Leech (1893) and others showed that it also depresses *smooth muscle structures*, and, as shown above, numerous investigators have demonstrated that it causes *relaxation of the blood vessels* by a direct action on the smooth muscles in the vascular walls. Doyon (1906) showed that it causes a similar relaxation of the *bronchial musculature*, and this was confirmed by Baehr and Pick (1913) in animals and by Nicholson (1939) in man. Barnes (1882), Steele (1883), and Soutar (1906) (all three quoted from Croft, 1928) found that inhalation of amyl nitrite depresses the *uterine contractions*, and this was confirmed by Croft (1928). Amyl nitrite also decreases the *contractility of the intestine*, as demonstrated by Holmes and Dresser (1928), Beams (1932), Beams and Barlow (1932), and others.

With respect to the *effect of amyl nitrite on the kidney function*, Chasis, Ranges, Goldring, and Smith (1938) found that the inhalation of amyl nitrite or the intravenous injection of sodium nitrite causes a reduction of the plasma flow and of the rate of filtration through the kidney when the blood pressure is reduced to 66/44 mm. Hg., which, in a later stage, may be followed by marked oliguria, presumably by an effect on the venous system. Herrick, Mann, and Sheehan (1939) noted an increased flow of blood through the kidneys



following inhalation of amyl nitrite. Hoffman (1872) and Sebold (1874) (quoted from Lewin, 1929) observed in rabbits, following the subcutaneous injection of amyl nitrite, diuresis, albuminuria, and glucosuria. The last named may result secondarily from the lowering of the blood pressure, because Tournade and Chabrol (1925) found that this gives rise to an increased output of epinephrine which, in turn, may be the cause of the glucosuria. Araki (1891) confirmed the findings of Hoffman (1872) and Sebold (1874) (quoted from Lewin, 1929) in that he found, following inhalation of amyl nitrite, glucose and lactic acid in the urine which he believed to be the result of reduced oxygen supply. It appears very likely that glucemia and increased permeability of the renal tissue may be involved in this phenomenon.

With regard to *toxic effects of inhalation of large doses of amyl nitrite in man*, these are usually characterized by rapid flushing of the face, pulsation in the head, and, in more severe cases, by cyanosis, confusion, vertigo, motor unrest, weakness, and yellow vision. The blood pressure may drop considerably, the pulse may become soft and thready and the victim may faint. Usually these symptoms are only of short duration and the patient recovers rapidly. Samelsohn (1875) reported on a patient who collapsed while standing, following the inhalation of amyl nitrite, but who recovered after a short time. Cadwallader (1896) described the case of a physician who had inhaled the vapors of amyl nitrite escaping from a leaking bottle while asleep, who was found in the morning unconscious and in collapse and who developed subsequently mental symptoms similar to those seen in certain cases of carbon monoxide poisoning; he died after several days from respiratory failure. As compared with the toxicological picture following the inhalation of amyl nitrite, the sequelae resulting from its ingestion are often less severe. Rosen (1888) noted, in a patient who had accidentally swallowed 12 to 15 gm. of amyl nitrite, no serious symptoms aside from nausea, vomiting, and delayed emptying of the stomach; and Shoemaker (1893) saw, in one patient who had taken one teaspoonful by mistake, only moderately flushed face, moderate headache, and rapid pulse. In spite of gastric lavage and emetics this patient later developed moderate cyanosis, shallow respiration, weak, slow, and irregular pulse, and clammy skin; however, he did not lose consciousness nor did he show twitchings or convulsions. With regard to the *local action of amyl nitrite*, Stasnik (1935) reported on a case where a 25-cc. ampoule of amyl nitrite exploded, spraying its contents into the eyes of a physician, the left eye also being injured by small fragments of glass. Whereas the right eye showed only hyperemia, the cornea of the left showed extensive corrosion and later a linear turbidity, presumably caused by nitrous



acid liberated from amyl nitrite. Repeated administration of amyl nitrite causes a certain amount of *tolerance*. According to Crandall, Leake, Lovenhart, and Muehlberger (1931), this can be developed within a few hours and may last, at most, 12 days.

Whereas no cases of *industrial poisoning from amyl nitrite* have been reported, Seggel (1936), as pointed out above, reported four cases of poisoning from inhalation of ethyl nitrite, a closely related compound.

With regard to the *treatment of amyl nitrite poisoning*, toxic effects from inhalation of small doses usually disappear rapidly upon discontinuation of the exposure. With more severe exposure attention has to be paid to the circulatory disturbances and to the methemoglobinemia. The lowering of the blood pressure, resulting in an inadequate supply of blood to the brain, is best combatted by placing the patient in a lying position. Because of the increased venous return and the increased burden on the heart, the patient should be kept at rest, and any physical exercise should be avoided. In case the respiration is shallow or otherwise impaired, artificial respiration may become necessary. This should, however, be performed with adequate precaution because of the possibility of pulmonary edema. The oxygenation of the blood may be increased by the administration of oxygen for 10 to 15 minutes, and this should be repeated if necessary. The circulation may be improved by the administration of caffeine-sodium salicylate or camphor. The methemoglobinemia, pulmonary edema, and peripheral stasis may necessitate venesection. It should be pointed out that although any chilling of the patient should be avoided, the administration of heat, as by hot baths or heating pads, should be avoided because the peripheral dilation resulting from such procedures may favor the onset of collapse.

## II. THE NITRIC ACID ESTERS OF MONOVALENT AND POLYVALENT ALIPHATIC ALCOHOLS

The nitric acid esters of aliphatic alcohols play an important role as explosives, and several members of this group are also used therapeutically as hypotensive agents. Whereas the nitric acid esters of monovalent alcohols—methyl and ethyl nitrate—are not used very extensively, the esters of the bivalent ethylene glycol, the trivalent glycerol, the quadrivalent erythritol, and some other polyvalent alcohols, to be described below, are extensively used as explosives and also as therapeutic agents. As pointed out by Marshall (1897-98), all nitric acid esters of aliphatic alcohols dilate the blood vessels and thus lower the blood pressure, and, in addition, they cause methemoglobinemia, but they differ with regard to the intensity and the duration of these effects.

## NITRIC ACID ESTERS OF MONOVALENT ALCOHOLS

## Methyl Nitrate

The lowest member of the monovalent aliphatic alcohol nitric acid esters is methyl nitrate,  $\text{CH}_3\text{ONO}_2$ , with a molecular weight of 77.04. It is a colorless liquid of the specific gravity 1.203 <sup>25°</sup> which boils at 65° C. and which readily explodes upon heating. It is insoluble in water but soluble in alcohol and ether.

Bradbury (1895) found that concentrations of 1:10,000 cause some but very little vasodilatation in perfused organs whereas concentrations of 1:1,000 have a distinct vasodilator effect. In rabbits, doses of 0.0125 gm. per kg. have practically no effect on the blood pressure and pulse rate and doses of 0.052 gm. per kg. have only a slight and transient effect, so that its action is considerably weaker than that of nitroglycerol and ethylene glycol dinitrate. Crandall, Leake, Loevenhart, and Muehlberger (1931) determined the minimal dose causing headache in man as 117 to 470 mg., and they found that the repeated administration of fractional doses may develop tolerance in 23 to 26 hours and that this may last for approximately 10 days.

## Ethyl Nitrate

Ethyl nitrate,  $\text{C}_2\text{H}_5\text{ONO}_2$ , has a molecular weight of 91.07. It is a colorless liquid of the specific gravity 1.100 at  $\frac{25^\circ}{4^\circ}\text{C.}$  which solidifies at -102° C. and boils at 87-88° C. At 55° C., 1.3 parts of ethyl nitrate dissolve in 100 parts of water and it is miscible with alcohol and ether.

Ethyl nitrate has anesthetic properties but is not used for this purpose because of its great explosiveness and its undesirable side actions. According to Lewin (1929) its inhalation causes headache, narcosis, vomiting, and stiffness of the musculature. Keeser (1939) found that it causes slowing and incomplete relaxation of the isolated frog heart and antagonizes the effects of epinephrine and sympatol but increases the effect of acetyl choline in that both the decrease of amplitude and slowing of the isolated heart are more marked. In cats, it causes a fall of the blood pressure and antagonizes the pressor action of epinephrine. It decreases the tone and the peristaltic waves of the isolated rabbit's intestine and paralyses the muscle of the leech and antagonizes in this test object the stimulant effect of acetyl choline. It is, therefore, evident that ethyl nitrate does not primarily affect the autonomic nerve system by increasing its excitability. Keeser (1939) found, further, that whereas ethyl nitrate does not affect the action of yeast-catalase in vitro, after its intravenous injection it inhibits the action of the blood-catalase in rabbits. Therefore, it interferes with

the destruction of hydrogen peroxide in the tissue which thus accumulates in the tissues and causes toxic effects. Premedication with sympatol, epinephrine, acetyl choline, and pilocarpine, or with thiourea, thiodiglycol, and cholic acid, did not prevent in guinea pigs the toxic effects of ethyl nitrate, and cysteine increased its toxicity. On the other hand, chronic ethyl nitrate poisoning of guinea pigs was favorably affected by glutathione and, to a greater extent, by thionine. Of other attempts to activate the catalase, inhibited by ethyl nitrate, the administration of ferrous sulfate was found to be ineffective whereas manganous sulfate and potassium iodide delayed the toxic action but did not attenuate the severity of the picture. Of other agents tested, ergotamine tartrate and hemin were found to be effective, and the same holds true to some extent for calcium lactate, but its effect is said to refer less to the activation of catalase than to the reduction of methemoglobin. It appears, therefore, that following the administration of ethyl nitrate certain reductive processes and the action of catalase are affected. Pulina (1942) found that the intraperitoneal injection of 400 mg. per kg. of ethyl nitrate, dissolved in olive oil, causes, in cats, after 10 minutes, unconsciousness, rapid respiration, fixation and maximal dilatation of the pupils, and, after 90 minutes, death from respiratory arrest, whereas smaller doses (300 mg. per kg.) were followed by recovery. According to Wilhelmi (1942) even comparatively large doses (125 to 250 mg. per kg.) cause in cats only moderate methemoglobinemia, but as also pointed out by Pulina (1942) they give rise to the formation of Heinz bodies which could not be observed with the corresponding nitrous acid ester.

The higher homologues of the monovalent aliphatic alcohol nitric acid esters evidently have not been studied toxicologically, and the paucity of data reported regarding the two lowest homologues allows no conclusions as to the relation between their physico-chemical data and their pharmacological and toxicological action.

## NITRIC ACID ESTERS OF BIVALENT ALCOHOLS

### Ethylene Glycol Mononitrate

Ethylene glycol mononitrate was studied by Wilhelmi (1942) with regard to its effect on the blood pigment and blood cells. She found that the intraperitoneal injection of doses corresponding to 100 mg. per kg. of the mononitrate in oil (based on the number of nitrate groups) causes, in cats, a moderate increase of the methemoglobin level in the blood but no injury of the blood cells, as indicated by the absence of Heinz bodies. The repeated intraperitoneal administration of 35 mg. per kg. had very little effect in cats, and upon autopsy the liver and spleen contained no destruction products of hemoglobin but the liver

showed central fatty necroses indicating a primary injury of the liver by ethylene glycol mononitrate. It was found that with respect to methemoglobin formation, ethylene glycol mononitrate is 10 to 20 times less effective than the ethylene glycol dinitrate.

### Ethylene Glycol Dinitrate

Introduction of a second nitro group into ethylene glycol mononitrate yields ethylene glycol dinitrate,  $\text{O}_2\text{NOCH}_2\text{CH}_2\text{ONO}_2$ , with a molecular weight of 152.07. It is a yellow liquid of the specific gravity 1.482  $\frac{21^\circ}{4^\circ}$  C. which solidifies at  $-20^\circ$  C. and boils (explodes) at  $114^\circ$  C. According to Gross, Bock, and Hellrung (1942) it dissolves in 100 cc. of water to the extent of 0.45 gm. at  $21^\circ$  C., in 100 cc. of olive oil to the extent of 12.2 gm., and mixes freely with 96 percent ethyl alcohol and diglycol monomethyl ether. According to the same authors the median vapor pressure at  $20^\circ$  C. is 0.045 mm. Hg. and the saturation value is 0.380 mg. ethylene dinitroglycol per liter of air.

The *determination of ethylene glycol dinitrate in air* may be performed by the method of Foulger (1936) which is a modification of Scoville's (1911) method for the determination of glycerol trinitrate. A measured volume of the ethylene glycol dinitrate-air mixture is passed through a U-tube, surrounded in a vacuum bottle by an acetone-dry ice freezing mixture. The ethylene glycol dinitrate is then dissolved in acetone, combined with the acetone washings of the U-tube, and made up to 100 cc. with acetone. One to 5 cc. of this acetone solution are transferred to a porcelain dish and the acetone is evaporated at room temperature. The remaining ethylene glycol dinitrate is thoroughly mixed with 2 cc. of phenolsulfonic acid, kept for 10 minutes, diluted with water, and transferred with the washings to a 100-cc. volumetric flask. It is carefully made alkaline with diluted ammonia water (1:1), cooled, and made up to 100 cc. with distilled water, and the intensity of the color is measured spectrophotometrically at 4900 A. Ethylene glycol dinitrate may also be determined by the procedure of Yagoda and Goldman (1943) which will be discussed with the determination of glycerol trinitrate and which is based on the nitration of m-xylenol in 62.5 percent sulfuric acid and the colorimetric determination of the nitroxylenol isolated by a simple steam distillation.

Ethylene glycol dinitrate is readily *absorbed* through the skin, the lungs, and the gastro-intestinal tract. According to Gross, Bock, and Hellrung (1942) the absorption through the skin occurs more readily than with glycerol trinitrate, and, in contrast to the latter, the inhalation of its vapors rapidly causes chronic poisoning and anemia.



Little is known regarding the *fate of ethylene glycol dinitrate in the organism* except that it is partly hydrolyzed with the formation of nitrate.

With regard to the *toxicity* of ethylene glycol dinitrate, Gross, Bock, and Hellrung (1942) found it to be about twice as toxic for cats as glycerol trinitrate, the certain fatal dose with subcutaneous injection being 100 mg. per kg. as compared with 200 mg. per kg. of the latter. For rabbits, which are less liable to form methemoglobin and are less sensitive, the corresponding values are 400 and 500 mg. per kg. body weight. The death of cats, observed after doses of 100 mg. per kg. or more, probably is largely due to methemoglobinemia. Other symptoms observed were lowering of the blood pressure, vomiting, weakness, dyspnea, and salivation. In contrast to observations made with glycerol trinitrate, cats poisoned acutely with ethylene glycol dinitrate did not show convulsions, these being seen only in chronic poisonings with this compound. With prolonged poisoning the animals became definitely anemic and some also jaundiced. Wilhelmi (1942) noted that in cats, after intraperitoneal injection of 20 to 24 mg. per kg. of ethylene glycol dinitrate in oil, the animals became distinctly sick, lost weight, became listless and later dyspneic, and died after 8 to 9 days. During the last day they voided a dark brown urine containing 3+ urobilin, 1+ urobilinogen, but no bilirubin. Even smaller doses caused severe anemia and other blood changes to be described below. According to Keeser (1939) the subcutaneous injection of 9 cc of a 4-percent suspension of ethylene glycol dinitrate causes, in dogs, severe poisoning mainly characterized by ataxia lasting for several days which is said to be overcome by large doses of ascorbic acid. With regard to the *toxicity of the vapors of ethylene glycol dinitrate*, Gross, Bock, and Hellrung (1942) found that exposure for 8 hours daily to concentrations of 0.013 mg. per liter (2 p. p. m.) over a period of 1,000 days caused moderate temporary blood changes which disappeared later without leaving any apparent after effects. Cats exposed in the same way to concentrations of 0.134 mg. per liter (21 p. p. m.) developed marked blood changes but otherwise behaved normally and showed no organic changes after an exposure for 1,000 days.

With regard to *circulatory changes* produced by ethylene glycol dinitrate, Bradbury (1895) found that in rabbits 12.5 mg. per kg. cause a marked fall of the blood pressure and an increase of the pulse rate. Smaller doses of 0.6 mg. caused a rapid but transient reduction of the pulse tension. In perfusion experiments with isolated organs he found that concentration of 1:10,000 caused distinct vasodilatation which was somewhat greater than that observed with glycerol dinitrate. In the opinion of Marshall (1897-98) the depressor effect of



ethylene glycol dinitrate is more marked but less lasting than than of glycerol trinitrate. Similarly, Wilhelmi (1942) found that with ethylene glycol dinitrate the fall of the blood pressure is more marked than with glycerol trinitrate, and she pointed out that the effect on the blood pressure and the methemoglobinemic action do not run parallel but that the former precedes the latter and returns to nearly normal values when the methemoglobinemia is still at its peak. As in the case of aliphatic nitrous acid esters, the fall of the blood pressure

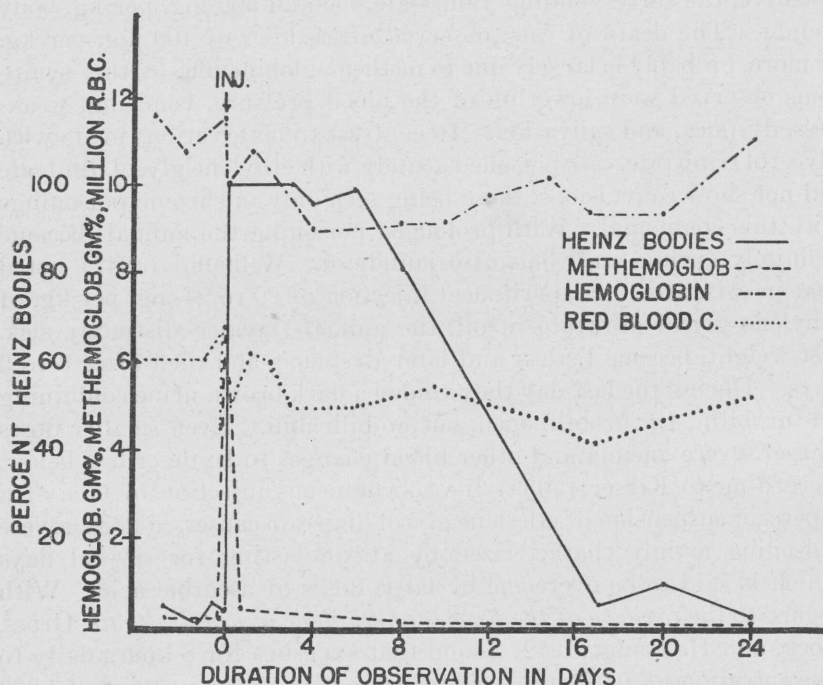


FIGURE 5.—Effect of subcutaneous injection of 5 mg. per kg. of ethylene glycol dinitrate on the formation of methemoglobin and Heinz bodies and its anemiagenic action as indicated by the reduction of red blood cells and hemoglobin.

is due to peripheral vasodilatation caused by a depression of the smooth muscles in the vascular walls.

In recent years the effect of ethylene glycol dinitrate on the blood pigment and on the blood cells has aroused much interest. Gross, Bock, and Hellrung (1942) showed that doses as small as 5 mg. per kg. given subcutaneously to cats are liable to cause methemoglobinemia in these animals, and that with doses of 60 mg. per kg. about 45 percent of the available hemoglobin is converted to methemoglobin and that it gives rise to the formation of Heinz bodies in the blood cells. As indicated in figure 5, the latter effects outlasts the methemoglobinemic action for a con-

siderable period of time. Wilhelmi (1942) studied in cats the methemoglobinemic action and Heinz body formation produced by ethylene glycol dinitrate following its intraperitoneal injection in oily solution. She found that in this respect it is four times as effective as glycerol trinitrate and 10 to 20 times as effective as ethylene glycol mononitrate and ethyl nitrate. Since this gradient does not parallel the rate of hydrolysis of these compounds resulting in liberation of nitrites, and from the ratio of ethylene glycol dinitrate given to the amount of methemoglobin formed, it appears that this is a molecular reaction in contrast to the methemoglobinemic action of aromatic nitro compounds which is presumably a catalytic process. In the opinion of Wilhelmi (1942) in acute poisoning ethylene glycol dinitrate causes an acute irreversible damage of the red blood cells and with chronic poisoning it causes anemia. The considerable decrease of the hemoglobin values (2 to 6 percent) suggests that the toxic action of ethylene glycol dinitrate does not stop at the methemoglobin formation but may lead to further destruction of the hemoglobin molecule and the formation of a large number of Heinz bodies. These were first small but gradually increased in size so that they finally covered one-fifth to one-fourth of the diameter of the red blood cells. In a later stage of the poisoning the large Heinz bodies became less numerous, the red blood cells then containing numerous small granules scattered through the cells or lumped together. This disappearance of the large Heinz bodies was paralleled by a sharp reduction of the hemoglobin, indicating that they were replaced by young blood cells with scattered granula. It appears that the formation of methemoglobin and of Heinz bodies does not run parallel but that the latter has a definite relation to the appearance of anemia.

The *pathological changes* observed in experimental animals as reported by Gross, Bock, and Hellrung (1942) and Wilhelmi (1942) may be summarized as follows: The heart muscle may show fine fat droplets (possibly due to anemia) and fatty degeneration. The liver may contain large amounts of blood pigment, and, with prolonged poisoning, it may show varying degrees of fatty infiltration and degeneration and hemorrhages. The spleen shows the typical picture seen in anemia, characterized by hemosiderosis and phagocytosis. The kidneys may show fatty infiltration and degeneration especially of the epithelium of the tubuli contorti, and, in acute poisoning, hemorrhagic inflammation. The mucosa of the stomach and upper intestine may show more or less marked inflammation. In chronic poisoning the bone marrow may show hyperplasia.

The *toxic symptoms in man* resulting from acute exposure to ethylene glycol dinitrate are essentially the same as those experienced with glycerol trinitrate, namely headache, nausea, vomiting, lowering of

the blood pressure, increase of the pulse rate, and cyanosis. Crandall, Leake, Loevenhart, and Muehlberger (1931) determined the minimal dose causing headache when applied to the skin as 1.8 to 3.5 cc. of a 1-percent alcoholic solution and found that the application of fractional doses totaling 170 mg. (16 cc.) caused tolerance within 24 to 36 hours, lasting for 10 to 13 days. Little information is available regarding the chronic toxicity of ethylene glycol dinitrate. Gross, Bock and Hellrung (1942) mentioned that a greater number of fatalities had occurred among explosives workers which were presumably due to exposure to ethylene glycol dinitrate, but these never were explained satisfactorily. They believed that the deaths of these men who died suddenly, evidently from circulatory failure after having had no exposure for 24 to 48 hours, were partly complicated by other factors such as the influence of alcohol and excessive physical exercise. In view of the changes of the cardiac muscle observed in experimental animals, the possibility of weakening of the cardiac muscle resulting from continued exposure appears to be within the realm of possibility.

## NITRIC ACID ESTERS OF TRIVALENT ALCOHOLS

### Glycerol Dinitrate

Glycerol dinitrate, dinitroglycerol,  $\text{O}_2\text{NOCH}_2\text{CH}(\text{OH})\text{CH}_2\text{ONO}_2$ , of the molecular weight 182.09, is a colorless to slightly yellow oil of the specific gravity 1.47<sup>15</sup>, which solidifies below  $-30^\circ\text{C}$ . and which boils at  $146^\circ\text{C}$ . 15 mm. Hg. without considerable decomposition. According to Will (1908) it is more soluble in water than glycerol trinitrate.

There is no information on the toxicity of glycerol dinitrate except a statement by Will (1908) that its absorption causes headache similar to that produced by glycerol trinitrate.

### Glycerol Trinitrate

Trinitroglycerol, glycerol trinitrate, nitroglycerin, or glonin,  $\text{O}_2\text{NOCH}_2\text{CHONO}_2\text{CH}_2\text{ONO}_2$ , has a molecular weight of 227.09 and is a colorless or yellowish oil of the specific gravity 1.601<sup>15</sup>. It exists in two solid forms, one melting at  $13.3^\circ\text{C}$ . and a less stable one melting at  $2.0^\circ\text{C}$ . It boils at  $160^\circ\text{C}$ . at 15 mm. Hg. and explodes at  $270^\circ\text{C}$ . At  $20^\circ\text{C}$ . 0.18 parts of nitroglycerin are soluble in 100 parts of water, 54 parts in 100 parts of absolute alcohol, and it is miscible with ether and olive oil. Gross, Bock, and Hellrung (1942) determined the vapor pressure of a purified sample of glycerol trinitrate as 0.00072 mm. Hg. at  $20^\circ\text{C}$ . They pointed out the variation in the values reported in the literature and believed 0.00038 mm. Hg. to be correct. They determined the saturation concentration in air as 0.009 mg. per liter, and

it appears, therefore, that the vapor pressure of ethylene glycol dinitrate is more than 100 times greater than that of glycerol trinitrate and that the ratio of the saturation concentration of ethylene glycol dinitrate to that of glycerol trinitrate is 76:1.

Glycerol trinitrate is used extensively as an explosive agent in dynamite and smokeless powder and as hypotensive agent in therapeutics. Upon saponification with an aqueous solution of potassium hydroxide it yields glycerol and nitrate. As found by Berl and Delpy (1909) (quoted from Rosenthaler, 1923), treatment with alcoholic potassium hydroxide yields nitrite, alkali cyanide, ammonia, glycerol dinitrate, mesoxalic acid, oxalic acid, and carbon dioxide. As shown by Crandall, Leake, Loevenhart, and Muehlberger (1929) and by Yagoda and von Oettingen (1944), its rate of hydrolysis is very rapid.

Glycerol trinitrate may be identified by the red color formed with a solution of brucine in sulfuric acid. Upon the addition of a solution of aniline in sulfuric acid it yields a red color which turns green upon dilution with water (Rosenthaler, 1923).

The *determination of glycerol trinitrate* in various materials may be made by determining the  $\text{NO}_2$  content by means of phenoldisulfonic acid as suggested by Scoville (1911); by determining the nitrogen content by the Kjeldhal method as advocated by Heyl and Staley (1914); or by following the procedure of the U. S. Pharmacopeia as outlined for the determination of amyl nitrite. For the determination of glycerol trinitrate in air the procedure developed by Foulger (1936) for the determination of ethylene glycol dinitrate may be followed. Yagoda and Goldman (1943) determined the concentration of glycerol trinitrate in air by drawing the air through a midget impinger containing 10 cc. of triethylene glycol or propylene glycol at a rate of 3 liters per minute, using at least 60 liters. For the analysis, a 5-cc. aliquot is transferred to a 250-cc. standard taper flask and diluted with an equal volume of water. To this mixture is added 0.1 cc. of a 1-percent solution of m-xylenol dissolved either in triethylene or propylene glycol, stoppered, and cooled under running water. Seventeen cc. of concentrated sulfuric acid are added dropwise while cooling so that the temperature does not exceed  $35^\circ \text{C}$ . The hydrolysis of glycerol trinitrate and the simultaneous nitration of m-xylenol are completed within 10 minutes after the addition of acid. After this time the mixture is diluted by the addition of 150 cc. of cold water and made ready for distillation. With careful distillation (1 cc. per minute) the distillate is collected in a water-cooled, graduated cylinder containing 1 cc. of 2-percent sodium hydroxide until the condensate reaches the 10-cc. mark in the receiver. After mixing, the material in the cylinder is used for colorimetric comparison. Blanks are prepared by reacting and distilling 5 cc. of the glycol, 5 cc. of water, 0.1 cc. of the 1-percent m-xylenol, and



17 cc. of sulfuric acid as described above. Standards are prepared in the same way by substituting 5 cc. of 0.002-percent potassium nitrate for 5 cc. of water in the reaction mixture.

The *absorption of glycerol trinitrate* takes place through the lungs, the skin, and the gastro-intestinal tract. Crandall, Leake, Loevenhart, and Muehlberger (1929) and Gross, Bock, and Hellrung (1942) studied its absorption through the skin, and according to the latter it is less readily absorbed than ethylene glycol dinitrate. Heppel (1941), von Oettingen and Donahue (1944 a, b), and others followed its absorption from the gastro-intestinal tract, and Gross, Bock, and Hellrung (1942) reported on its absorption through the lungs. At present the question whether the absorption through the skin or through the lungs is more important for the evaluation of industrial exposure has not been definitely answered. However, it has been definitely proved that the former route of entry is of great importance.

With regard to the *fate of glycerol trinitrate in the organism*, the first students of its action (Hay, 1883, Cagnoli, 1885, and Atkinson, 1887-88) found that glycerol trinitrate liberates nitrite in the organism. Crandall, Leake, Lovenhart, and Muehlberger (1929) showed that in dogs, following its intravenous administration, glycerol trinitrate disappears rapidly from the circulation, and that 1 minute after the injection an average of 13.9 percent and after 20 minutes no significant amounts were detectable. Crandall (1933) demonstrated in perfusion experiments that various tissues and also blood may destroy glycerol trinitrate, a varying percentage of which may be accounted for as nitrite. He found further that in habituated animals the decomposition of glycerol trinitrate by blood was decreased, which was paralleled by a decreased physiological response to a standard dose of nitrite. In contrast to the rapid decomposition of glycerol trinitrate in blood and other tissues it appears that it is not decomposed in the stomach, as indicated by the report of Pond (1902) who was able to isolate 9.5 mg. of glycerol trinitrate from the stomach of an embalmed cadaver which had been buried for 2 months.

Concerning the *excretion of glycerol trinitrate*, Atkinson (1887-88) stated that after large doses it is excreted partly unchanged and partly as nitrite with the urine, whereas with smaller doses it is excreted mostly as nitrite in the urine (Marshall, 1897-98) but also with the saliva (Várady and Szántó, 1940).

The *effect of glycerol trinitrate on the central nervous system* differs between cold and warm blooded animals. Bruel (1876) reported that its administration to frogs causes convulsions of central origin, and similar observations were reported by Hay (1883) and Cagnoli (1885). In contrast to these findings in frogs, Hay (1883) saw in mammals



little evidence of a primary central stimulation but predominantly a depression of the central nervous system which later was followed by convulsions of asphyxial character. This difference in response may be explained by the methemoglobinemia produced by glycerol trinitrate, the effects of which on the central nervous system are more marked in warm than in cold blooded animals. In addition, it should be remembered that according to Langendorff (1891) and Schübel (1936) glycerol itself causes in frogs a primary stimulation of the central nervous system. With intramuscular injection of large doses (1 to 0.4 gm. per kg.) Orestano (1936) saw immediately in rabbits (an animal which does not readily form methemoglobin) severe tonic convulsions of long duration. Pilcher and Sollmann (1915) found that glycerol trinitrate usually causes stimulation of the vasomotor center, presumably because of anemia and anoxemia caused by the fall of the blood pressure. Meyer (1926) noted that under the influence of glycerol trinitrate the pulmonary ventilation is first increased and then decreased, and that during the latter phase the oxygen consumption is reduced. With regard to the effect of glycerol trinitrate on the sensory organs, Lewin and Guillery (1913) stated that following its administration the eyes become bright and sensitive to light and the acuity of the vision is sometimes reduced, perhaps on account of anoxia resulting from methemoglobinemia or from lowering of the blood pressure.

With regard to the *effect of glycerol trinitrate on the circulation*, Marshall (1897-98) showed that it effects the isolated heart in the opposite way from that observed with digitalis and that it reduces the tension of the pulse. MacNider (1908) believed that glycerol trinitrate does not depress the heart muscle but that it improves its action by lowering its burden on account of peripheral vasodilatation. Dossin (1911) showed that glycerol trinitrate decreases the amplitude of the heart and increases the heart rate during the time of reduced blood pressure. Plumier (1905) believed that this increase of the heart rate is not due to a direct effect on the heart but is the functional result of the lowered blood pressure. In the opinion of Pirrie (1912) small doses of glycerol trinitrate may weaken the cardiac action, as was also observed by Leech (1893) with nitrites, but with low concentrations this effect is counterbalanced by the increase of the pulse rate produced simultaneously whereas with more concentrated solutions the increase of the pulse rate is followed by a decrease and finally by a reversible cardiac arrest. In the opinion of Meyer (1926) glycerol trinitrate differs from amyl nitrite in that it produces a diminution of amplitude before affecting the blood pressure but after 3 to 5 minutes causes an increase of amplitude, dirotism, and moderate bradycardia.

Lauber and Brauch (1930) studied the cardiac efficiency following varying doses of glycerol trinitrate and found that with therapeutic doses this was first increased and then decreased. In their experience, doses of 0.3 to 2.0 mg. cause marked reduction of the blood pressure and reduction of the beat volume, the minute volume, and the cardiac efficiency. With smaller doses of 0.2 mg. the maximal effect was observed earlier and the return to normal sooner. Starr, Gamble, Margolies, Donal, Joseph, and Eagle (1937) found that doses of 1.2 mg. placed on the tongue caused a marked increase in cardiac output. With regard to the effect of glycerol trinitrate on the electrocardiogram, Hewlett (1917) claimed that doses of glycerol trinitrate causing definite vascular changes had no noteworthy effect on the electrocardiogram. However, large doses (100 mg.) may cause lengthening of the PR interval and heart block (as shown by Schott, 1920) which may be due in part to anoxia on account of methemoglobin formation. Evans and Hoyle (1933) noted no significant effects on the electrocardiogram of normal persons from 0.6 to 5.0 mg. of glycerol trinitrate, but in patients with an inverted T wave this was raised in the majority of the cases, this effect being explained by the improved blood supply to the cardiac muscle.

With regard to the *effect of glycerol trinitrate on the blood vessels*, Bradbury (1895), Wallace and Ringer (1909), Stewart (1915), and others showed that it causes vasodilatation due to a peripheral mechanism. Hewlett (1917) found that 1 to 2 drops of a 1-percent solution placed on the tongue caused swelling of the arm, increase of the size and volume of the pulse, a change toward a more pointed collapsing pulse, and disappearance of smaller secondary pulse waves, but no marked alterations of the blood flow through the arm. He believed that alterations of the pulse should be attributed not only to vasodilatation but also in part to changes in the cardiac output or to vascular changes in other parts of the organism. Walton and Kehr (1928) studied the effect of glycerol trinitrate on the turgidity of the nasal membrane following its application to the skin. They noted a primary decrease and subsequent increase of the nasal volume, the former preceding the fall of the blood pressure and occurring also in the absence of any other objective or subjective symptoms.

Meyer (1912) showed that glycerol trinitrate causes a dilatation of the *coronary arteries* of the dog heart in situ which is of longer duration than observed with amyl nitrite; and Voegtlin and Macht (1913) noted the same effect in isolated coronary arteries, this effect being more marked than with amyl nitrite and less lasting than observed with erythritol tetranitrate. Similar results were reported by Kountz (1932), Kountz, Pearson, and Koenig (1934), and Katz, Lind-

ner, Weinstein, Abramson, and Jochim (1938). As illustrated in figure 6, Schloss (1913) showed in cats that the vasodilator action on the coronary arteries precedes and outlasts the lowering of the blood pressure and that during the time of reduced blood pressure the coronary flow is decreased. Similar results were reported by Krantz, Carr, Forman, and Ellis (1939 b) following the administration of isomannide dinitrate. Smith (1921) studied in dogs the effect of glycerol trinitrate on the collateral circulation between distal branches of the left coronary artery and noted definite evidence of vasodilatation in 5 out of 15 experiments.

With regard to the *effect of glycerol trinitrate on the cerebral blood vessels*, Wallace and Ringer (1909) found that these were dilated. Leake, Kammer, and Hitz (1929) made quantitative measurements of the dilatation of the pial blood vessels in trepanized dogs and noted

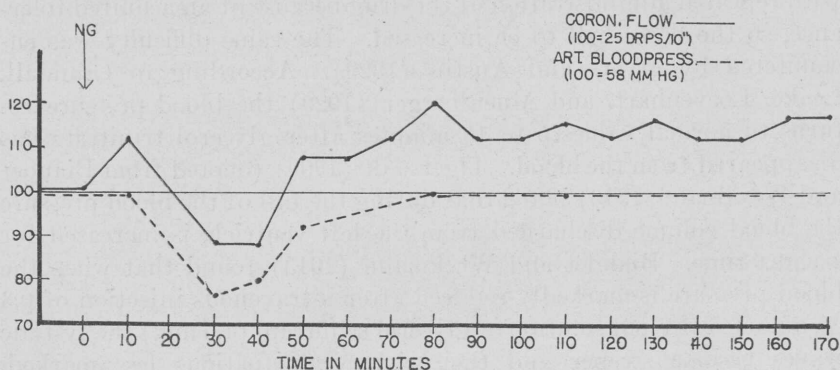


FIGURE 6.—Effect of an intravenous injection of glycerol trinitrate (1 drop of 1-percent solution) on the coronary circulation and arterial blood pressure of cats.

an average increase of 21 percent of their diameter following the administration of glycerol trinitrate. Schmidt and Hendrix (1938) measured in rabbits the parietal and cerebral blood flow and found this to be increased following the intra-arterial injection of glycerol trinitrate.

Concerning the *effect of glycerol trinitrate on the capillaries*, Schickler and Mayer-List (1923) found that in glycerol trinitrate hyperemia, as under the influence of heat, rhythmic contractions of the capillaries of the nailbed may be noted, beginning with stasis and leading to temporary narrowing and arrest of the blood flow; they considered this contracture to be of autogenous character. On the other hand, Kauffman (1924) noted within a few seconds after the administration of glycerol trinitrate distinct dilatation of the capillaries of the nailbed, paralleled by an increase of the rate of blood flow lasting 1 to 1½ hours.

The effect of *glycerol trinitrate* on the blood pressure has been studied extensively. Bruel (1876), Murrell (1879), Atkinson (1887-88), Wallace and Ringer (1909), Dossin (1911), and others showed that glycerol trinitrate causes a fall of the blood pressure which, as shown by Capps and Matthews (1913) and Pilcher and Sollmann (1915), is of peripheral origin. Dmitrenko (1909) claimed that in 16 out of 23 persons therapeutic doses of glycerol trinitrate caused a fall of the pressure in the brachial artery and a simultaneous rise in the arteries of the finger. Matthew (1909) found that in patients who had been given doses of 120 and in some instances 180 mg. the blood pressure started to fall after an average of 60 seconds, the maximal fall of 28 mm. Hg. was reached after 5 minutes, and the blood pressure returned to normal values after 20 to 28 minutes had elapsed. He found it difficult to maintain the blood pressure at a low level even with repeated administration of the drug because of an acquired tolerance, so the doses had to be increased. The same difficulty was encountered by Myers and Austin (1929). According to Crandall, Leake, Loevenhart, and Muehlberger (1929) the blood pressure returns to normal values 5 to 10 minutes after glycerol trinitrate has disappeared from the blood. Tigerstedt (1909) (quoted from Biaudet and Weckmann, 1913) noted that during the fall of the blood pressure the blood volume discharged from the left ventricle is increased for a short time. Biaudet and Weckmann (1913) found that when the blood pressure is markedly reduced after intravenous injection of 0.3 cc. of an 0.5-percent solution of glycerol trinitrate (1.5 mg.) the systolic waves become steeper and the primary fluctuations less marked. Loomis (1905) (quoted from Miller, 1910), Hewlett (1906 a) using amyl nitrite, and Dmitrenko (1909) saw in man, and MacNider (1908), Miller (1910), and Matthew (1909) saw in dogs, a secondary rise of the blood pressure. In the opinion of Wiggers (1911 a, b) the fall of the blood pressure produced by glycerol trinitrate mobilizes two compensatory mechanisms, namely increase of the rate and of the amplitude of the heart beat, which tend to alleviate and may even surpass the fall.

The effect of *glycerol trinitrate* on the pulmonary arterial pressure is characterized by a rise during the fall of the systemic blood pressure as was also observed with amyl nitrite. As pointed out by Wood (1901-02) this may be due to a stasis of the blood flow in the small circuit, resulting from a greater return of blood to the right heart, or it may be due to an active contraction of the pulmonary arteries, the latter alternative being considered the more likely. There is, however, no conclusive evidence that the response of pulmonary arteries to glycerol trinitrate is different from that of other arteries, and Plumier (1905) showed that in the perfused lung glycerol trinitrate causes



only vasodilatation. He and Wiggers (1911 a) were of the opinion that Wood's assumption is not correct, and he showed that the rise of the pressure in the pulmonary artery occurs during the fall of the pressure in the carotid artery and that it is paralleled by a less marked rise in the left auricle and an increase of the pulse rate. He believed that the rise of pressure in the pulmonary artery is the outcome of the effect of glycerol trinitrate on the general circulation, resulting in a greater return of blood to the heart. Wiggers (1911 a) pointed out that the fall of the systemic arterial blood pressure decreases the blood supply to the medulla, resulting in a depression of the cardio-inhibitory center and thus in an increase of the heart rate and an increased amplitude of the heart beat. Both effects result in an increased minute output of the heart and thus antagonize the effects of the peripheral vasodilatation and increase the pressure in the pulmonary artery and vein. This explanation is not in accordance with a constriction of the pulmonary arteries as cause of the rise of the pulmonary arterial pressure.

With regard to the *effect of glycerol trinitrate on the venous pressure*, Capps and Matthews (1913) found that in dogs the intravenous injection of 0.4 to 1.2 mg. causes a fall of the arterial and venous pressures, but following the oral administration of large doses (2 mg. per kg.) von Oettingen and Donahue (1944 b) noted a rise of the venous pressure. Payan and Giraud (1925) found that in man glycerol trinitrate caused a fall of the venous pressure in 50 percent of the subjects which was preceded in 60 percent by a temporary rise. It appears, therefore, that the response of the venous pressure may vary, that with a severe fall of the arterial pressure it may be increased, and that with moderate vasodilatation sufficient to enhance the venous return it may be decreased.

With regard to the *effect of glycerol trinitrate on the spinal pressure*, von Oettingen and Donahue (1944 b) found in dogs that the oral administration of 2 mg. per kg. is followed by a much less spectacular rise than observed after 5 mg. per kg. of erythritol tetranitrate or pentaerythritol tetranitrate, possibly because with such doses the animal rapidly went into collapse.

The *effect of glycerol trinitrate on the blood* is characterized by methemoglobin formation and, with sufficiently heavy exposure, by anemia. Bruel (1876) was evidently the first to describe the discoloration of the blood and he mentioned also that the pigment formed was unable to maintain the oxygenation of the blood. Later this pigment was recognized as methemoglobin, but in the opinion of Heubner and Rhode (1923) nitrous oxide hemoglobin may also be formed. According to Gross, Bock, and Hellrung (1942) the methemoglobinemic action is less marked than that of ethylene glycol di-



nitrate although it is somewhat more persistent. They found that in cats the subcutaneous injection of 15 mg. per kg. (given in oily solution) causes only a moderate methemoglobinemia (4.3 percent) within 3 hours, 30 mg. per kg. causes 20 percent within 2 hours, and 75 mg. per kg. causes 40 percent after 7 hours. The formation of Heinz bodies and the anemiagenic action are also somewhat less marked than with ethylene glycol dinitrate. Similar results were reported by Wilhelmi (1942) with intraperitoneal injection in cats. In the opinion of Gross, Bock, and Hellrung (1942) the anemia produced by glycerol trinitrate is not due to destruction of red blood cells on account of the formation of Heinz bodies. In animal experiments Orestano (1936) found that even with comparatively large doses the methemoglobinemia disappeared within 12 to 24 hours.

Like other vasodilator drugs, *glycerol trinitrate has a definite effect on the kidney function.* Murrell (1879) noted that it had a diuretic action resulting in the excretion of urine of lower specific gravity than normal. Kauffmann (1924) who made a detailed study of this effect found that it varies considerably with different individuals and that increased diuresis is the exception rather than the rule.

With regard to the *toxicity of glycerol trinitrate for animals*, not much information is available. Bokorny (1896) claimed that it is little toxic for lower animals and plants. As shown in table 4 which gives the experimental observations of Dossin (1911), Oltman and Crandall (1931), and Gross, Bock, and Hellrung (1942) the toxic dose varies with the route of administration and with the species of the animal used. Following intravenous injection of fatal doses Oltman and Crandall (1931) noted in rabbits immediate stimulation of the respiration which was closely followed by slowing of the heart beat, muscular twitchings, and clonic and tonic convulsions. In between the convulsions the heart rate was accelerated and the respiratory rate decreased, and the animals died from respiratory paralysis. In contrast to the comparatively low acute toxicity of glycerol trinitrate (with subcutaneous injection of 200 mg. per kg. cat and 500 mg. per kg. rabbit) the repeated subcutaneous injection of much smaller doses may cause toxic effects as demonstrated by Gross, Bock, and Hellrung (1942). These investigators found that 1 cat died 4 days after 40 daily injections of 0.1 mg. per kg. after having lost its appetite, lost weight, and developed anemia and fatty degeneration of the liver. Other animals were evidently more resistant. One animal showed similar symptoms after 50 injections of 7.5 and even 15 mg. per kg. but survived, whereas 2 other cats died after 20 and 10 such doses. On account of its lower vapor pressure, glycerol trinitrate is less liable to cause toxic effects from inhalation of its vapors than ethylene glycol dinitrate. Gross, Bock, and Hellrung (1942) found

that the maximal concentration they could produce was of the order of 0.005 mg. per liter (0.6 p. p. m.) and that exposure of cats to such concentrations for 68 days caused no distinct toxic effects except a moderate lymphocytosis. Only one animal exposed for 156 days developed a marked anemia with signs of increased regeneration of red blood cells but without showing appreciable methemoglobinemia or more marked formation of Heinz bodies.

**Table 4.—TOXICITY OF GLYCEROL TRINITRATE FOR VARIOUS SPECIES OF ANIMALS WITH VARIOUS ROUTES OF ADMINISTRATION**

| ORAL ADMINISTRATION         |                  |                    |                                   |
|-----------------------------|------------------|--------------------|-----------------------------------|
| Species                     | Dose mg./kg.     | Symptoms           | Author                            |
| Dog.....                    | 1, 3, 5, 10..... | Not fatal.....     | Dossin (1911).                    |
| SUBCUTANEOUS ADMINISTRATION |                  |                    |                                   |
| Dog.....                    | 3, 10, 30.....   | Tolerated.....     | Dossin (1911).                    |
| Cat.....                    | 200.....         | Certain fatal..... | Gross, Bock, and Hellrung (1942). |
| Rabbit.....                 | 500.....         | do.....            | Gross, Bock, and Hellrung (1942). |
| INTRAVENOUS ADMINISTRATION  |                  |                    |                                   |
| Dog.....                    | 3, 10, 30.....   | Not fatal.....     | Dossin (1911).                    |
| Rabbit.....                 | 45.....          | Fatal.....         | Oltman and Crandall (1931).       |

Although the picture of glycerol trinitrate poisoning is similar to that of sodium nitrite poisoning it appears that the toxic action of the former cannot be solely explained on the basis of its nitrite action because the minimal fatal dose of glycerol trinitrate with intravenous administration to rabbits is 45 mg. per kg. whereas that of sodium nitrite is 80 to 90 mg. per kg. As may be seen from this description, the toxicological picture of glycerol trinitrate poisoning is similar to that of asphyxiation which may either be due to the fall of the blood pressure or may be caused by methemoglobinemia.

With regard to *habituation to exposure to glycerol trinitrate*, this plays an important role in the therapeutic use of the drug and also in industrial exposure, as will be shown below. Animal experiments on this question were reported by Crandall (1933) who showed that tolerant dogs destroyed glycerol trinitrate less readily than normal animals. Gross, Bock, and Hellrung (1942) showed that in rabbits tolerance, at least regarding the pressor action of glycerol trinitrate, is rapidly developed but is of short duration. Their experiments on the anemiagenic action of aliphatic nitrates also show that with repeated subcutaneous injections the animals developed a tolerance in

this respect which, however, did not extend to the formation of Heinz bodies.

Reports on *pathological findings in animals exposed to glycerol trinitrate* are not numerous. Gross, Bock, and Hellrung (1942) noted in cats, following 10 daily subcutaneous injections of 15 mg. per kg. albumin and bile pigments in the urine, icterus and hemorrhages in the dura, cerebellum, heart, liver, and spleen, and pigment deposits in the two latter organs. Similar pigment deposits were described by Wilhelmi (1942). It may be significant that with prolonged cutaneous administration hemorrhages in the heart muscle were also seen in one animal. Wilhelmi (1942) noted central fatty changes in the liver of one animal, and Gross, Bock, and Hellrung (1942) noted fatty and degenerative changes in liver and heart in cats treated with repeated subcutaneous injections. It appears, therefore, that continued exposure to glycerol trinitrate of sufficient intensity may lead to permanent injury of the heart muscle.

*Poisonings from glycerol trinitrate in man* may be classified as medicinal, accidental, and occupational poisonings. Cases of medicinal poisoning resulting from an overdose of glycerol trinitrate were reported by Meyer (1926) and Noer (1887) whose patient suffered from collapse characterized by prostration, pale, cold, and clammy skin, perspiration and slow pulse, and slow respiration. Miller (1910) pointed out the great differences in the therapeutic doses as given by different clinicians and he reported that following administration of 0.6 mg. of glycerol trinitrate, 20 percent of the patients complained of headache which occurred during recovery from the maximal fall of the blood pressure. In the experience of Prodger and Ayman (1932), 4 out of 110 patients who had received therapeutic doses of glycerol trinitrate developed alarming symptoms characterized by a fall of the blood pressure, slowing of the pulse rate, anxiety, restlessness, cold perspiration, and pallor, and one showed heart block.

Accidental poisonings from the ingestion of glycerol trinitrate were reported by Huseman (1867) (quoted from Meixner and Mayrhofer, 1921); Holst (1870); Maschka (1873) and Wolff (1878) (both quoted from Meixner and Mayrhofer, 1921); Reynalds (1898); and Meixner and Mayrhofer (1921) the majority of these subjects died in collapse or, if they survived, showed cerebral symptoms such as unconsciousness or confusion, headache, dizziness, or moderate fever (Reynalds, 1898, and Signorino, 1936). The patients of Signorino (1936) who suffered from severe cyanosis developed a secondary anemia and urobilinuria.

Toxic effects resulting from the handling of glycerol trinitrate and dynamite were reported by G. C. Laws (1898), C. E. Laws (1910),

Evans (1912), Pirrie (1912), Elbright (1914), Heitz (1924), Aperia (1936), and others. Exposure to glycerol trinitrate results within a few minutes in flushing of the face and neck and headache which starts in the frontal region but later extends toward the occiput and the neck. According to Crandall, Leake, Loevenhart, and Muehlberger (1931) the minimal effective dose of glycerol trinitrate with cutaneous absorption is 1 to 1.8 cc. of a 1-percent solution, corresponding to 10 to 18 mg. The headache is frequently associated with the fall of the blood pressure, but it has also been observed that headache may set in when the blood pressure is again on the increase. Matthew (1909) observed headache in patients who did not respond to glycerol trinitrate by a fall of the blood pressure. The headache is often associated with nausea and vomiting and occasionally with diarrhea. During headache the heart action is increased, later slowed, but as a rule the blood pressure stays within normal limits (Elbright, 1914, and Aperia, 1936). According to Heitz (1924) the diastolic pressure is affected more and longer than the systolic. In the opinion of Aperia (1936) the amplitude may be reduced. He found that exposure to concentrations of 5 mg. of glycerol trinitrate per cubic meter (0.6 p. p. m.) increases the minute volume of the cardiac output by 30 to 50 percent, but that higher concentrations will reduce the minute volume. However, it does not appear to be established to what extent his patients may have absorbed glycerol trinitrate through the skin. Moderate exposure may result in insomnia (Heitz, 1924); if more severe it may cause excitement and render the victim irrational (Laws 1910; Heitz, 1924). In women, exposure to glycerol trinitrate may result in menstrual disturbances (Laws, 1898). Severe and prolonged exposure may result in weakness and prostration (Laws, 1898), and occasionally it may cause jaundice (Pirrie, 1912) and edema of the legs in the absence of cardiac disturbances and injury of the kidneys (no albuminuria) (Pirrie, 1912). Exposure to glycerol trinitrate may occasionally be followed by diuresis and the secretion of light colored urine (Laws, 1910). According to the same author, glycerol trinitrate headache may occasionally be preceded by temporary loss of vision, and Pirrie (1912) observed one case of bilateral optical atrophy following exposure to glycerol trinitrate. Prosser White (1928) stated that workers handling glycerol trinitrate occasionally suffer from ulcers under the nails and on the fingertips which do not heal readily. Laws (1898) reported eruptions of the skin similar to poison ivy poisoning. The toxic effects of glycerol trinitrate are aggravated by the ingestion of alcohol, by perspiration, and by hyperpnea (Evans, 1912).

With regard to late effects resulting from prolonged exposure to glycerol trinitrate, Laws (1910) mentions dyspnea and tachycardia



upon exertion and enlargement of the left heart, and according to Aperia (1938) continued exposure for many years may result in a great difference between the arterial and venous pressures.

Rabinowitch (1944) emphasized the forensic importance of such symptoms of glycerol trinitrate exposure as drowsiness, insomnia, languor, and, especially, mental confusion, dizziness, mental excitement, pugnaciousness, hallucinations, and maniacal manifestations.

Since the introduction of glycerol trinitrate as a therapeutic agent, as for instance in angina pectoris, it was recognized that patients rapidly developed a *tolerance* to the drug necessitating an increase of the dose to produce the desired effect (Murrell, 1879, and Stewart, 1905). This may lead to the intake of extremely high doses such as 150 mg. within 24 hours without apparent side actions, as reported by Himmelsbach (1893). Matthew (1909) had to increase the dose in some cases to 60 minims (3.914 gm.) in 24 hours. Myers and Austin (1929) showed that premedication of rabbits with sodium nitrite causes tolerance to the depressor action of glycerol trinitrate; however, this tolerance is rapidly lost. In the opinion of Crandall, Leake, Loevenhart, and Muehlberger (1931) tolerance to headache is more easily established than tolerance to the effect on the blood pressure and pulse rate. They believed that there is a complete cross-tolerance between the different members of this group and that it is more readily produced by amyl nitrite than by nitric acid esters, and that there is a parallelism between the ease of hydrolysis by alkali and tolerance.

There is very little information available with regard to *pathological changes in glycerol trinitrate poisoning of man*. Holst (1870) saw, in a patient who died after the ingestion of glycerol trinitrate, marked hyperemia of the meninges, hyperemia and edema of the lungs, flaccid heart, hyperemia of the mucosa of the stomach with ecchymoses, and marked hyperemia of the kidneys.

With regard to the *prophylaxis of glycerol trinitrate poisoning*, greatest cleanliness of the operation is of paramount importance. It has been stated above that Aperia (1936) found that concentrations of 5 mg. of glycerol trinitrate per cubic meter (0.5 p. p. m.) had some effect on the circulation by increasing the minute volume of the cardiac output by 30 to 50 percent, which indicates that the pollution of the air has to be carefully controlled. On the other hand, it should be emphasized that glycerol trinitrate may be readily absorbed through the skin, so that any contact with dust carrying glycerol trinitrate should be avoided by proper protective garments and greatest cleanliness of the operation. Persons who continue to suffer from headache, nausea, and vomiting and who develop no tolerance to the exposure should be removed from operations in which glycerol trinitrate is handled. Periodic medical examinations with regard to blood pres-



sure changes and stability of the blood pressure and of the pulse rate with exercise may be helpful in detecting incipient injury of the circulatory apparatus. Red blood cell counts may be helpful in detecting existing hypoxemia as indicated by an increase of the red blood cells and of the hemoglobin.

With regard to the *treatment of glycerol trinitrate poisoning*, any ingested material should be removed by gastric lavage and any other symptoms should be treated symptomatically; in case of cyanosis the administration of oxygen may prove helpful in overcoming the hypoxemia. In case toxic effects result from the inhalation of dust and vapors containing glycerol trinitrate, discontinuation of the exposure and—in case of absorption through the skin—thorough cleansing of the skin with sodium thiosulfate solution, soap and water are of paramount importance. Moderate poisonings may require no other medication but fresh air and rest; in more severe cases oxygen and analeptics (sodium caffeine benzoate) may be indicated. Mild headache, as frequently experienced by glycerol trinitrate workers, usually disappears upon continuation of the exposure. According to Rabinowitch (1944) only 2 to 3 percent of the exposed workers fail to become hardened. Severe headache may improve upon the application of cold, and according to Rabinowitch (1944) bromides are ineffective and coal tar derivatives such as acetanilide and depressants of the central nervous system are contraindicated. According to the same author, occasionally belladonna has been found to be of benefit.

## NITRIC ACID ESTERS OF QUADRIVALENT ALCOHOLS

### Erythritol Tetranitrate

Of the nitric acid esters of quadrivalent alcohols, erythritol tetranitrate, nitroerythrite,  $O_2NOCH_2(CHONO_2)_2CH_2ONO_2$ , has found therapeutic application and for this reason has been studied repeatedly with regard to its pharmacological action. It has the molecular weight 302.12 and forms leaflets which melt at 60° C., explode at higher temperature, and are insoluble in cold water but soluble in alcohol and ether.

Very little is known with regard to its *absorption and excretion and its fate in the organism*. In contrast to the nitric acid esters of mono-, bi-, and trivalent alcohols, absorption of erythritol tetranitrate through the skin is of minor importance. It is very likely that it is absorbed from the respiratory tract when inhaled as dust, although experimental studies on the mode of entry have not been reported. As shown by Lawton, Yagoda, and von Oettingen (1944) it is readily (although more slowly than glycerol trinitrate) absorbed from the

gastro-intestinal tract of rats; 66 percent of the material administered being absorbed in 2 hours and approximately 80 percent after 4 to 6 hours. With regard to its fate in the organism, it is at least partly hydrolyzed with the formation of nitrate which in turn is reduced to nitrite. According to Yagoda and von Oettingen (1944) it is more readily hydrolyzed by blood than glycerol trinitrate both in vitro and in vivo (dogs) (fig. 7).

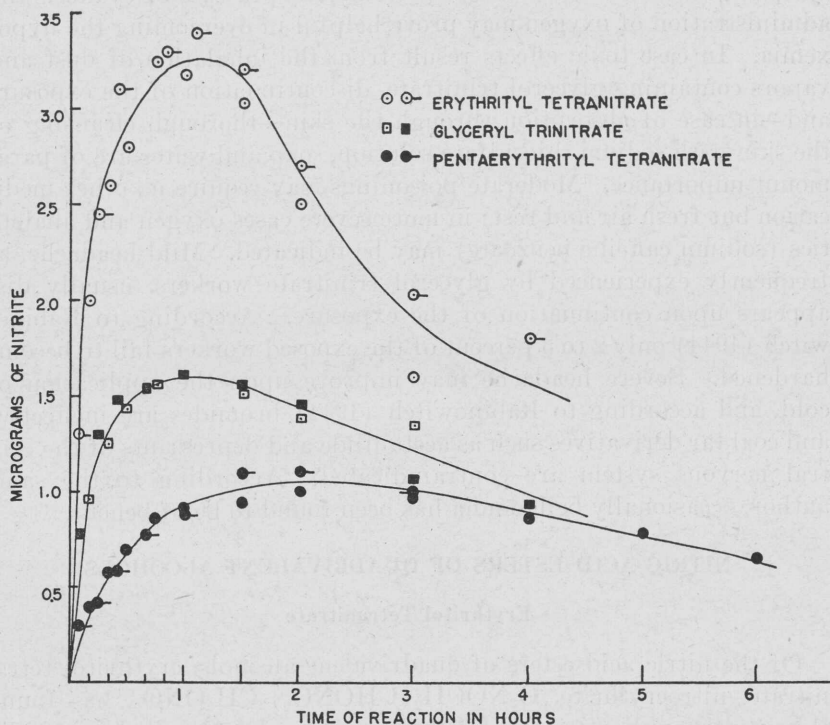


FIGURE 7.—Rate of hydrolysis of erythritol tetranitrate, glycerol trinitrate, and pentaerythritol tetranitrate by dog blood.

With regard to the *effect of erythritol tetranitrate on the circulation*, Bradbury (1895) found that concentrations of 1:10,000 cause *vaso-dilatation* in perfused organs, being more effective than glycerol trinitrate and less active than mannitol hexanitrate. Voegtlin and Macht (1913) found that its dilator action on the coronary arteries was more marked and more lasting than that of glycerol trinitrate. According to Bradbury (1895), 0.05 gm. per kg. causes in rabbits a definite and lasting fall of the blood pressure which was confirmed by Marshall (1897-98). Von Oettingen and Donahue (1944, b) found that in dogs 5 mg. per kg. of erythritol tetranitrate given orally causes a fall

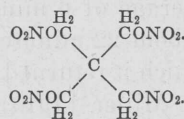
of the arterial blood pressure, lowering of the venous pressure after a primary rise, and a distinct and lasting rise of the spinal pressure. Matthew (1909) studied its effect in man and found that after the administration of 60 mg. the blood pressure started to fall after an average of 6 minutes until the maximal fall of 34 mm. was reached (about 22 minutes) at which level it remained for 1 to 2 hours, after which it returned very slowly to normal values. From data published by Miller (1910) it appears that there are considerable individual variations with regard to the pressure response to erythritol tetranitrate. He found that of 10 patients examined the administration of 30 mg. caused a fall of 30 mm. or more in 5, of 15 mm. in 2, of 5 mm. in 2, and no fall in 1. One of his patients collapsed but recovered after 15 minutes. In the opinion of Nicholson and Moseley (1941) erythritol tetranitrate is an effective diuretic in subacute glomerulonephritis.

With regard to *toxic manifestations of erythritol tetranitrate*, Miller (1910) stated that following the administration of 30 mg., of 10 patients 6 complained of sudden onset of severe headache. This reaction was observed more frequently after administration of erythritol tetranitrate than after glycerol trinitrate. As in the case of the latter it appears that headache and hypotensive action occur independently. Crandall, Leake, Loevenhart, and Muehlberger (1931) determined the minimal dose of erythritol tetranitrate that causes headache as 45 mg. They found that headache lasted for about 8 hours and was not associated with the fall of the blood pressure and changes of the pulse rate. They also showed that the repeated administration of fractional doses of 30 mg. every 3 hours for 60 hours caused tolerance to the headache dose which gradually disappeared in the course of 12 days. Hueper and Landsberg (1940) reported that daily feeding of massive doses of erythritol tetranitrate over a period of 30 weeks caused in rats atheromatous and arteriosclerotic changes of the vascular walls. These changes were found mostly in the blood vessels of the head, neck, brain, and meninges; with larger doses they were also found in the coronary arteries, and with massive doses were found in the splanchnic region as well. Donahue and Monaco (1944) fed rats daily doses of 2 mg. per kg. body weight over a period of 1 year without noticing any untoward effects on their growth, blood picture, lungs, liver, kidney, spleen, femur or brain, and, specifically, no injury of the vascular walls.

### Pentaerythritol Tetranitrate

Pentaerythritol tetranitrate, PETN, penthrite, Hasethrol, is a powerful explosive. It is the nitric acid ester of the quadrivalent alcohol pentaerythritol and has the structural formula:

It has the molecular weight 316.05 and represents a white crystalline material with a specific gravity of 1.67 and a melting point of 140° C. It is insoluble in water, slightly soluble in alcohol, and soluble in acetone.



With regard to its *absorption, excretion, and fate in the organism*, Lawton, Yagoda, and von Oettingen (1944) found that it is absorbed very slowly from the gastro-intestinal tract of rats, 15 to 18 percent being absorbed within 6 hours as compared with 70 to 80 percent of erythritol tetranitrate. Von Oettingen, Donahue, and Valaer (1944) found no evidence that pentaerythritol tetranitrate is absorbed through the skin to any considerable extent, but as shown by von Oettingen and Donahue (1944 a) it is absorbed through the lungs when inhaled as dust. Like other nitric acid esters, pentaerythritol tetranitrate is hydrolyzed with the formation of nitrite. But according to Yagoda and von Oettingen (1944) the rate of hydrolysis by blood in vitro is much slower than with glycerol trinitrate and erythritol tetranitrate, and the same holds true for the hydrolysis in vivo (dogs).

With regard to the *effect of pentaerythritol tetranitrate on the circulation*, von Oettingen and Donahue (1944 b) found that following the oral administration of 5 mg. per kg. to dogs it causes a fall of the blood pressure, little effect on the venous pressure, and a rise of the spinal pressure, being in these respects much less effective than erythritol tetranitrate and glycerol trinitrate (fig. 8). The oral administration of 64 mg. to healthy individuals caused no changes in the blood pressure, the heart rate, or the concentration of nitrite in the blood.

With regard to *other toxic manifestations*, patch tests indicate that pentaerythritol tetranitrate is neither a primary irritant nor a strong sensitizer. Experiments reported by Donahue and Monaco (1944) show that daily feeding of 2 mg. per kg. to rats over a period of 1 year causes no untoward effects on the growth, the blood picture, or on the lungs, liver, kidney, spleen, femur, or brain of these animals, and specifically no injury of the vascular walls.

It appears, therefore, that in comparison with other nitric acid esters, pentaerythritol tetranitrate is comparatively nontoxic and that the customary methods of good housekeeping should be sufficient to prevent injurious effects in workers handling this material.

## NITRIC ACID ESTERS OF POLYVALENT ALCOHOLS

## Mannitol Hexanitrate

The most common nitric acid ester of the hexavalent alcohol mannitol is mannitol hexanitrate,  $C_6H_8(ONO_2)_6$ , of the structural formula

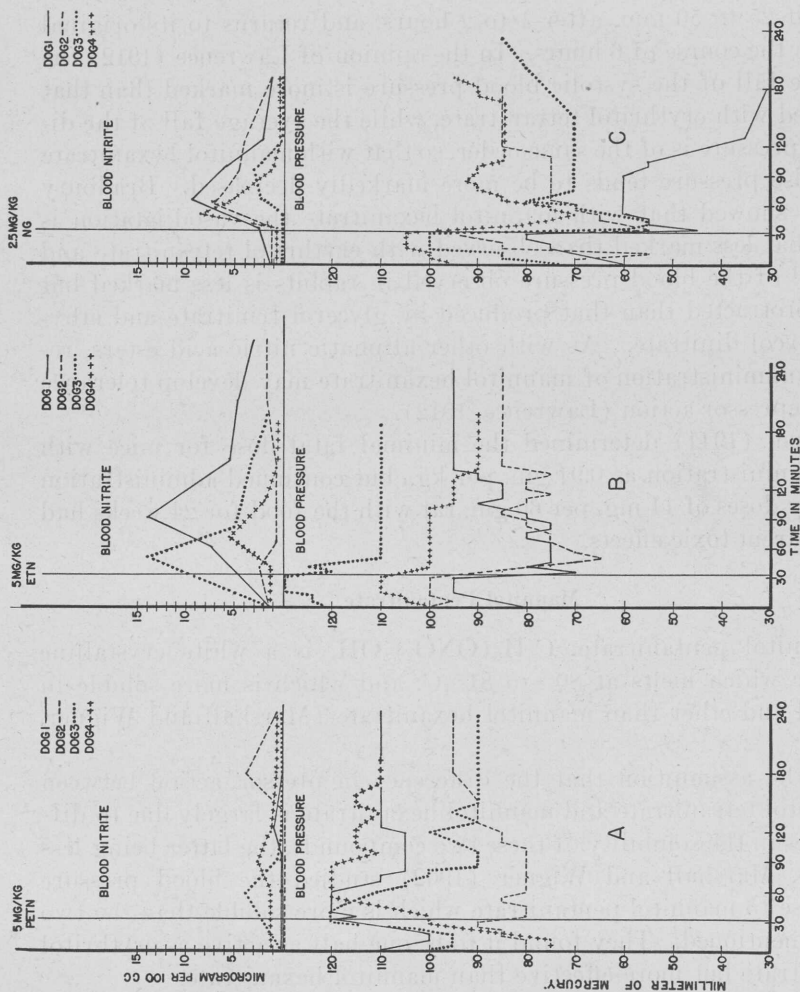
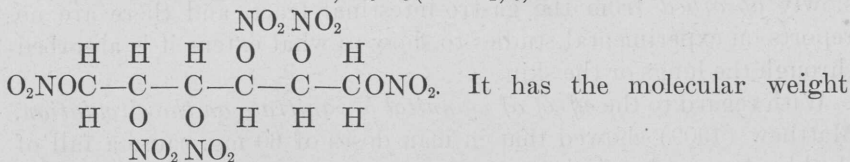


FIGURE 8.—Liberation of nitrite in dogs after oral administration of 5 mg. per kg. of pentaerythrithol tetranitrate (A), 5 mg. per kg. of erythritol tetranitrate (B), and 2.5 mg. per kg. of glycerol trinitrate (C), and the changes of the blood pressure produced by these nitric acid esters.



452.17 and represents needles of the specific gravity 1.604<sup>4</sup> ° C. which melt at 112 to 113° C. and which explode at higher temperature. It is insoluble in water but soluble in 100 parts of alcohol to the extent of 2.9 parts at 13° C. and in ether to the extent of 4 parts at 9° C.

As shown by Heppel (1941), in rats mannitol hexanitrate is very slowly *absorbed* from the gastro-intestinal tract, and there are no reports on experimental studies to show to what extent it is absorbed through the lungs or the skin.

With regard to the *effect of mannitol hexanitrate on the circulation*, Matthew (1909) showed that in man doses of 60 mg. cause a fall of the blood pressure which starts after 8 to 16 minutes, reaches its maximum of 25 to 50 mm. after 1 to 2 hours, and returns to its original level in the course of 6 hours. In the opinion of Lawrence (1912) the average fall of the systolic blood pressure is more marked than that observed with erythritol tetranitrate, while the average fall of the diastolic pressure is of the same order, so that with mannitol hexanitrate the pulse pressure tends to be more markedly decreased. Bradbury (1895) showed that with mannitol hexanitrate the vasodilatation is somewhat less marked than observed with erythritol tetranitrate and the fall of the blood pressure observed in rabbits is less marked but more protracted than that produced by glycerol trinitrate and ethylene glycol dinitrate. As with other aliphatic nitric acid esters, repeated administration of mannitol hexanitrate may develop tolerance to its depressor action (Lawrence, 1912).

Heppel (1941) determined the minimal fatal dose for mice with oral administration as 0.91 gm. per kg., but continued administration of daily doses of 11 mg. per 60 gm. rat with the food for 24 weeks had no apparent toxic effects.

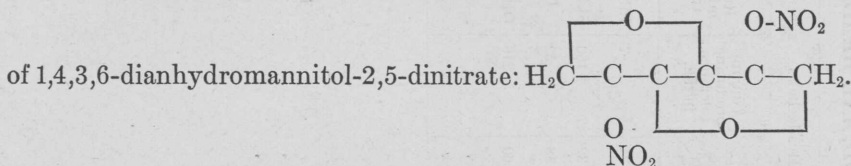
#### Mannitol Pentanitrate

Mannitol pentanitrate,  $C_6H_8(ONO_2)_5OH$ , is a white crystalline powder which melts at 80° to 81° C. and which is more soluble in alcohol and ether than mannitol hexanitrate (Marshall and Wigner, 1902).

On the assumption that the difference in pressor action between erythritol tetranitrate and mannitol hexanitrate is largely due to differences in the solubility of these two compounds, the latter being less soluble, Marshall and Wigner (1902) studied the blood pressure response to mannitol pentanitrate which is more soluble than the two esters mentioned. They found it to be one-half as active as erythritol tetranitrate but more effective than mannitol hexanitrate.

## 1,4,3,6-Dianhydromannitol-2,5-dinitrate

Krantz, Carr, Forman, and Ellis (1939 a) studied the pharmacology



They found it 10 times less effective than erythritol tetranitrate with regard to its effect on the blood pressure. In man doses of 100 mg. caused a rapid fall of the blood pressure which remained low for 5 to 6 hours. The continued administration of 10 mg. daily for 20 days to rats weighing 125 to 200 gm. caused no toxic effects. In spite of the marked depressor action neither methemoglobin nor nitrite could be detected in the blood nor was there any evidence of hydrolytic cleavage during treatment for 1 hour with 0.2 normal sodium hydroxide at 37° C. This appears to indicate that not the free nitrite but the entire molecule is responsible for the effect on the blood pressure.

#### THE COMPARATIVE EFFECT OF VARIOUS ALIPHATIC NITRIC ACID ESTERS

It has been shown that the different aliphatic nitric acid esters have qualitatively the same effect on the various physiological functions. They differ, however, with regard to the speed and intensity of their action. This is illustrated in table 5 which gives the effects of the more important compounds of this group.

Data concerning the *absorption of these esters* are scanty and not strictly quantitative, and for this reason are not listed in this table. All are absorbed from the gastro-intestinal tract. Lawton, Yagoda, and von Oettingen (1944) confirmed the findings of Heppel (1941) that glycerol trinitrate is readily absorbed by this route. The former found that erythritol tetranitrate is absorbed slowly and the latter found that mannitol hexanitrate is absorbed even less readily. With regard to the absorption through the skin, Gross, Bock, and Hellrung (1942) stated that ethylene glycol dinitrate is more readily absorbed through the skin than glycerol trinitrate, but data on the cutaneous absorption of erythritol tetranitrate and mannitol hexanitrate are not available; however, this is presumably of a very low order. As to their absorption through the lungs, methyl nitrate evidently is readily absorbed, as indicated by its rapid action, reported by Crandall, Leake, Loevenhart, and Muehlberger (1931). According to Gross, Bock, and Hellrung (1942), ethylene glycol dinitrate is more readily absorbed by this route than glycerol trinitrate, and as shown by von Oettingen and Donahue (1944 a) pentaerythritol tetranitrate is absorbed slowly.

**Table 5.—EFFECT OF ALIPHATIC NITRIC ACID ESTERS ON BLOOD VESSELS, BLOOD PRESSURE, BLOOD, AND HEADACHE**

| Compound                       | Vasodilatation <sup>1</sup> | Blood pressure                                                                        |                              |                         |                                 |                           |                           | Blood                   |                      | Headache             |                                             |                                   |                         |
|--------------------------------|-----------------------------|---------------------------------------------------------------------------------------|------------------------------|-------------------------|---------------------------------|---------------------------|---------------------------|-------------------------|----------------------|----------------------|---------------------------------------------|-----------------------------------|-------------------------|
|                                |                             | Dose in mg.                                                                           | Beginning of fall in minutes | Maximal fall in mm. Hg. | Time of maximal fall in minutes | Duration of fall in hours | Return to normal in hours | Methemoglobin formation | Heinz body formation | Headache dose in mg. | Time required to produce tolerance in hours | Amount producing tolerance in mg. | Route of administration |
| Methyl nitrate.....            | Hardly any.....             | -----                                                                                 | -----                        | -----                   | -----                           | -----                     | -----                     | -----                   | -----                | ?                    | 24                                          | ?                                 | Inhaled.                |
| Ethyl nitrate.....             | -----                       | -----                                                                                 | -----                        | -----                   | -----                           | -----                     | -----                     | ±                       | ±                    | -----                | -----                                       | -----                             | -----                   |
| Ethylene glycol dinitrate..... | 9-23.....                   | -----                                                                                 | -----                        | -----                   | -----                           | -----                     | -----                     | 4+                      | 4+                   | 35                   | 32                                          | 160                               | Skin.                   |
| Glycerol trinitrate.....       | 9-19.....                   | $\left\{ \begin{array}{c} 2 \text{ }^{2} 130 \\ (2 \text{ mm.}) \end{array} \right\}$ | 1                            | 28                      | 4½                              | -----                     | ½                         | +                       | +                    | 18                   | 38                                          | 52                                | Do.                     |
| Erythritol tetranitrate.....   | 13-35.....                  | $\left\{ \begin{array}{c} 65 \\ (1 \text{ gr.}) \end{array} \right\}$                 | 5½                           | 35                      | 22                              | 1-2                       | 5-6                       | -----                   | -----                | 45                   | 60                                          | 216                               | Orally.                 |
| Mannitol hexanitrate.....      | 6-11.....                   | $\left\{ \begin{array}{c} 65 \\ (1 \text{ gr.}) \end{array} \right\}$                 | 12                           | 35                      | 100                             | 1-2                       | 5-16                      | -----                   | -----                | -----                | -----                                       | -----                             | -----                   |

<sup>1</sup> Increased flow in cc./min. by perfusion with concentrations of 1:10,000.

<sup>2</sup> Dose refers to spirits of glycerol nitrates=1.3 mg.

As illustrated in table 5, the *vasodilator action of aliphatic nitric acid esters* as studied by Bradbury (1895) shows considerable differences. Whereas methyl nitrate is hardly effective, ethylene glycol dinitrate is a potent vasodilator agent; glycerol trinitrate and mannitol hexanitrate are less potent, the latter being the least effective. It may be noted that in this series the vasodilator action of erythritol tetranitrate is apparently more marked than that of the four other esters studied, and Voegtlin and Macht (1913) also state that erythritol tetranitrate causes a more lasting dilatation than glycerol trinitrate.

With regard to the *effect of aliphatic nitric acid esters on the blood pressure*, Bradbury (1895) stated that in rabbits methyl nitrate in comparatively large doses has only a slight and transient effect; ethylene glycol dinitrate and glycerol trinitrate have a marked effect (the latter being slightly less effective), and erythritol tetranitrate and mannitol hexanitrate are much less effective but cause a much more prolonged fall than observed with the other esters. These findings were confirmed by Matthew (1909) in humans with regard to glycerol trinitrate, erythritol tetranitrate, and mannitol hexanitrate, as illustrated in table 5. This was confirmed by Miller (1910) with regard to the action of glycerol trinitrate and erythritol tetranitrate. The greater effect of ethylene glycol dinitrate on the blood pressure as compared with that of glycerol trinitrate was also observed by Wilhelmi (1942), and von Oettingen and Donahue (1944 b) showed that erythritol tetranitrate and, to a greater extent, pentaerythritol tetranitrate are inferior to glycerol trinitrate in this respect.

Quantitative data on the *methemoglobinemic action of aliphatic nitric acid esters* are comparatively recent. Such studies were published by Gross, Bock, and Hellrung (1942) and Wilhelmi (1942). According to the latter the methemoglobinemic action of ethyl nitrate is only moderate and that of ethylene glycol mononitrate is not very marked. Both investigators agree that ethylene glycol dinitrate is much more effective (according to Wilhelmi, 1942, four times more effective) than glycerol trinitrate which is also a powerful methemoglobin former.

Similar relations were reported with regard to the *effect of aliphatic nitric acid esters on the formation of Heinz bodies*. As shown by Gross, Bock, and Hellrung (1942) and Wilhelmi (1942), ethylene glycol dinitrate is more effective in this respect than glycerol trinitrate, whereas according to the latter ethylene glycol mononitrate is ineffective and ethyl nitrate little effective in this respect.

The most outstanding side action of aliphatic nitric acid esters is their capacity to produce more or less severe *headache*. This action was studied by Crandall, Leake, Loevenhart, and Muehlberger (1931) as illustrated in table 5. Since these authors used different routes of

administration it is difficult to evaluate exactly the efficiency of the different compounds studied, but these data indicate that erythritol tetranitrate is definitely less effective than ethylene glycol dinitrate and glycerol trinitrate.

These findings show that there is no direct relation between the number of nitric acid radicals of the aliphatic nitric acid esters and their pharmacological and toxicological action, and it appears that several physico-chemical factors are involved in their action.

#### THE MECHANISM OF ACTION OF ALIPHATIC NITRIC ACID ESTERS

Hay (1883), Leech (1893), and others suggested that the vasodilator effect of aliphatic nitric acid esters was essentially due to *liberation of nitrite* in the organism. Herrman, Leake, Loevenhart, and Muehlberger (1926) determined the rate of hydrolysis of several aliphatic nitric acid esters (using  $\frac{1}{10}$  normal alcoholic solutions based on the number of nitric acid radicals) by normal sodium hydroxide solution and compared this with their depressant effect on the blood pressure of dogs following the intravenous injection of 0.5 cc. of  $\frac{1}{100}$  normal alcoholic solutions. Their results as given in table 6 show a striking parallelism between rate of hydrolysis and depressor action. Crandall, Leake, Loevenhart, and Muehlberger (1931) showed that tolerance to certain aliphatic nitric acid esters was paralleled by a decrease of their hydrolytic cleavage by blood. Krantz, Carr, Forman, and Ellis (1939 b) in their study of the pharmacological action of isomannide dinitrate pointed out that the fall of the blood pressure produced by this compound was not paralleled by the presence of detectable quantities of nitrite in the blood. Rath and Krantz, (1942 a, b) claimed that neither the intravenous nor the oral administration of erythritol tetranitrate, isomannide dinitrate and glycerol trinitrate resulted in the liberation of nitrite in the blood, and they believed that there is little evidence that the depressor action of aliphatic nitric acid esters is due to the liberation of nitrite. Yagoda and von Oettingen (1944), using the method of Yagoda (1944), determined the concentration of nitrite in the blood of dogs fed, by stomach tube, doses of 5 mg. per kg. of pentaerythritol tetranitrate and erythritol tetranitrate and 2.5 mg. per kg. of glycerol trinitrate, and followed simultaneously the changes in the blood pressure of these animals. As illustrated in figure 8, the administration of these substances is followed by the liberation of nitrites, but this has no apparent relation to the onset and the intensity of the fall of the blood pressure. This holds true especially for the administration of glycerol trinitrate (fig. 8, C). Krantz, Carr, Forman, and Cone (1940) measured the rate of hydrolysis and percentage of nitrite formed from isomannide, erythritol tetranitrate, glycerol trinitrate, and mannitol hexanitrate,



as illustrated in table 7, and they determined their depressant effect on the blood pressure of dogs before and after hydrolysis. As shown in table 7, the depressant effect of erythritol tetranitrate, glycerol trinitrate, and mannitol hexanitrate parallels the amount of nitrite liberated by hydrolysis, and this effect was practically abolished when their hydrolysates were tested; however, isomannide dinitrate, which does not readily split off nitrite, maintains its depressor effect, indi-

**Table 6.—RATE OF HYDROLYSIS AND DEPRESSANT EFFECT ON THE BLOOD PRESSURE OF ALIPHATIC NITRIC ACID ESTERS**

(Herrman, Leake, Loevenhart, and Muehlberger, 1926)

| Compounds                      | Depression of blood pressure | Percent of total nitrogen recovered as nitrite at the end of— |             |           |            |            |             |          |
|--------------------------------|------------------------------|---------------------------------------------------------------|-------------|-----------|------------|------------|-------------|----------|
|                                |                              | 10 sec-onds                                                   | 30 sec-onds | 1 min-ute | 3 min-utes | 5 min-utes | 10 min-utes | 2½ hours |
| Methyl nitrate.....            | Practically 0.....           | -----                                                         | -----       | -----     | -----      | -----      | -----       | 0.1      |
| Ethylene glycol dinitrate..... | +                            | -----                                                         | -----       | -----     | -----      | 0.1        | 0.3         | 51.8     |
| Glycerol trinitrate.....       | ++                           | -----                                                         | 13.1        | 17.5      | 29.3       | 34.3       | 37.8        | 46.0     |
| Mannitol hexanitrate.....      | +++                          | 29.3                                                          | 32.3        | 32.2      | 32.2       | 31.8       | 32.2        | 35.5     |

**Table 7.—FACTORS INVOLVED IN THE DEPRESSOR ACTION OF ALIPHATIC NITRATES**

(Krantz, Carr, Forman, and Cone, 1940)

| Compound                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          | Degree of hydrolysis    |                        | Effect on blood pressure of dogs |                                         |                                  |                                         | Partition coefficient oil/water <sup>1</sup> x 10 <sup>2</sup> |
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                                                               | Percent alkali consumed | Percent nitrite formed | Before hydrolysis                |                                         | After hydrolysis                 |                                         |                                                                |
|                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   |                         |                        | Original blood pressure, mm. Hg. | Maximal fall of blood pressure, mm. Hg. | Original blood pressure, mm. Hg. | Maximal fall of blood pressure, mm. Hg. |                                                                |
| <div><div><div><div><div></div><div>O</div><div></div></div><div><div><div><div></div><div>H</div><div></div></div><div>C</div><div><div><div><div></div><div>O</div><div></div></div><div>NO<sub>2</sub></div></div></div><div><div><div><div></div><div>H</div><div></div></div><div>C</div><div><div><div><div></div><div>O</div><div></div></div><div>NO<sub>2</sub></div></div></div><div><div><div><div></div><div>H</div><div></div></div><div>C</div><div><div><div><div></div><div>O</div><div></div></div><div>NO<sub>2</sub></div></div></div><div><div><div><div></div><div>H</div><div></div></div><div>C</div><div><div><div><div></div><div>O</div><div></div></div><div>NO<sub>2</sub></div></div></div><div><div><div><div></div><div>H</div><div></div></div><div>C</div><div><div><div><div></div><div>O</div><div></div></div><div>NO<sub>2</sub></div></div></div><div><div><div><div></div><div>H</div><div></div></div><div>C</div><div><div><div><div></div><div>O</div><div></div></div><div>NO<sub>2</sub></div></div></div><div><div><div><div></div><div>H</div><div></div></div><div>C</div><div><div><div><div></div><div>O</div><div></div></div><div>NO<sub>2</sub></div></div></div><div><div><div><div></div><div>H</div><div></div></div><div>C</div><div><div><div><div></div><div>O</div><div></div></div><div>NO<sub>2</sub></div></div></div><div><div><div><div></div><div>H</div><div></div></div><div>C</div><div><div><div><div></div><div>O</div><div></div></div><div>NO<sub>2</sub></div></div></div><div><div><div><div></div><div>H</div><div></div></div><div>C</div><div><div><div><div></div><div>O</div><div></div></div><div>NO<sub>2</sub></div></div></div><div><div><div><div></div><div>H</div><div></div></div><div>C</div><div><div><div><div></div><div>O</div><div></div></div><div>NO<sub>2</sub></div></div></div><div><div><div><div></div><div>H</div><div></div></div><div>C</div><div><div><div><div></div><div>O</div><div></div></div><div>NO<sub>2</sub></div></div></div><div><div><div><div></div><div>H</div><div></div></div><div>C</div><div><div><div><div></div><div>O</div><div></div></div><div>NO<sub>2</sub></div></div></div><div><div><div><div></div><div>H</div><div></div></div><div>C</div><div><div><div><div></div><div>O</div><div></div></div><div>NO<sub>2</sub></div></div></div><div><div><div><div></div><div>H</div><div></div></div><div>C</div><div><div><div><div></div><div>O</div><div></div></div><div>NO<sub>2</sub></div></div></div><div><div><div><div></div><div>H</div><div></div></div><div>C</div><div><div><div><div></div><div>O</div><div></div></div><div>NO<sub>2</sub></div></div></div><div><div><div><div></div><div>H</div><div></div></div><div>C</div><div><div><div><div></div><div>O</div><div></div></div><div>NO<sub>2</sub></div></div></div><div><div><div><div></div><div>H</div><div></div></div><div>C</div><div><div><div><div></div><div>O</div><div></div></div><div>NO<sub>2</sub></div></div></div><div><div><div><div></div><div>H</div><div></div></div><div>C</div><div><div><div><div></div><div>O</div><div></div></div><div>NO<sub>2</sub></div></div></div><div><div><div><div></div><div>H</div><div></div></div><div>C</div><div><div><div><div></div><div>O</div><div></div></div><div>NO<sub>2</sub></div></div></div><div><div><div><div></div><div>H</div><div></div></div><div>C</div><div><div><div><div></div><div>O</div><div></div></div><div>NO<sub>2</sub></div></div></div><div><div><div><div></div><div>H</div><div></div></div><div>C</div><div><div><div><div></div><div>O</div><div></div></div><div>NO<sub>2</sub></div></div></div><div><div><div><div></div><div>H</div><div></div></div><div>C</div><div><div><div><div></div><div>O</div><div></div></div><div>NO<sub>2</sub></div></div></div><div><div><div><div></div><div>H</div><div></div></div><div>C</div><div><div><div><div></div><div>O</div><div></div></div><div>NO<sub>2</sub></div></div></div><div><div><div><div></div><div>H</div><div></div></div><div>C</div><div><div><div><div></div><div>O</div><div></div></div><div>NO<sub>2</sub></div></div></div><div><div><div><div></div><div>H</div><div></div></div><div>C</div><div><div><div><div></div><div>O</div><div></div></div><div>NO<sub>2</sub></div></div></div><div><div><div><div></div><div>H</div><div></div></div><div>C</div><div><div><div><div></div><div>O</div><div></div></div><div>NO<sub>2</sub></div></div></div><div><div><div><div></div><div>H</div><div></div></div><div>C</div><div><div><div><div></div><div>O</div><div></div></div><div>NO<sub>2</sub></div></div></div><div><div><div><div></div><div>H</div><div></div></div><div>C</div><div><div><div><div></div><div>O</div><div></div></div><div>NO<sub>2</sub></div></div></div><div><div><div><div></div><div>H</div><div></div></div><div>C</div><div><div><div><div></div><div>O</div><div></div></div><div>NO<sub>2</sub></div></div></div><div><div><div><div></div><div>H</div><div></div></div><div>C</div><div><div><div><div></div><div>O</div><div></div></div><div>NO<sub>2</sub></div></div></div><div><div><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|                         |                        |                                  |                                         |                                  |                                         |                                                                |

<sup>1</sup> Krantz, Carr, Forman, and Ellis (1939, b).

cating that the liberation of free nitrite is not necessary for the depressor action of this compound.

Krantz, Carr, Forman, and Ellis (1939 a) determined the *partition coefficient oil over water* of these compounds as given in the last column of table 7, but it appears from a comparison of these values and the depressor action of the various compounds that this is not the determining factor, at least not with the nitric acid esters of the polyvalent alcohols. Gross, Bock, and Hellrung (1942) determined the solubility of ethylene glycol dinitrate and glycerol trinitrate in oil and found that the latter, which is less effective both with regard to the depressant effect on the blood pressure and the methemoglobinemic action, is miscible with oil whereas the former dissolves in 100 parts of oil to the extent of 12.2 gm. at 21° C. Forman, Carr, and Krantz (1941) synthesized a number of nitrates of glycolic acid esters and Krantz, Carr, Forman, and Cone (1940) reported on their depressor action and their partition coefficient oil over water, as given in table 8. As indicated by this table, the depressor action of these substances (as indicated by their molar concentration causing a fall of 10 mm. Hg.) and their solubility in oil go parallel. They also showed that the nitrate of the disodium salt of malic acid, which is soluble in water, caused no depressor response in molar concentrations. Similarly, the sodium salt of the nitrate of lactic acid produced only a minimal depressor response in molar concentration. If, however, these compounds were rendered oil soluble by esterification of the carboxylic group with ethyl and amyl alcohol they exhibited strong pressor action.

Krantz, Carr, Forman, and Ellis (1939 a) studied the depressor action of a number of nitrated polyalcohols with an internal ether linkage as illustrated in table 9. As indicated by a comparison of the

• Table 8.—THE DEPRESSOR RESPONSE TO NITRATES OF GLYCOLLIC ACID ESTERS

(Krantz, Carr, Forman, and Cone, 1940)

| Compound                         | Nitrogen           |                    | Number of experiments | Effective depressive molar concentration 10 mm. Hg. | Partition coefficient oil/water |
|----------------------------------|--------------------|--------------------|-----------------------|-----------------------------------------------------|---------------------------------|
|                                  | Calculated         | Percent            |                       |                                                     |                                 |
| $O_2NO-CH_2-COONa$ .....         | <sup>1</sup> 121.5 | <sup>1</sup> 121.4 | 24                    | 0.10                                                | 0.9                             |
| Sodium glycocollate nitrate..... |                    | 121.8              |                       |                                                     |                                 |
| $O_2NO-CH_2-COOC_2H_5$ .....     | 9.40               | 9.51               | 10                    | .013                                                | 17                              |
| Ethyl glycocollate nitrate.....  |                    | 9.57               |                       |                                                     |                                 |
| $O_2NO-CH_2-COOC_3H_7$ .....     | 8.59               | 8.30               | 27                    | .008                                                | 24                              |
| Propyl glycocollate nitrate..... |                    | 8.40               |                       |                                                     |                                 |
|                                  |                    | 8.29               |                       |                                                     |                                 |
| $O_2NO-CH_2COOC_4H_9$ .....      | 7.91               | 7.74               | 18                    | .003                                                | 108                             |
| Butyl glycocollate nitrate.....  |                    | 7.76               |                       |                                                     |                                 |
|                                  |                    | 7.83               |                       |                                                     |                                 |
| $O_2NO-CH_2COOC_7H_{15}$ .....   | 6.39               | 5.24               | 7                     | .001                                                | 142                             |
| Heptyl glycocollate nitrate..... |                    | 5.48               |                       |                                                     |                                 |
|                                  |                    | 5.16               |                       |                                                     |                                 |

<sup>1</sup> Neutralization equivalent on the acid.

Table 9.—EFFECT OF ALIPHATIC NITRIC ACID ESTERS ON THE BLOOD PRESSURE

(Krantz, Carr, Forman, and Ellis, 1939 a)

| Compound                                                                                                                                                                                                                                                                                                                                                                                 | Original blood pressure, mm. Hg. | Maximum fall of blood pressure, mm. Hg. | Average duration of action, minutes | Number of experiments |
|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------|-----------------------------------------|-------------------------------------|-----------------------|
| $  \begin{array}{cccc}  \text{NO}_2 & \text{NO}_2 & \text{NO}_2 & \text{NO}_2 \\    &   &   &   \\  \text{HC} & -\text{C}- & \text{C}- & \text{CH} \\    &   &   &   \\  \text{H} & \text{H} & \text{H} & \text{H}  \end{array}  $ <p>Erythritol tetranitrate</p>                                                                                                                        | 131                              | 42                                      | 4                                   | 9                     |
| $  \begin{array}{ccccccc}  & & \text{O} & & & & \\  & &   & & & & \\  \text{H}_2\text{C} & -\text{C}- & \text{H} & -\text{C}- & \text{H} & -\text{C}- & \text{CH}_2 \\  &   & &   & &   & \\  & \text{O} & & \text{O} & & \text{O} & \\  &   & &   & &   & \\  & \text{NO}_2 & & \text{NO}_2 & & \text{NO}_2 &  \end{array}  $ <p>Isomannide dinitrate</p>                               | 156                              | 34                                      | 11                                  | 22                    |
| $  \begin{array}{ccccccc}  & & \text{O} & & & & \\  & &   & & & & \\  \text{H}_2\text{C} & -\text{C}- & \text{H} & -\text{C}- & \text{H} & -\text{C}- & \text{CH}_2 \\  &   & &   & &   & \\  & \text{NO}_3 & & \text{NO}_3 & & \text{NO}_3 & \\  &   & &   & &   & \\  & \text{H} & & \text{H} & & \text{H} &  \end{array}  $ <p>Isosorbide dinitrate</p>                               | 108                              | 31                                      | 10                                  | 10                    |
| $  \begin{array}{ccccccc}  & & \text{O} & & & & \\  & &   & & & & \\  \text{H}_2\text{C} & -\text{C}- & \text{H} & -\text{C}- & \text{H} & -\text{C}- & \text{CH}_2 \\  &   & &   & &   & \\  & \text{NO}_3 & & \text{NO}_3 & & \text{NO}_3 & \\  &   & &   & &   & \\  & \text{H} & & \text{H} & & \text{H} & \\  & & & & & & \text{NO}_2  \end{array}  $ <p>Styrcitol tetranitrate</p> | 97                               | 38                                      | 10                                  | 5                     |
| $  \begin{array}{ccccccc}  \text{NO}_2 & \text{NO}_2 & & \text{NO}_2 & \text{NO}_2 & & \\    &   & &   &   & & \\  \text{H}_2\text{C} & -\text{C}- & \text{H} & -\text{C}- & \text{C}- & \text{C}- & \text{CH}_2 \\    &   & &   &   & & \\  \text{H} & \text{H} & & \text{H} & \text{H} & & \\  & & & & & & \text{O}  \end{array}  $ <p>Polygalitol tetranitrate</p>                    | 100                              | 40                                      | 13                                  | 3                     |
| $  \begin{array}{ccccccc}  & & \text{O} & & & & \\  & &   & & & & \\  \text{H}_2\text{C} & -\text{C}- & \text{H} & -\text{C}- & \text{H} & -\text{C}- & \text{CH}_2 \\  &   & &   & &   & \\  & \text{O} & & \text{O} & & \text{O} & \\  &   & &   & &   & \\  & \text{NO}_2 & & \text{NO}_2 & & \text{NO}_2 &  \end{array}  $ <p>Mannitan tetranitrate</p>                              | 109                              | 48                                      | 9                                   | 8                     |
| $  \begin{array}{ccccccc}  \text{NO}_2 & \text{NO}_2 & & \text{NO}_2 & \text{NO}_2 & & \\    &   & &   &   & & \\  \text{H}_2\text{C} & -\text{C}- & \text{H} & -\text{C}- & \text{C}- & \text{C}- & \text{CH} \\    &   & &   &   & & \\  \text{H} & \text{H} & & \text{H} & \text{H} & &  \end{array}  $ <p>Sorbitan tetranitrate<sup>1</sup></p>                                      | 117                              | 55                                      | 5                                   |                       |
| $  \begin{array}{ccccccc}  & & \text{O} & & & & \\  & &   & & & & \\  \text{H}_2\text{C} & -\text{C}- & \text{H} & -\text{C}- & \text{H} & -\text{C}- & \text{CH}_2 \\  &   & &   & &   & \\  & \text{NO}_2 & & \text{NO}_2 & & \text{NO}_2 & \\  &   & &   & &   & \\  & \text{H} & & \text{H} & & \text{H} &  \end{array}  $ <p>Erythritan dinitrate</p>                               | 106                              | 27                                      | 16                                  | 5                     |

The position of the oxide group being uncertain.

effect of erythritol tetranitrate and erythritan tetranitrate, the presence of an internal ether linkage reduces somewhat the depressor action but prolongs the duration of their action. Such compounds are said to be less oil soluble and more water soluble than the nitrates of dehydrated compounds.

It appears, therefore, that the importance of the partition coefficient oil over water for the depressor action of aliphatic nitric acid esters may vary with different compounds.

With the volatile aliphatic nitric acid esters the *vapor tension* may be a *determining factor* in determining the intensity of their action. Gross, Bock, and Hellrung (1942) showed that the vapor pressure of ethylene glycol dinitrate is more than 100 times greater than that of glycerol trinitrate and that the former is much more effective with regard to its methemoglobinemic action, Heinz body formation, and its depressor action on the blood pressure. From the determinations of Crater (1929) it appears further that up to 35° C. the vapor tension of ethylene glycol dinitrate increases more rapidly with the rising temperature than is the case with glycerol trinitrate, which also would tend to increase the potential dangers of the former.

It appears, therefore, that the pharmacological and toxicological action of aliphatic nitric acid esters does not depend exclusively on the liberation of nitrite groups. There is evidence that with some of the compounds the action is produced by the molecule in toto. With those compounds which are not readily hydrolyzed their solubility in oil may be of importance, and with volatile aliphatic nitric acid esters their vapor tension may have a definite influence on the intensity of their action.

### III. APPRAISAL OF THE POTENTIAL DANGERS OF ALIPHATIC NITROUS AND NITRIC ACID ESTERS

It has been shown above that there is a close resemblance between the pharmacological action of aliphatic nitrous and nitric acid esters, and this holds true also for the effect of sodium nitrite which appears to be puzzling in the light of the fact that the depressant effect of aliphatic nitric acid esters on the blood pressure and their methemoglobinemic action are not paralleled by an increase of the nitrite concentration in the blood. Generally the effects of sodium nitrite are less marked, and the maximal effect is produced more slowly but is of longer duration than is the case with organic nitrites and nitrates (Dossin, 1911, Weiss and Ellis, 1933, and others). There may be other quantitative differences, as indicated by the observation of Starr, Gamble, Margolies, Donal, Joseph, and Eagle (1937) that sodium nitrite does not affect the cardiac output to the same extent as observed with aliphatic nitrous and nitric acid esters. But it appears that observations made following the administration of sodium nitrite may

be utilized in the appraisal of the potential dangers of aliphatic nitrites and nitrates.

The *acute toxicity of nitrous and nitric acid esters* is due mainly to their vasodilator action (resulting in a fall of the blood pressure) and to their methemoglobinemic action; their effect on the circulation appears to deserve special consideration. Since of the nitrous acid esters only amyl nitrite, and of the nitric acid esters only glycerol trinitrate have been studied more closely in this respect, the following discussion is largely based on findings made with these compounds.

Both amyl nitrite and glycerol trinitrate *cause an increase of the pulse rate*, as found by Guthrie (1859), Cash and Dunstan (1894), and Hewlett (1906 a, b) with amyl nitrite, and by Plumier (1905) and others with glycerol trinitrate. As pointed out by Hewlett (1906 a, b) for the former and by Plumier (1905) for the latter, this is probably due to a decrease in vagus tone on account of a reduced blood supply to the central nervous system, as had been shown by Mayer and Friedrich (1875) and Filehne (1879). With the more volatile compound (amyl nitrite) this effect may be complicated by a temporary primary reflex stimulation of the medullary centers over the trigeminal nerve endings in the nose, as demonstrated by Dautrebande (1933):

The effect on the *cardiac output* varies with the dose. Small doses which do not lower the blood pressure may increase the output, as observed by Lindhard (1915) with amyl nitrite and by Starr, Gamble, Margolies, Donal, Joseph, and Eagle (1937) with glycerol trinitrate, but large doses cause a reduction, as observed by Lindhard (1915) with amyl nitrite and by Lauber and Brauch (1930) with glycerol trinitrate. Similarly, large doses of both compounds decrease the *beat volume* as shown by Lindhard (1915) and Lauber and Brauch (1930). As pointed out by Winkler (1898), this may lead to an *increase of the pressure in the left auricle*.

Both substances and all nitrous and nitric acid esters studied cause a *peripheral vasodilatation* by direct depression of the muscles in the vascular walls. This results in a shift of the blood to the splanchnic area. This *vasodilator* action extends also to the vessels *in the brain*, as was shown by Schüller (1874), Pick (1876), Mosso (1881), and Hürthle (1889) (both quoted from Wolff, 1929), Hirschfelder (1915), Raphael and Stanton (1919), and Wolff (1929) for amyl nitrite and by Wallace and Ringer (1909), Leake, Kammer, and Hitz (1929), and Schmidt and Hendrix (1938) for glycerol trinitrate. But in spite of this cerebral vasodilatation the blood supply to the brain may be inadequate because of the shift of the mass of blood to the splanchnic region. Like other blood vessels the *coronary arteries are also dilated* by amyl nitrite and glycerol trinitrate (Loeb, 1903, Meyer, 1912, Voegtlin and Macht, 1913, Schloss, 1913, Kountz, 1932, and Katz,



Lindner, Weinstein, Abramson, and Jochim, 1938). As found by Carrier (1922) and Schulmann and Marek (1926) for amyl nitrite, by Kauffmann (1924) for glycerol trinitrate, and by Deusch and Liepelt (1928) for sodium nitrite, these compounds also cause dilatation of the capillaries. According to the latter the dilator action is presumably due to a reflex mechanism from the arterioles.

The vasodilator action of aliphatic nitrous and nitric acid esters is the main cause of the *fall of the arterial blood pressure* produced by these compounds as observed by Berger (1874), Mayer and Friedrich (1875), Bradford and Dean (1894), Hewlett (1906 a, b), Pilcher and Sollmann (1915), and others with amyl nitrite and by Bruel (1876), Murrell (1879), Atkinson (1887-88), Wallace and Ringer (1909), Dossin (1911), Capps and Matthews (1913) and others with glycerol trinitrate. In the case of amyl nitrite the fall may be preceded by a primary rise due to reflex stimulation of the vasomotor center as observed by Berger (1874), Wessely (1915), and Dautrebande (1932 a, b). In the case of glycerol trinitrate there may be a secondary rise, as reported by Loomis (1905) (quoted from Miller, 1910), Hewlett (1906 a, b), Dmitrenko (1909), MacNider (1908), Miller (1910), and Matthew (1909). The diastolic and systolic pressures may not respond to the same extent. They may vary with the dose and also (according to Sollmann, 1932) with the mechanism of the fall of the blood pressure. If the vasomotor tone and the heart rate are affected most, the reduction of the diastolic pressure is greater than that of the systolic pressure, and the reverse holds true if cardiac force and blood volume are predominantly changed. Therefore the *pulse pressure* may be either increased or decreased, depending on these conditions.

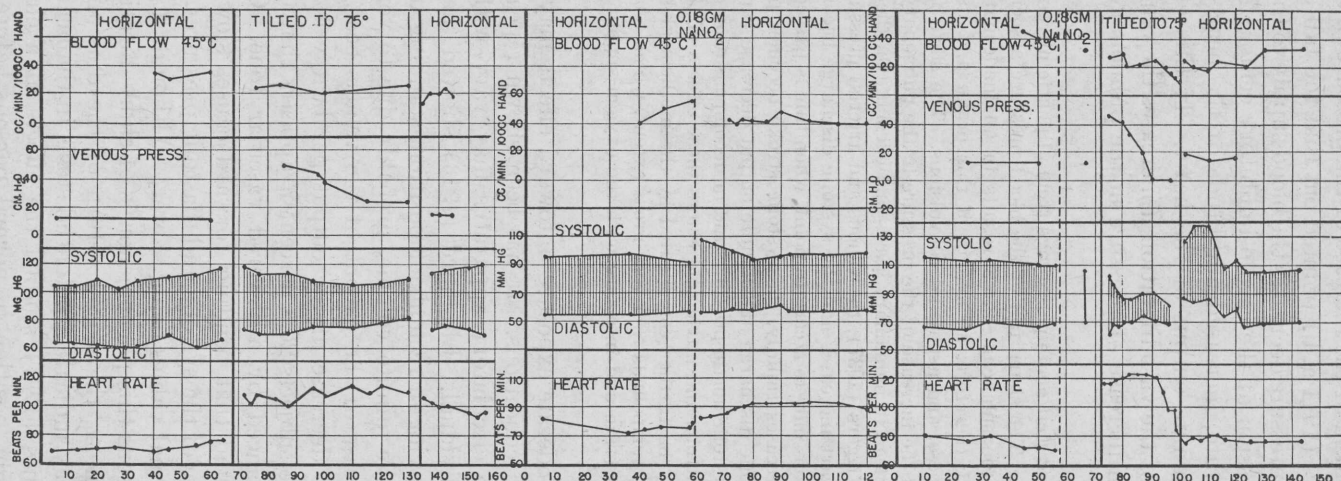
The behavior of the *venous pressure* may vary with the condition of the circulatory apparatus. Burton-Opitz and Wolf (1910) and Payan and Giraud (1925) noted a fall of the venous pressure and McDowall (1922-23) a primary rise and subsequent fall following administration of amyl nitrite; whereas with glycerol trinitrate, Capps and Matthews (1913) saw a fall, Payan and Giraud (1925) a primary fall, and von Oettingen and Donahue (1944 b) a rise when the animal was in collapse.

The behavior of the *blood pressure in the pulmonary artery* appears to be especially significant for the explanation of certain accidents in acute poisoning from aliphatic nitrous and nitric acid esters. As shown by von Openchowski (1889), Bradford and Dean (1894), Plumier (1905 and 1906) and Frey (1909) for amyl nitrite and by Wood (1901-02), Plumier (1905) and Wiggers (1911, a) for glycerol trinitrate, this is increased as the systemic arterial blood pressure is reduced. As pointed out by Winkler (1898) the rise of pressure in the pulmonary artery and in the left auricle leads to a decreased return of blood to the arterial system and to pulmonary stasis and edema. With

regard to the mechanism of the increase of the pressure in the pulmonary artery, Wiggers (1911 a, b) pointed out that the fall of the systemic arterial blood pressure decreases the blood supply to the medulla, resulting in a depression of the cardio-inhibitory center and thus in an increased heart rate, increased amplitude of the heart beat, and thus to increased minute output of the heart. If this effect overshadows the effects of the vasodilatation it increases the venous return and thus leads to an increase of pressure in the pulmonary vein and artery.

It is, therefore, evident that acute poisonings from aliphatic nitrous and nitric acid esters are similar. The sharp fall of the blood pressure associated with a shift of the blood to the splanchnic area results in an inadequate supply of blood to the brain and, therefore, an anoxia of this organ and its sequelae—headache, unconsciousness, and asphyxial convulsions. The shift of the distribution of the blood and the increase of the pressure in the pulmonary artery and in the left auricle may lead to pulmonary stasis and edema. On the other hand, if, as pointed out by Wiggers (1937), the venous and arterial pressures rise, the diastolic size of the heart increases and the systolic discharge becomes greater. This mechanism functions only to a certain point, when this is passed the diastolic ventricular size increases rapidly, the systolic stroke becomes progressively smaller, and both systolic discharge and minute output decrease rapidly, resulting in a rapid fall of systolic aortic pressure and in decompensation of the heart. With some aliphatic nitrous and nitric acid esters these effects of these circulatory complications may be further complicated by the methemoglobinemia produced by some of these compounds to a very great extent, which tends to aggravate the anoxia of the brain and interfere with the oxygen supply to the heart and other vital organs.

But even with moderate exposure untoward effects may be anticipated under certain conditions. It has been shown repeatedly that the reaction of the circulatory apparatus may vary with different postures of the individual. Lindhard (1913) showed that, as compared with the behavior in recumbent position, standing up causes a slight decrease in the minute volume, probably due to venous stasis and a greater decrease of the output of the venous beat. Since in female subjects the decrease of the output per beat was around 31 percent and in male subjects 20.8 percent it appears that this venous stasis may be enhanced by a lowered vascular tone. Olmer and Jacques (1928) found that bending forward causes slowing of the heart beat and a rise of the systolic and diastolic pressures, the return of the latter to normal values being slower. Although it was found that in normal persons this phenomenon is of short duration, lasting 30 seconds at the most, the slower return of the diastolic pressure to normal values may indicate a potential stasis in the heart. Weiss, Wilkins, and Haynes (1937) studied the effects of a change from recumbent to upright position in normal persons before and after administration of small doses (0.18 gm.) of sodium nitrite. As illustrated in figure 9, the change from horizontal to upright position



EFFECT OF TILTING TO 75° ON THE BLOOD FLOW THROUGH THE HAND AT 45°C, ON THE VENOUS PRESSURE IN THE FOOT, THE ARTERIAL BLOOD PRESSURE, AND THE HEART RATE.

EFFECT OF 0.18 GM.  $\text{NaNO}_2$  ON THE BLOOD FLOW THROUGH THE HAND AT 45°C, ON THE ARTERIAL BLOOD PRESSURE, AND THE HEART RATE.

EFFECT OF THE ADMINISTRATION OF 0.18 GM.  $\text{NaNO}_2$  FOLLOWED BY TILTING TO 75° ON THE BLOOD FLOW THROUGH THE HAND AT 45°C, ON THE VENOUS PRESSURE IN THE FOOT, THE ARTERIAL BLOOD PRESSURE, AND THE HEART RATE. AFTER 29 MIN. AT 75° THE SUBJECT FANED.

FIGURE 9.—Circulatory response of a normal person to a change from a recumbent to an upright position, and the response to 0.18 gm. sodium nitrite in recumbent position and in upright position.

causes in a normal person no essential change of the blood flow through the hand, a slight reduction of the pulse pressure, and a moderate primary increase of the venous pressure in the foot, followed by a progressive fall to slightly above the normal hydrostatic level of the heart, an increase of the pulse rate, but no subjective symptoms. The administration of 0.18 gm. of sodium nitrite in a horizontal position causes a slight, questionable decrease of the flow through the hand, no essential changes of the systolic and diastolic blood pressures or of the pulse pressure, a very slight rise of the heart rate, and no subjective symptoms. If, however, the position of the person is changed to upright after the ingestion of sodium nitrite a train of very spectacular phenomena is observed after 5 minutes have elapsed. After this time the person starts to yawn—this becoming gradually more frequent, the respiration becomes deeper and assumes the character of sighing, the subject becomes restless, the intestinal peristalsis is increased, warm and later cold perspiration sets in, the skin turns slightly cyanotic and later ashen grey, the subject becomes drowsy, the pupils become dilated, and then the subject falls into vasomotor collapse. The maximal flow through the hand is at first slightly reduced, but falls later to zero and becomes more and more influenced by respiratory changes. The pulse pressure is markedly reduced, mainly on account of a sharp fall in systolic pressure, and the pulse is small and thready, but when the subject yawns or sighs it improves temporarily. The venous pressure falls rapidly to below the hydrostatic level of the right auricle. The heart rate is rapid until syncope sets in, then it falls to 80 beats per minute at which time the blood pressure cannot be obtained. Upon return of the subject to horizontal position he rapidly regains consciousness and all symptoms disappear. With regard to the mechanism of these phenomena, Weiss, Wilkins, and Haynes (1937) believed that the elevation of the cardiac rate and the vasoconstrictor response are due to the stimulating effect of the lowered systolic pressure on the cardiac sinus and the aortic depressor reflex mechanism. They assumed that the later effects are due to a progressive ischemia of the medullary centers and a simultaneous stimulation of the sympathetic and the para-sympathetic nerves followed later by predominant stimulation of the latter. Wilkins, Haynes, and Weiss (1937) studied the response of the venous system to sodium nitrite. They found that after small doses of nitrite there is a distinct loss of tone of the vascular bed so that with upright position a considerable portion of the total blood accumulates in the lower extremities, resulting in a disproportion between the total blood and the volume in the vascular bed. They believed that sodium nitrite has a specific action on the veins, inasmuch as after its administration the same increase in pressure distends the vein 20 to 40 percent more than



normally and that the venous tone is decreased in the presence of a normal arteriolar tone. They believed further that this arteriolar constriction can be explained as a secondary response to and not as the primary essential pathological physiology of collapse, and that this marked arteriolar constriction, coupled with a small arterial pressure, may result in such a low flow to the tissue that a dangerous degree of anoxemia is produced. When this anoxemia involves the vital centers, these may be damaged to such an extent that a vicious circle is established and the collapse becomes deeper and finally causes the death of the patient. Although these studies were made after the administration of sodium nitrite, it appears likely that similar conditions may result from the absorption of aliphatic nitrous and nitric acid esters and that persons exposed to these compounds may suffer similar disturbances when changing their posture or when performing vigorous physical exercise.

Little information is available with regard to the effect of continued exposure to aliphatic nitrous and nitric acid esters. Gross, Bock, and Hellrung (1942) mentioned a number of fatalities with signs of acute circulatory failure in workers handling ethylene glycol dinitrate and glycerol trinitrate which occurred after strenuous physical exercise 24 to 48 hours after discontinuation of the exposure. The reason for these accidents is not understood. It appears possible that alcoholic excesses, as assumed by these authors, may be partly responsible, but they certainly are not their primary cause. Aperia (1936) stated that persons exposed for several years to glycerol trinitrate showed a greater difference in arterial and venous pressure than normal persons, and he noted no signs of hypertension in such persons ranging from 41 to 63 years of age. This may indicate that in such persons the peripheral vascular tone is lower than normal. Laws (1898) stated that workers handling glycerol trinitrate may suffer sometimes from a hypertrophy of the left ventricle, and Laws (1910) stated that the area of dullness over the heart is frequently increased and that such workers may suffer from shortness of breath upon any undue exertion.

It is not quite clear whether the cardiac enlargement is due to a true hypertrophy or to a dilatation. As pointed out before, aliphatic nitrous and nitric acid esters are methemoglobin formers, and since this pigment is unable to carry oxygen to the tissue a condition may exist which is similar to subacute carbon monoxide poisoning. It was shown by Campbell (1932), in mice, and by Valchera (1936), in rabbits, that exposure to subtoxic concentrations of carbon monoxide results in an increase of the heart weight (true hypertrophy), and Gey (1924) saw hypertrophy of the cardiac muscle in 4 out of 19 cases of human protracted carbon monoxide poisoning. Therefore, it is pos-



sible that a true hypertrophy of the heart may be observed also in subacute poisoning from aliphatic nitrous and nitric acid esters.

The fatal accidents mentioned by Gross, Bock, and Hellrung (1942) might be explained by the following mechanism. It has been shown that under the influence of aliphatic nitrous and nitric acid esters the tone of the blood vessels is decreased. It is to be expected that with continued exposure the circulatory apparatus adjusts itself to this condition so that this and the resulting reduction of the blood pressure represent the normal status. If, however, the exposure to the esters is discontinued and their effect wears off so that the vascular tone and the blood pressure approach normal values, such an individual resembles a person suffering from hypertension. This effect may be greatly aggravated by a peripheral vasoconstriction such as is produced by low temperatures or by an increase of pressure in the abdominal cavity as by filling of the stomach or contraction of the abdominal muscles. It was shown by Rein (1931) (quoted from Wiggers, 1937) that cooling of the body surface causes peripheral vasoconstriction resulting in a shift of the blood to the splanchnic system, so that the condition which will be suggested in the following becomes more aggravated. The increased venous pressure, resulting from the increased pressure in the abdominal cavity, results in a sudden increase of the venous return. This, in turn, causes an increase of the pressure in the right auricle, in the right ventricle, in the pulmonary circuit, and in the left auricle. Since, as pointed out above, under the conditions in question the heart has to press the blood against a relatively higher resistance, it suffers from a double load which may easily lead to acute cardiac failure, stasis in the pulmonary circulation, and possibly to pulmonary edema. Such a crisis is especially liable to occur if the cardiac muscle is weakened.

It has been shown experimentally by Gross, Bock, and Hellrung (1942) that continued exposure to low concentrations of ethylene glycol dinitrate and to a lesser extent of glycerol trinitrate may result in anemia. Unfortunately, no blood studies have been reported in workers handling these materials, therefore it is difficult to appraise the practical importance of this observation.

The methemoglobinemic action of aliphatic nitrous and nitric acid esters is of importance in the acute toxicity of these compounds, especially of those such as ethylene glycol dinitrate with which this effect is marked. This methemoglobinemia may lead to an anoxic condition in vital organs, especially the brain and the heart muscle, and in association with the circulatory changes produced by these compounds may have serious effects. Aside from this direct effect on the oxygen carrying capacity of the blood the presence of methemo-

globin interferes with the dissociation of oxyhemoglobin as was shown by Darling and Roughton (1942) and Lester and Greenberg (1944), so that in the case of greater oxygen demand, as produced by vigorous exercise, the anoxia may actually be greater than indicated by the methemoglobin content of the blood. Marshall and Marshall (1945) pointed out that the methemoglobinemic action of nitrites is characterized by an induction period, a reactionary period, and a stationary period, and in their opinion first methemoglobin and subsequently hemoglobin and NO hemoglobin are formed, the latter reaction being enhanced by the presence of reducing agents.

It has been mentioned that Keeser (1939) found that ethyl nitrate inhibits the action of catalase in rabbits. It is not known whether this effect exists with other nitrous and nitric acid esters or whether it extends to other enzymes. Therefore, at present it is not possible to evaluate the clinical importance of this observation.

It has been pointed out that the circulatory disturbances produced by aliphatic nitrous and nitric acid esters may be aggravated by injury of certain vital organs and of the blood vessels. No human autopsy findings have been reported which might prove this point and the existing literature on experimental studies of this subject allows no definite conclusions. Hueper and Landsberg (1940) found, in rats fed comparatively large doses of erythritol tetranitrate and sodium nitrite over a long period of time, degenerative vascular and parenchymatous lesions in heart, lungs, brain, kidney, and testes after several months. In their opinion the vessels of the head, neck, brain, and meninges are most susceptible in this respect whereas the coronaries and the vessels of the splanchnic region are less susceptible. Donahue and Monaco (1944), feeding smaller doses of erythritol tetranitrate and penta-erythritol tetranitrate over a period of 1 year, saw no such vascular changes and were unable to confirm the findings of Hueper and Landsberg (1940). Since, however, erythritol tetranitrate and penta-erythritol tetranitrate rank among the least toxic aliphatic nitric acid esters it is quite possible that changes similar to those observed by Hueper and Landsberg (1940) may be produced by much smaller doses of glycerol trinitrate and especially of ethylene glycol dinitrate. If this should prove true, the relative hypertension (postulated for workers handling these compounds) would gain additional importance inasmuch as it might lead to rupture of the affected blood vessels, especially upon vigorous exercise, and to apoplectic accidents.

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**THE GENERA *BOOPHILUS*, *RHIPICEPHALUS*,  
AND *HAEMAPHYSALIS* (IXODIDAE)  
OF THE NEW WORLD**

**By**

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## INTRODUCTION

The present paper, the last in the monographic series<sup>1</sup> on NEW WORLD ticks prepared at the Rocky Mountain Laboratory, treats of the genera *Boophilus*, *Rhipicephalus*, and *Haemaphysalis*, and is based mainly on the numerous specimens which have accumulated in this laboratory through collections made by field workers from the laboratory, and through correspondence with individuals in this country and in other countries. Since sufficient material was available, the species from both North America and South America are included.

Much more attention should be directed to the study of ticks in all the Americas south of the United States. There are probably many species new to science in southern Mexico, Central America, and especially in South America south of the 25th meridian where only one species, *Ixodes uriae*, has been recorded.

Grateful acknowledgment is made to Dr. E. W. Price and to Mr. Allen McIntosh of the Bureau of Animal Industry, United States Department of Agriculture for authentic specimens of *Boophilus annulatus* which date back to the time when this tick was of importance because it is the vector of Texas fever in cattle; to Dr. Henrique de Beaurepaire Aragão of the Oswaldo Cruz Institute, Rio de Janeiro, Brazil, for authentic specimens; to Mr. L. H. Dunn, Ovid, New York, and to Dr. G. B. Fairchild, Gorgas Memorial Laboratory, Panama, for essential specimens of *Haemaphysalis* from Central America.

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<sup>1</sup> The Genera *Dermacentor* and *Octocentor* (Ixodidae) in the United States, with studies in variation, R. A. Cooley. National Institute of Health Bulletin No. 175. Government Printing Office, 1938.

The Genus *Amblyomma* (Ixodidae) in the United States, R. A. Cooley and Glen M. Kohls. Journal of Parasitology, **30**: 77-111, 1944.

The Argasidae of North America, Central America and Cuba, R. A. Cooley and Glen M. Kohls. American Midland Naturalist, Monograph No. 1, 1944.

The Genus *Ixodes* in North America, R. A. Cooley and Glen M. Kohls. National Institute of Health Bulletin No. 184. Government Printing Office, 1945.



## MEDICAL AND VETERINARY IMPORTANCE

Late in the last century epoch-making discoveries were announced by Theobald Smith and F. L. Kilbourne. According to Herms (1939, p. 2), "Taking rank with Laveran's discovery of the malaria parasite is the discovery by Theobald Smith in 1889 (Smith and Kilbourne, 1893) of the causative organism (*Babesia bigemina*) of Texas cattle fever, also a red-blood-corpuscle-inhabiting protozoön. Associated with Smith in the investigation of the disease was F. L. Kilbourne, and together in 1893 they made the second great fundamental discovery in the field of medical entomology, namely, that the cattle tick, *Boophilus annulatus* (Say) (*B. bovis* Riley), is the necessary intermediary host of the causal agent of the disease."

These findings led to the subsequent discovery of numerous similar associations of ticks and organisms pathogenic to man and animals.

*Boophilus microplus* is more prevalent on cattle in the New World than is *B. annulatus*. The former is also a carrier of *Babesia bigemina*, as shown by the writings of Parodi (1916), Clark (1918), Guy (1919), Fonseca and Braga (1923), Lignieres (1924), Rosenbusch (1927), and Jimenez de Asua, Dios, Zuccarini, and Kuhn (1928).

*Rhipicephalus sanguineus* is probably the most cosmopolitan tick in the world and in the New World occurs in many parts of North and South America where its range is being rapidly extended by transportation on dogs, its most important host. In the Old World, it is the transmitting agent of fièvre boutonneuse among dogs and to man. This disease is not known to occur in the New World, but it cannot be safely assumed that it has not been introduced. A rickettsia which is at least very closely related to the pathogenic agent of fièvre boutonneuse is prevalent in *Amblyomma maculatum* in southern United States. While pathogenic for guinea pigs, this rickettsia has not been associated with disease in man or any native animal (Parker, Kohls, Cox, George W., and Davis, 1939; Parker and Cox, H. R., 1939).

*R. sanguineus* is also the vector of canine piroplasmosis which is well-known in Europe, Asia, and Africa. Eaton (1934) recognized the presence of this disease in dogs in Florida. Hagen (1943) states that it "has been reported in Puerto Rico and in Brazil," and the same author states, "It was seen by Clark in Panama in imported hunting dogs," and "the disease occurs in Texas."

Parker, Philip, and Jellison (1933) showed that under experimental conditions, *R. sanguineus* will harbor and transmit the infectious agent of

Rocky Mountain spotted fever. Since this tick is found on dogs in association with the proved vectors of this disease, it is hardly conceivable that *R. sanguineus* has not already become a transmitting agent among dogs. Fortunately this tick seldom bites man in this country. It does occasionally do so when dwellings are infested; particularly when standards of living are low, and there is a close association between dogs and occupants. Under such conditions, occasional instances of the transmission of Rocky Mountain spotted fever to man appear to be a definite future possibility; in fact, there is evidence which suggests that such transmission has already occurred in Mexico.

Drs. P. Regendanz and Julio Muniz (1935) in laboratory experiments on guinea pigs were able to demonstrate stage-to-stage transmission in *R. sanguineus* of "exanthematic typhus" of São Paulo, now known to be the same as Rocky Mountain spotted fever.

As a parasite, *R. sanguineus* is not only directly injurious to the dog but is a continual annoyance, calling for repeated removal of the ticks and treatment of kennels and other bedding places.

Dogs that are kept in dwellings or that have access to them, commonly cause infestations difficult to eradicate and, in large cities, provide frequent jobs for the commercial pest exterminators.

Parker (1923) discovered *Haemaphysalis leporis-palustris* to be a natural vector of Rocky Mountain spotted fever. While this tick rarely attacks man, its passage of the disease from animal to animal is of primary importance in the maintenance of the disease in nature.

Parker (1924) also showed that *H. leporis-palustris* is a natural vector of tularemia among rabbits and certain ground-frequenting birds in North America. It is possible that this tick is of considerable importance also in the natural maintenance of tularemia.

Larson, Shillinger, and Green (1936) reported the transmission of rabbit papillomatosis by *H. leporis-palustris* in laboratory experiments.

Todd (1919) reported a fatal case of tick paralysis in a child (age 10?) due to a single *Haemaphysalis cinnabarina* (now *chordeilis*) attached to the head of the child.

Parker, Philip, and Davis (1932) have published data which suggest that *H. cinnabarina* may be a transmitting agent of tularemia among ground-frequenting birds. If it is, this tick, too, is of possible significance in the incidence of tularemia in man resulting from contact with game birds.



# EXPLANATION OF TERMS

FIGURE 1

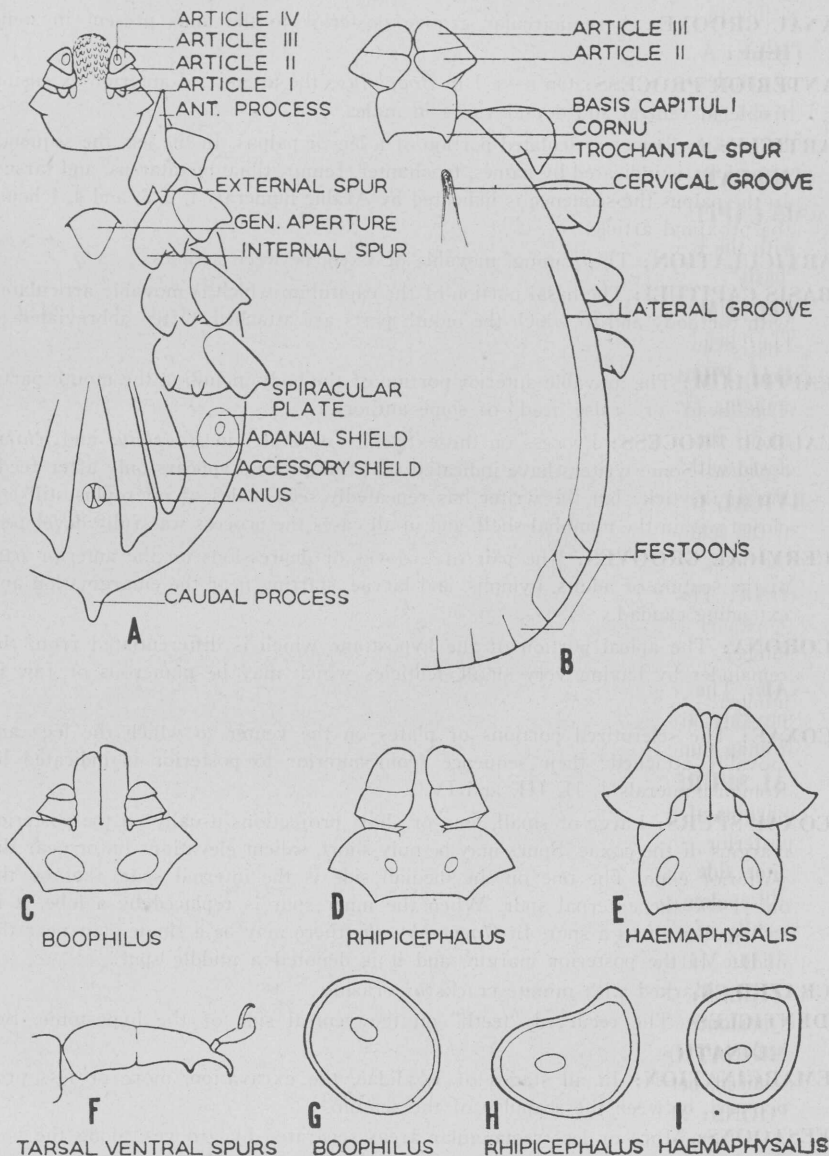


FIGURE 1. A. From male *Boophilus*, venter. B. From male *Haemaphysalis*, dorsum. C,D,E. Female capitulum, dorsal. F. From *Boophilus*. G,H,I. Spiracular plates.

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**ACCESSORY SHIELDS:** Paired shields outside the adanal shields in males of *Boophilus* and *Rhipicephalus*.

**ADANAL SHIELDS:** Paired ventral shields near the anus in males of *Boophilus* and *Rhipicephalus*.

**ANAL GROOVE:** A semicircular groove posterior to the anus present in some genera.

**ANTERIOR PROCESS:** On coxa I in *Boophilus*; the attenuated anterior extension visible in ventral and dorsal views in males.

**ARTICLE:** A distinct articulated portion of a leg or palpus. In the leg, the sequence of articles is indicated by names, trochanter, femur, tibia, metatarsus, and tarsus. In the palpus the sequence is indicated by Arabic numerals 1, 2, 3, and 4, 1 being the proximal article.

**ARTICULATION:** The joining, movable or fixed, between articles.

**BASIS CAPITULI:** The basal portion of the capitulum which is movably articulated with the body and to which the mouth parts are attached. Often abbreviated to "basis".

**CAPITULUM:** The movable anterior portion of the body including the mouth parts. The "head" or "false head" of some authors.

**CAUDAL PROCESS:** Process on the extremity of males in *Boophilus* and *Rhipicephalus*. Some writers have indicated that this process appears only after feeding of the tick; but the writer has repeatedly seen males of *microplus* still enclosed within the nymphal shell, and in all cases the process was fully developed.

**CERVICAL GROOVES:** The pair of grooves or depressions on the anterior part of the scutum of adults, nymphs, and larvae, starting near the emargination and extending caudad.

**CORONA:** The apical portion of the hypostome which is differentiated from the remainder by having very small denticles which may be numerous or few in number.

**COXAE:** The sclerotized portions or plates on the venter to which the legs are movably attached; their sequence from anterior to posterior is indicated by Roman numerals I, II, III, and IV.

**COXAL SPURS:** Large or small, long or short projections usually on the posterior margins of the coxae. Spurs may be only short, salient elevations on or near the posterior edge. The one on the median side is the internal spur, that on the outer side the external spur. When the inner spur is replaced by a lobe, it is still spoken of as a spur. In *Haemaphysalis* there may be a single spur near the middle of the posterior margin, and it is denoted a middle spur.

**CRAZED:** Marked with minute cracks or crazing.

**DENTICLES:** The recurved "teeth" on the ventral side of the hypostome. See hypostome.

**EMARGINATION:** In all stages of Ixodidae, the excavation, more or less pronounced, between the scapulae of the scutum.

**FESTOONS:** More or less rectangular areas separated by grooves, along the posterior submarginal area of the dorsum in both sexes. In *Haemaphysalis*, present also on nymphs and larvae.

**GENITAL APERTURE:** The external evidence of the genital organs found on the ventral median line posterior to the capitulum. Absent in nymphs and larvae.

**GENITAL GROOVE:** A long groove beginning near the genital aperture and extending toward, or to the posterior margin.

**HYPOSTOME:** The median ventral mouth part which is immovably attached to the basis capituli. Its dentition is expressed by a ratio. Thus 3/3 means that there are three longitudinal files on each half of the hypostome. Measurement is made from the base to the anterior extremity.

**LATERAL CARINAE:** On the scutum in all stages, paired lineal elevations outside the cervical grooves, and usually more declivitous on their inner sides, and sometimes so faint as to be indefinite. Often absent.

**LATERAL GROOVES:** In males, grooves at the sides of the scutum.

**LEGS:** The sequence of the legs from anterior to posterior is indicated in Roman numerals I, II, III, and IV. Measurements of the articles are made on the dorsal side since they "telescope" on the ventral side.

**LIGULA:** A tongue-shaped retrograde, sclerotized projection on the venter of palpal article 1.

**MARGINAL GROOVES:** In females, grooves at the sides of the body posterior to the scutum.

**MEDIAN GROOVE:** A dorsal body groove, on the middle line back of the scutum.

**PALPI:** Paired, articulated appendages on the basis capituli lying parallel with the hypostome and chelicerae. Four articles were originally present in all ticks, but the proximal article 1 may be obsolescent or absent. In the genus *Boophilus*, palpal articles 2, 3, and 4 are plainly visible, and all articulations are movable, though between 2 and 3 the movement is only slight. The articulation between the basis capituli and article 1 and that between 1 and 2 is confusing by reason of the fact that 1 has been much reduced or may be absent. If present at all, 1 is represented only by a sclerotized spot, or plate, on the inner side (toward the hypostome) which may be very indefinite, a distinct inner edge, or a retrograde extension, which, because of its shape, may be called a "ligula." (See fig. 5B.) The palpi in this genus do not have a fixed length as may be observed in occasional specimens which have one palpus distinctly longer than the other.

In *Rhipicephalus*, article 1 is present and movably attached to 2. On the venter there is a large triangular plate on the inner side while on the dorsum a small plate on the inner side may be present or absent.

In all species of *Haemaphysalis* known to the writer palpal article 1 is absent and the articulation between 2 and 3 is fused.

**POROSE AREAS:** A pair of pitted areas, usually depressed, on the dorsum of the basis capituli, present in all females (of Ixodidae), absent in males, nymphs and larvae.

**POSTANAL MEDIAN GROOVE:** Ventral, longitudinal groove posterior to the anus.

**POSTEROLATERAL GROOVES:** A pair of dorsal body grooves outside the median groove.

**PUNCTATIONS:** Pits in the surface of the exoskeleton, frequent on the scutum and sometimes present on the basis capituli. The pit may or may not have a hair in it, and in some the "floor" is level.

**SALIENT:** An edge that extends, in contradistinction to a margin that terminates by a rounded edge.

**SCLEROTIZATION:** The hardening, and incidentally, darkening of the body wall or its parts by the deposit of nonchitinous substances in the exocuticula.

**SCUTUM:** The sclerotized plate covering all or most of the dorsum in males, and the anterior plate in females, nymphs, and larvae.

**SPIRACULAR PLATE:** Paired plates situated behind legs IV, the external evidence of the respiratory system.

**TROCHANTAL SPUR:** Retrograde projection near the distal end of the trochanter. Both dorsal and ventral spurs may be present in a species.

**VENTRAL CORNUA:** Retrograde horns on the posterior margin of ventral surface of the basis, similar to the cornua on the dorsal surface.

**VENTRAL TARSAL SPURS:** Spurs on the ventral side of the tarsus designated as terminal and subterminal ventral spurs.

## GENUS *BOOPHILUS* CURTICE 1891

1890. *Boophilus* Curtice. Name *Boophilus* used without description.  
1891. *Boophilus* Curtice, p. 317. Original description.  
1901. *Boophilus* Curtice: Salmon and Stiles, p. 419. Description, synonymy, and bibliography.  
1907. *Margaropus* Karsch: Neumann, p. 223. Makes *Boophilus* a synonym of *Margaropus*.  
1908. *Margaropus* Karsch: Banks, p. 35.  
1911. *Boophilus* Curtice: Nuttall and Warburton, p. 124.  
1912. *Margaropus* Karsch: Hooker et al., p. 111.  
1934. *Boophilus* Curtice: Minning, p. 11.  
1941. *Boophilus* Curtice: Osorno-Mesa, p. 428.  
1945. *Boophilus* Curtice: Mackie et al., p. 536.

Lightly sclerotized. Inornate, with eyes but without festoons. Anal groove obsolete in female, faint in male. Basis capituli definitely hexagonal or in male sometimes lacking lateral points (angles); anterior portion (in front of the lateral points) never marginate at the sides. Hypostome with dentition 3/3 or 4/4. Palpi short, when apposed not meeting over the chelicerae; articles 2 and 3 with transverse ridges continuous on dorsal and lateral surfaces. Palpal article 1 absent or reduced to sclerotized spot or plate, which, if present, is on the inner edge; hairs absent on the sclerotized spots, present or absent on the small plates; or with a small, retrograde ligula. All palpal articulations movable (not fixed) though the degree of movement between 2 and 3 is slight. The articulation between basis and article 1, as well as that between articles 1 and 2, is a band of soft tissue causing the palpus to become mildly protrusile. Female scutum small. Male scutum extending the full length of the body. Adanal and accessory shields present. Coxa I with one or two spurs and with a long anterior process (in male); coxae II, III, and IV with two, one, or no spurs. Spiracular plate oval or subcircular. All tarsi with terminal ventral spurs. Male caudal process present or absent.

**Genotype:** *Boophilus annulatus* (Say).

The genus *Boophilus* (and the distinct genus *Margaropus*) are one-host ticks and probably originated on ungulates long before the coming of modern man. We should not be too much influenced by the less important records of these ticks taken from animals other than ungulates. They constitute a rather distinct phylogenetic group and show relationships to *Rhipicephalus* and *Anocentor*.

Our knowledge of the systematics of the Ixodoidea is far from complete, and emphasis should be placed on facilitating, not confusing, future studies of these ticks. To continue to use questionable new genera,



subgenera, and the species and subspecies of this group could retard development and lead to probable errors. The present author has decided to disregard for the present the new subgenera of Minning, synonymize *cyclops* (from Central America), and suspend judgment on new species and subspecies from other countries. Some of these species and subspecies are lacking in our collection.

## BOOPHILUS ANNULATUS (SAY), 1821

### FIGURES 2 AND 3

1821. *Ixodes annulatus* Say, pp. 75-76. Original description.  
1844. *Haemaphysalis rosae* Koch, p. 237.  
1869. *Ixodes bovis* Riley, p. 168. (Type host, *Bos taurus*, type locality, Missouri.)  
1869. *Ixodes indentatus* Gamgee, p. 121.  
1891. *Boophilus bovis* (Riley): Curtice, pp. 313-319.  
1897. *Rhipicephalus annulatus* (Say): Neumann, p. 407.  
1901. *Boophilus annulatus* (Say): Salmon and Stiles, pp. 420-426, bibliography.  
1901. *Ixodes annulatus* Say: Stiles and Hassall, p. 3, as a synonym of *Boophilus annulatus* (Say).  
1907. *Margaropus annulatus* (Say): Neumann, p. 224.  
1908. *Margaropus annulatus* (Say): Banks, pp. 36-37. Follows Neumann in making *Boophilus* a synonym of *Margaropus*.  
1911. *Margaropus annulatus annulatus* (Say): Neumann, 1911, p. 47. Synonymy, hosts.  
1912. *Margaropus annulatus* (Say): Hooker et al., p. 111.  
1934. *Boophilus annulatus* (Say): Minning, p. 11.  
1936. *Margaropus annulatus* (Say): Brumpt, p. 1174.  
1941. *Boophilus annulatus* (Say): Travis, pp. 465-467.

### FEMALE

**Body.** Length (unengorged), 2.75<sup>1</sup>; width, 1.40. Scutum occupying about half the length. Edges of spiracular plates usually visible from above. Dorsal surface outside the scutum and venter with numerous fine short hairs. Median and posterolateral grooves present. Hairs absent in all grooves. Fully engorged female large, oval, with both ends equally thick dorsoventrally. Leg I distant from the capitulum. Length and width may be as much as 12.00 by 8.00.

**Capitulum.** Length from tips of palpi to posterior margin, about 0.48; greatest width of basis, 0.72. Basis about twice as wide as long. Cornua faint, sometimes absent. Porose areas large, subcircular or oval, mildly concave, size variable. Palpal hairs small and few.

In ventral view, basis is subreniform in shape. Palpal article 1 obliterated or very faint as a sclerotized spot on the inner edge; never with hairs on its inner edge. Articles 2 and 3 with a few palpal hairs on their inner edges.

**Hypostome.** Slightly longer than the palpi, at the base narrower than

<sup>1</sup> All measurements in this paper are in millimeters.

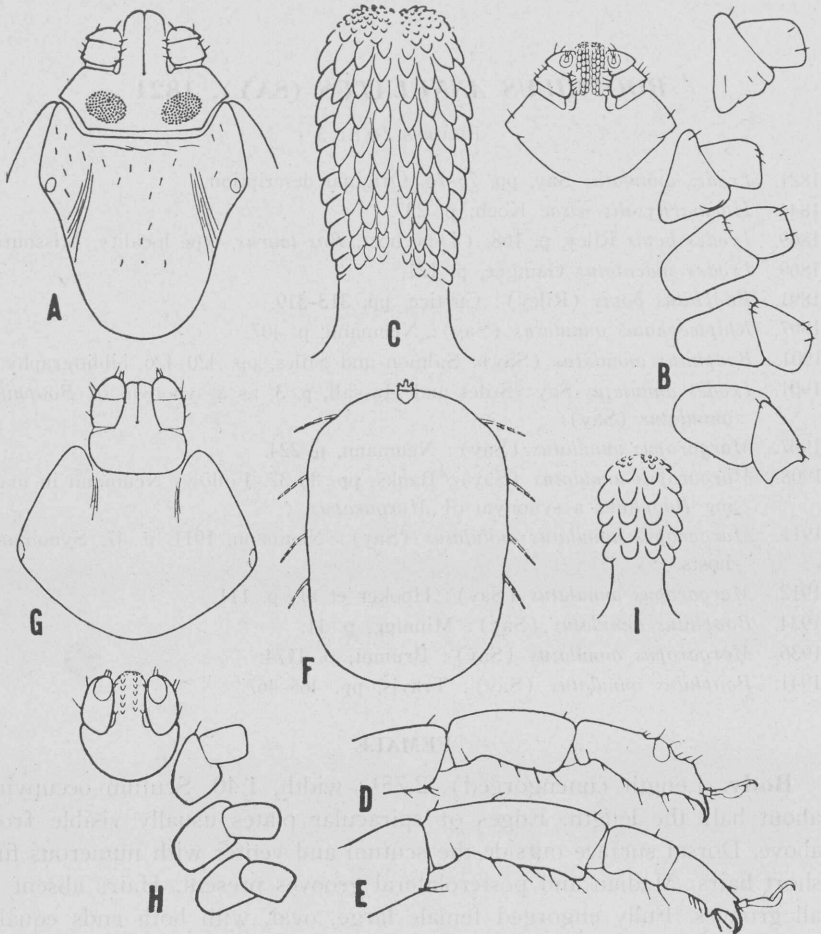


FIGURE 2. *Boophilus annulatus* (Say). A. Female capitulum and scutum, dorsum. B. Female capitulum and coxae, venter, and variant. C. Female hypostome. D. Female metatarsus and tarsus, leg I. E. Female metatarsus and tarsus, leg IV. F. Engorged female. G. Larva capitulum and scutum, dorsum. H. Larva capitulum and coxae, venter. I. Larva hypostome.

the combined width of the chelicerae, apex rounded, denticles seven or eight in each file and covering the apical two-thirds. Length, about 0.36.

**Scutum.** Length, 1.20; width, 1.38. Widest in front of the eyes, posterolateral margins straight or mildly convex, scapulae long, very widely separated, emargination deep. Cervical grooves faint or absent just posterior to the emargination, distinct and broad near the posterolateral margins. Punctations absent. Hairs few, scattered, sometimes absent.

**Legs.** Long, moderately thin, longer than in the male. All tarsi tapering gradually. Tarsi II, III, and IV with subapical ventral spurs. Pulvilli

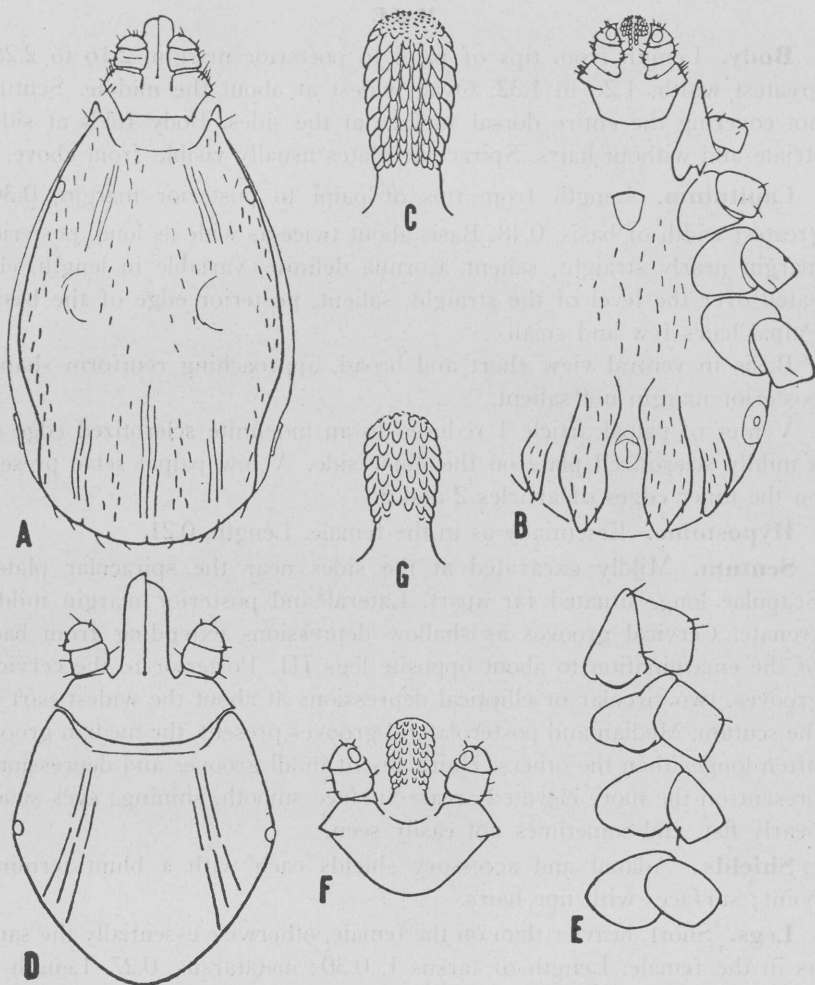


FIGURE 3. *Boophilus annulatus* (Say). A. Male capitulum and scutum, dorsum. B. Male capitulum, coxae, and shields, venter. C. Male hypostome. D. Nymph capitulum and scutum, dorsum. E. Nymph coxae, venter. F. Nymph capitulum, venter. G. Nymph hypostome.

small. Length of tarsus I, 0.48; metatarsus, 0.42. Length of tarsus IV, 0.51; metatarsus, 0.63.

**Coxae.** All about equal in size, in nearly parallel rows, mildly convex, hairs few and short. (Coxae II, III, and IV longer and narrower than in *microplus*.) Coxa I with a broad, short, rounded, external spur; internal spur absent; anterior process short, not free anteriorly. Coxae II, III, and IV without spurs.

**Genital aperture.** At about the level of the intervals between coxae I and II.

**MALE**

**Body.** Length from tips of palpi to posterior margin, 2.16 to 2.28; greatest width, 1.26 to 1.32. Oval, widest at about the middle. Scutum not covering the entire dorsal surface at the sides. Body folds at sides striate and without hairs. Spiracular plates usually visible from above.

**Capitulum.** Length from tips of palpi to posterior margin, 0.36; greatest width of basis, 0.48. Basis about twice as wide as long, posterior margin nearly straight, salient. Cornua definite, variable in length, elevated over the level of the straight, salient, posterior edge of the basis. Palpal hairs few and small.

Basis in ventral view short and broad, approaching reniform shape; posterior margin not salient.

Venter of palpal article 1 reduced to an indefinite sclerotized edge or a mildly sclerotized plate on the inner side. A few palpal setae present on the inner edges of articles 2 and 3.

**Hypostome.** Essentially as in the female. Length, 0.21.

**Scutum.** Mildly excavated at the sides near the spiracular plates. Scapulae long, situated far apart. Lateral and posterior margin mildly crenate. Cervical grooves as shallow depressions extending from back of the emargination to about opposite legs III. Posterior to the cervical grooves, two circular or elliptical depressions at about the widest part of the scutum. Median and posterolateral grooves present, the median groove often longer than the others. Hairs absent in all grooves and depressions, present on the more elevated areas. Surface smooth, shining; eyes small, nearly flat, and sometimes not easily seen.

**Shields.** Adanal and accessory shields each with a blunt terminal point; surfaces with fine hairs.

**Legs.** Short, heavier than on the female, otherwise essentially the same as in the female. Length of tarsus I, 0.30; metatarsus, 0.27. Length of tarsus IV, 0.33; metatarsus, 0.45.

**Coxae.** Coxa I with internal and external spurs about equal in length; internal spur a little wider and more rounded, variable; anterior process long, extending beyond the scapula and visible from above, free terminally. Coxae II, III, and IV without spurs. Hairs on coxae small and few.

**Genital aperture.** Between coxae II.

**NYMPH**

**Body.** When engorged, wider in front, sides constricted at the spiracular plates, which are usually visible from above. (No unengorged nymphs available.)

**Capitulum.** Length, tip of palpi to posterior margin, 0.24; greatest width of basis, 0.45. Cornua absent. Palpi much as in adults but less sclerotized. In ventral view, article 1 not in evidence. Palpal hairs few.



**Hypostome.** Denticles 3/3. About seven denticles in each file. Length about 0.16.

**Scutum.** Length and width about equal, each 0.48 to 0.57. Subpentagonal; eyes faint. Cervical grooves as broad shallow valleys, divergent posteriorly. Surface smooth, shining; punctations and hairs absent.

**Legs.** Short, moderately heavy.

**Coxae.** Small, convex; one or two hairs on each coxa. Coxae I, II, and III each with one short, broad, rounded spur; IV with spurs absent.

### LARVA

**Body.** Length from tips of palpi to posterior margin, 0.63; width, 0.45. Short oval. Scutum occupying about half the length of the body.

**Capitulum.** Length from tips of palpi to posterior margin, 0.15; width, 0.15. Lateral profile lines of basis convex; posterior margin mildly convex or straight. Cornua absent. Palpi short, broader and with hairs shorter than in *microplus*; article 1 absent. In ventral view, basis broadly rounded behind.

**Hypostome.** Short, broad, with about five denticles in each file. Dentition 2/2. Length about 0.08.

Out of the old genus *Boophilus* Curtice, Minning (1934) made 3 subgenera as follows: *Boophilus* including 6 species and subspecies, *Palpobophilus* including one species, and *Uroboophilus* including 11 species. Of the total of 18 species and subspecies appearing in the paper, 12 are described as new. In his studies, Minning had before him numerous lots of specimens collected from many parts of the world, but he showed little evidence of having given adequate attention to the effect of variation in speciation and to the obvious desirability of pointing out distinct correlating criteria in erecting new subgenera, species, and subspecies. His very small figures are not convincing.

### HOSTS AND DISTRIBUTION

*Boophilus annulatus* (Say) was described from deer, "*Cervus virginianus*", Florida and *Ixodes bovis* Riley, a synonym, was described from *Bos taurus* Missouri. This tick was widely distributed in the Southern States at about the beginning of this century; but because it was the vector of the very destructive Texas cattle fever, eradication measures were applied and have reduced its numbers to, or near, extinction in the United States. At its worst, it was present in all or parts of 16 States as follows: Texas, Oklahoma, Arkansas, Missouri, Louisiana, Tennessee, Mississippi, Alabama, Kentucky, Virginia, North Carolina, South Carolina, Georgia, Florida, and California. In Mexico, this tick was present and still occurs there.

The program of eradication is still being continued, especially along the areas in Texas and California that border on Mexico, where there is danger that *B. annulatus* may be brought in again, and in Florida and Puerto Rico. In recent years, there have been a few instances of re-introduction of this tick, but prompt action has stamped out the infestations.

Cattle are the usual hosts of this tick, but other hosts listed by the early writers include deer, horse, mule, sheep, goat, and buffalo.

## BOOPHILUS MICROPLUS (CANESTRINI), 1887

### FIGURES 4 AND 5

1887. *Haemaphysalis micropla* Canestrini, p. 104. Original description.  
1890. *Rhipicephalus micropla* (Canestrini), p. 493.  
1897. *Rhipicephalus annulatus* (Say): Neumann, p. 407.  
1899. *Rhipicephalus australis* Fuller, pp. 389-394.  
1901. *Boophilus australis* (Fuller): Stiles and Hassell, pp. 2-3.  
1901. *Boophilus australis* (Fuller): Salmon and Stiles, p. 426.  
1901. *Rhipicephalus annulatus* var. *microplus* (Canestrini): Neumann, p. 280.  
1911. *Margaropus micropla* (Canestrini): Neumann, p. 49. Synonymy and says the species is very probably *M. annulatus australis*.  
1912. *Margaropus annulatus australis* (Fuller): Hooker et al., p. 117.  
1913. *Margaropus annulatus australis* (Fuller): Bishopp, p. 366.  
1934. *Uroboophilus cyclops* Minning, p. 35. Original description.  
1934. *Uroboophilus microplus* (Canestrini): Minning, p. 33.  
1941. *Boophilus (Uroboophilus) microplus* (Canestrini): Osorno-Mesa, p. 429.  
1941. *Boophilus annulatus microplus* (Canestrini): Travis, pp. 465-467.  
1943. *Boophilus microplus* (Canestrini): Fairchild, p. 586.

### FEMALE

**Body.** Unengorged, length from tip of palpi to posterior margin, from 2.34 to 2.85; width from 1.14 to 1.50. Long oval. Scutum occupying about half the total length. Median and posterolateral grooves present. Marginal groove absent. Venter with genital and postanal median grooves present. Hairs present on dorsal and ventral surfaces but absent in all grooves. Fully engorged specimens may be as large as 13.0 by 9.0 and are oval, wider and thicker behind.

**Capitulum.** Length from tip of palpi to posterior margin, about 0.45; width from 0.62 to 0.66. (The palpi are mildly protrusile, hence measurements of length are not entirely dependable.) Hexagonal. Cornua as rounded corners (variable). Dorsal surface with two longitudinal valleys which traverse the porose areas. Porose areas oval, mildly convex, with longer axes diagonal. Palpal article 1 not visible above. Inner edge of article 2 either in one continuous convex curve or mildly notched near the middle, the notch when present leading to a transverse dorsal crease, which may or may not extend to the outer side of the article.

In ventral view, basis is subreniform with posterior margin salient. Palpal article 1 absent. Articles 2 and 3 with the posterior salient edges continuing into the median margin; postero-inner edges of 2 and 3 sometimes extended into mild diagonal lobes.

**Hypostome.** Short, broad, mildly notched apically; denticles, five or six in each file and occupying about three-fifths of the total length. Length, about 0.30.

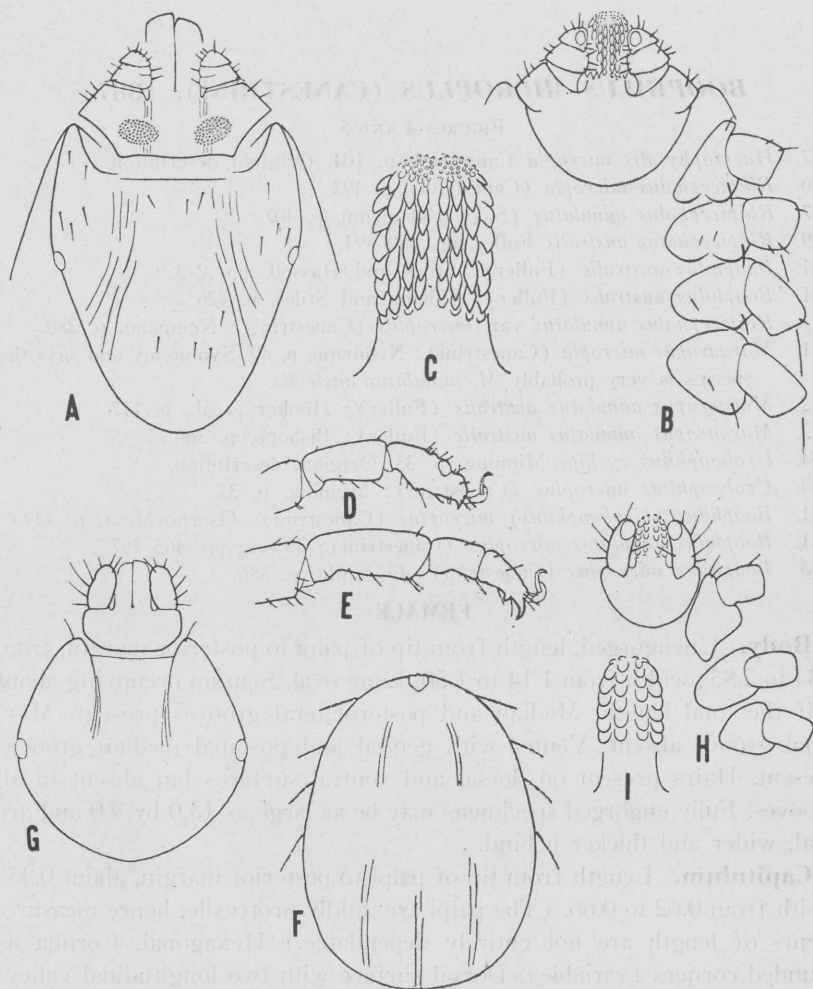


FIGURE 4. *Boophilus microplus* (Canestrini). A. Female capitulum and scutum, dorsum. B. Female capitulum and coxae, venter. C. Female hypostome. D. Female metatarsus and tarsus, leg I. E. Female metatarsus and tarsus, leg IV. F. Engorged female. G. Larva capitulum and scutum, dorsum. H. Larva capitulum and coxae, venter. I. Larva hypostome.

**Scutum.** Length, from 0.96 to 1.02; width, from 0.75 to 0.80; longer than wide. Scapulae long, blunt; the interval deep, very wide. Eyes distinct. Cervical grooves as broad divergent valleys, terminating at postero-lateral margins. Hairs few, scattered, absent in the valleys. Punctations absent.

**Legs.** Long, all about equally heavy. Terminal ventral spurs on tarsus I, and both terminal and subterminal spurs on II, III, and IV present.

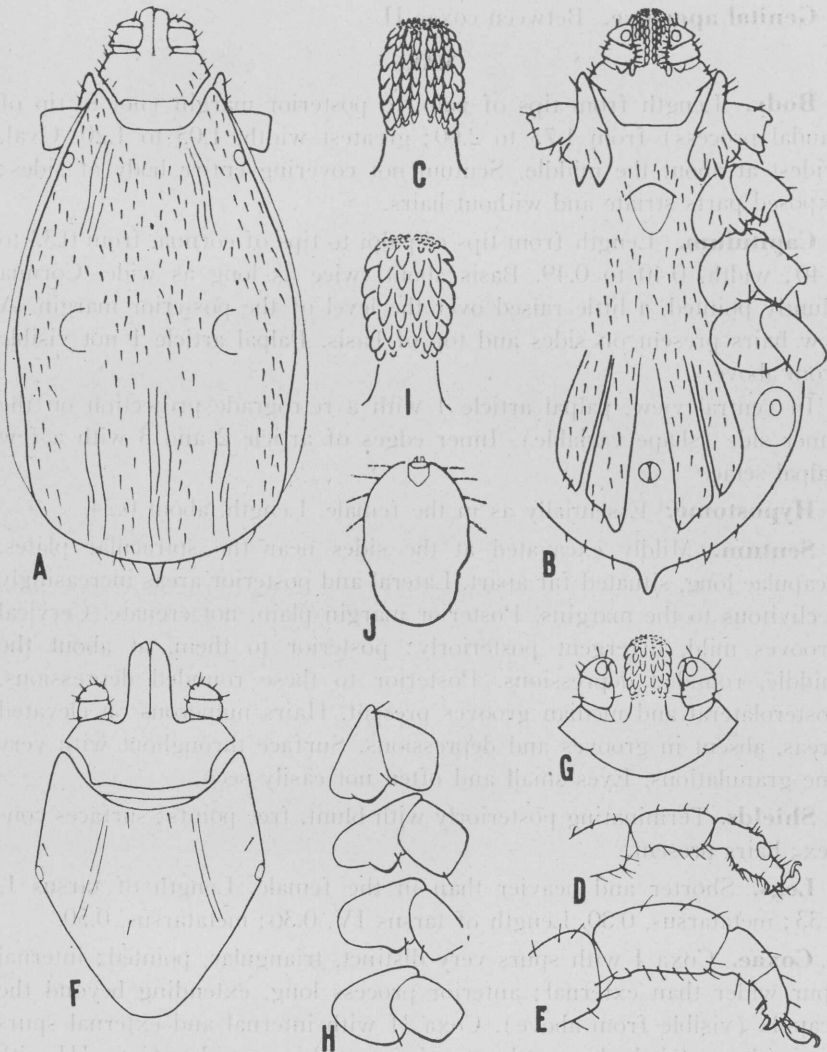


FIGURE 5. *Boophilus microplus* (Canestrini). A. Male capitulum and scutum, dorsum. B. Male capitulum, coxae, and shields, venter. C. Male hypostome. D. Male metatarsus and tarsus, leg I. E. Male metatarsus and tarsus, leg IV. F. Nymph capitulum and scutum, dorsum. G. Nymph capitulum, venter. H. Nymph coxae, venter. I. Nymph hypostome. J. Engorged nymph.

Length of tarsus I, 0.36; metatarsus, 0.30. Length of tarsus IV, 0.39; metatarsus, 0.495.

**Coxae.** Coxae I and II with spurs broadly rounded, about equal, wider than long. Coxa III, outer spur, smaller than on II; internal spur absent. Coxa IV, external spur very short, scarcely more than a salience; internal spur absent. Hairs few.



**Genital aperture.** Between coxae II.

#### MALE

**Body.** Length from tips of palpi to posterior margin (not to tip of caudal process) from 1.75 to 2.00; greatest width, 1.05 to 1.20. Oval, widest at about the middle. Scutum not covering entire body at sides; exposed parts striate and without hairs.

**Capitulum.** Length from tips of palpi to tips of cornua, from 0.33 to 0.40; width, 0.40 to 0.49. Basis about twice as long as wide. Cornua bluntly pointed, a little raised over the level of the posterior margin. A few hairs present on sides and top of basis. Palpal article 1 not visible from above.

In ventral view, palpal article 1 with a retrograde projection on the inner side (shape variable). Inner edges of article 2 and 3 with a few palpal setae.

**Hypostome.** Essentially as in the female. Length, about 0.24.

**Scutum.** Mildly excavated at the sides near the spiracular plates. Scapulae long, situated far apart. Lateral and posterior areas increasingly declivitous to the margins. Posterior margin plain, not crenate. Cervical grooves mild, divergent posteriorly; posterior to them, at about the middle, rounded depressions. Posterior to these rounded depressions, posterolateral and median grooves present. Hairs numerous on elevated areas, absent in grooves and depressions. Surface throughout with very fine granulations. Eyes small and often not easily seen.

**Shields.** Terminating posteriorly with blunt, free points; surfaces convex; hairs present.

**Legs.** Shorter and heavier than in the female. Length of tarsus I, 0.33; metatarsus, 0.30. Length of tarsus IV, 0.36; metatarsus, 0.50.

**Coxae.** Coxa I with spurs very distinct, triangular, pointed; internal spur wider than external; anterior process long, extending beyond the scapula (visible from above). Coxa II with internal and external spurs distinctly rounded, shorter than on I, internal spur wider. Coxa III with a still shorter, rounded, external spur; internal spur as a rounded salience (similar to that on II). Coxa IV with spurs absent. Hairs few.

**Genital aperture.** Situated between coxae II.

#### NYMPH

**Body.** Length well-engorged, 2.40 to 2.70; width, 1.60 to 1.80. Wide in front, narrow behind, mildly constricted at the spiracular plates; edges of the spiracular plates usually visible from above. (No unfed nymphs available.)

**Capitulum.** Length from tips of palpi to posterior margin, 0.24; greatest width, 0.30. Basis with posterior salient margin convex. Cornua

faint or absent. Palpi short, poorly sclerotized; transverse ridges faint. Sheaths of the chelicerae very long, fully twice as long as the palpi. Palpal hairs few, not conspicuous.

**Hypostome.** Short and broad. Denticles  $3/3$  with about five in each file. Length about 0.14.

**Scutum.** Pentagonal. Length and width equal, each about 0.45. Cervical grooves as shallow valleys, divergent posteriorly. Eyes small, oval, faint. Surface smooth, shining. Punctations absent. Hairs few.

**Legs.** Short, moderately heavy.

**Coxae.** Small, convex. Coxa I with a short, broad, rounded, external spur; II and III about as in I but progressively smaller; IV, spurs absent.

### LARVA

**Body.** Length from tips of palpi to posterior margin of body, 0.60; width, 0.42. Short oval. Scutum occupying about three-fifths of the length of the body.

**Capitulum.** Length from tips of palpi to posterior margin, 0.15; width of basis, 0.18. Lateral profile lines of basis convex; posterior margin nearly straight. Cornua absent. Palpal article 1 absent. In ventral view, basis broadly rounded behind. Palpi with relatively long hairs.

**Hypostome.** Short, broad, with about six broad, short, rounded denticles in each file. Dentition  $2/2$ . Length about 0.065.

**Scutum.** Length, 0.31; width, 0.42. Cervical grooves shallow, short, converging posteriorly. Surface smooth, shining, impunctate, and with hairs absent.

**Coxae.** Coxa I with a short, broad, internal spur; II and III without spurs.

### HOSTS AND DISTRIBUTION

*Boophilus microplus* (Canestrini) was described as *Haemaphysalis microplæ* from specimens that came from Paraguay. Salmon and Stiles (1901) referred to this tick as *australis* Fuller and mentioned that species as present on cattle in this country. Aragão, in various papers has mentioned the species as present on cattle, deer (*Mazama* sp.) horse, and man from Argentina, and from deer, ox, and horse from Brazil. Osorno-Mesa (1941, p. 429) mentions it as present in Colombia. Dunn (1923, p. 21) under the name *Margaropus annulatus australis*, records the tick from cattle in Panama; and Fairchild (1943, p. 586) states it is common on cattle in Panama and is also found on horse, dog, goat, and deer. Valadez (1923, p. 202) mentions the tick under the name *Margaropus annulatus australis* as present in Mexico.

Travis (1941, p. 466) gave a report of special interest because his survey gave records from numerous mammals and birds. One hundred and fifty-five mammals of twelve species were examined and ticks of various kinds were found, but *B. microplus* was found only on deer, and on only 13 out of 22 examined. Eighty-seven birds, including twenty-one species, were examined and showed various other ticks present but none of *B. microplus*.

In the Rocky Mountain Laboratory collection are the following: from Argentina, 1 lot; from Peru, 1 lot; from Ecuador, 1 lot from cattle; from Colombia, 33 lots from cattle, horse, and dog; from Brazil, 17 lots from cows; from Venezuela, 1 lot from cows; Costa Rica, 2 lots from ox and tapir; from Honduras, 3 lots from cattle; from Guatemala, 2 lots from cows; and from Mexico, 5 lots from cows.

## GENUS *RHIPICEPHALUS* KOCH, 1844

1844. *Rhipicephalus* Koch, p. 238. Original description.  
1889. *Phantoixodes* Berlese, p. 289.  
1897. *Rhipicephalus* Koch: Neumann, p. 384.  
1901. *Rhipicephalus* Koch: Salmon and Stiles, p. 415. Description, bibliography, and synonymy.  
1908. *Rhipicephalus* Koch: Banks, p. 34. Description.  
1911. *Rhipicephalus* Koch: Nuttall and Warburton, p. 122. Description and figures.  
1945. *Rhipicephalus* Koch: Mackie et al., p. 536.

Usually inornate, with eyes and festoons. Sexual dimorphism slight. Anal and median postanal grooves present. Males with adanal shields and usually a pair of accessory shields. Basis capituli usually hexagonal with anterior portion (in front of the lateral points) not emarginated laterally. Palpi short, articulation between 1 and 2 movable, between 2 and 3 slightly movable. In dorsal view, article 1 either visible or not; in ventral view, 1 distinct as a subtriangular plate. Coxa I bifid, deeply divided. Male caudal process present or absent. Spiracular plate bluntly or elongate comma-shaped.

**Genotype:** *Rhipicephalus sanguineus* (Latreille).

Neumann (1911, p. 38) mentions *Rhipicephalus bursa* Can. and Fanz. as present in Curaçao, Cuba, Haiti, and Vera Cruz. This species, by its greater size and by other features, is readily separated from *R. sanguineus* and it is surprising that *bursa* has not been reported again by collectors. *R. bursa* is present in many countries in the Mediterranean area, and includes among its hosts cow, horse, sheep, hog, and dog. Such a tick could have been introduced easily into the New World by the shipment of domestic animals. However, because of the absence of specimens and the lack of additional evidence of its occurrence in the Western Hemisphere, the species is not included in this paper.

## **RHIPICEPHALUS SANGUINEUS (LATREILLE), 1806**

FIGURES 6 AND 7

1806. *Ixodes sanguineus* Latreille, p. 157. Original description.  
1844. *Rhipicephalus sanguineus* (Latreille): Koch, pp. 238-239.  
1873. *Rhipicephalus stigmaticus* Gerstaecker, p. 496.  
1897. *Rhipicephalus sanguineus* (Latreille): Neumann, p. 385. Description and synonymy.  
1908. *Rhipicephalus texanus* Banks, p. 34.  
1911. *Rhipicephalus sanguineus* (Latreille): Neumann, p. 35. Synonymy, hosts, and distribution.  
1912. *Rhipicephalus sanguineus* (Latreille): Hooker et al., p. 102. Description, hosts, distribution.

### **FEMALE**

**Body.** Length (unfed) tips of scapulae to posterior margin, from 2.40 to 2.70; width, 1.44 to 1.68. Either elliptical or oval; when oval, wider behind. Scutum occupying about half the length of the body. Lateral profile lines of postscutal area diverging from the scutum opposite the eyes. Median and posterolateral grooves usually present and conspicuous. Marginal grooves distinct. Engorged specimens may become as large as 11.50 long by 7.50 wide and are oval; slightly wider, and a little thicker dorsoventrally behind.

**Capitulum.** Length from tips of palpi to posterior corners, 0.62 to 0.68. Extreme width of basis, 0.77 to 0.79. Posterior margin of basis nearly straight, salient. Cornua as salient corners. Porose areas small, elliptical, depressed, with their longer axes longitudinal. Surface smooth, shining; with a few punctations; hairs absent. Palpal article 1 visible as a small sharp point on the inner side. (Seen better if the palpi are separated. Often obscure.)

In ventral view, basis smooth, shining, impunctate; posterior margin not salient, with faint sclerotized posterolateral corners. Palpal article 1 with a subtriangular plate on the inner side. Inner edge of article 1 with conspicuous, curved, parallel hairs which are irregularly branched; and on 2, similar hairs on the inner edge; 3 with two or three fine, simple hairs.

**Hypostome.** Clavate, long, with a distinct corona. Dentition 3/3. All denticles about equal in size, with 9 or 10 in each file. Length, about 0.39.

**Scutum.** Length, from 1.14 to 1.44; width, from 1.02 to 1.14. Oval, broad and broadly rounded behind. Scapulae long, rounded. Eyes large, oval, mildly convex; situated at about the widest part of the scutum. Cervical grooves short, deepest in front, and fading out posteriorly. Lateral carinae faint. Punctations, large and small, more numerous in median



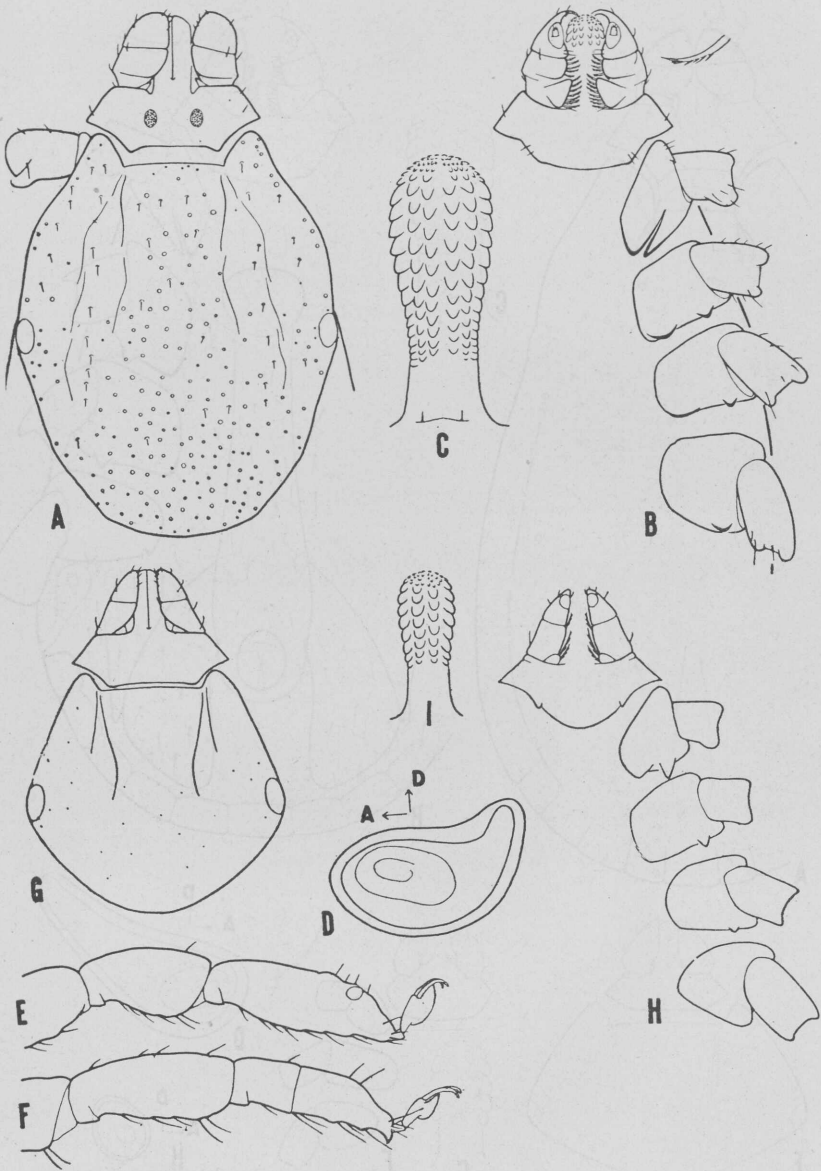


FIGURE 6. *Rhipicephalus sanguineus* (Latreille). A. Female capitulum and scutum, dorsum. B. Female capitulum and coxae, venter, and palpal hair enlarged. C. Female hypostome. D. Female spiracular plate. E. Female metatarsus and tarsus, leg I. F. Female metatarsus and tarsus, leg IV. G. Nymph capitulum and scutum, dorsum. H. Nymph capitulum and coxae, venter. I. Nymph hypostome.

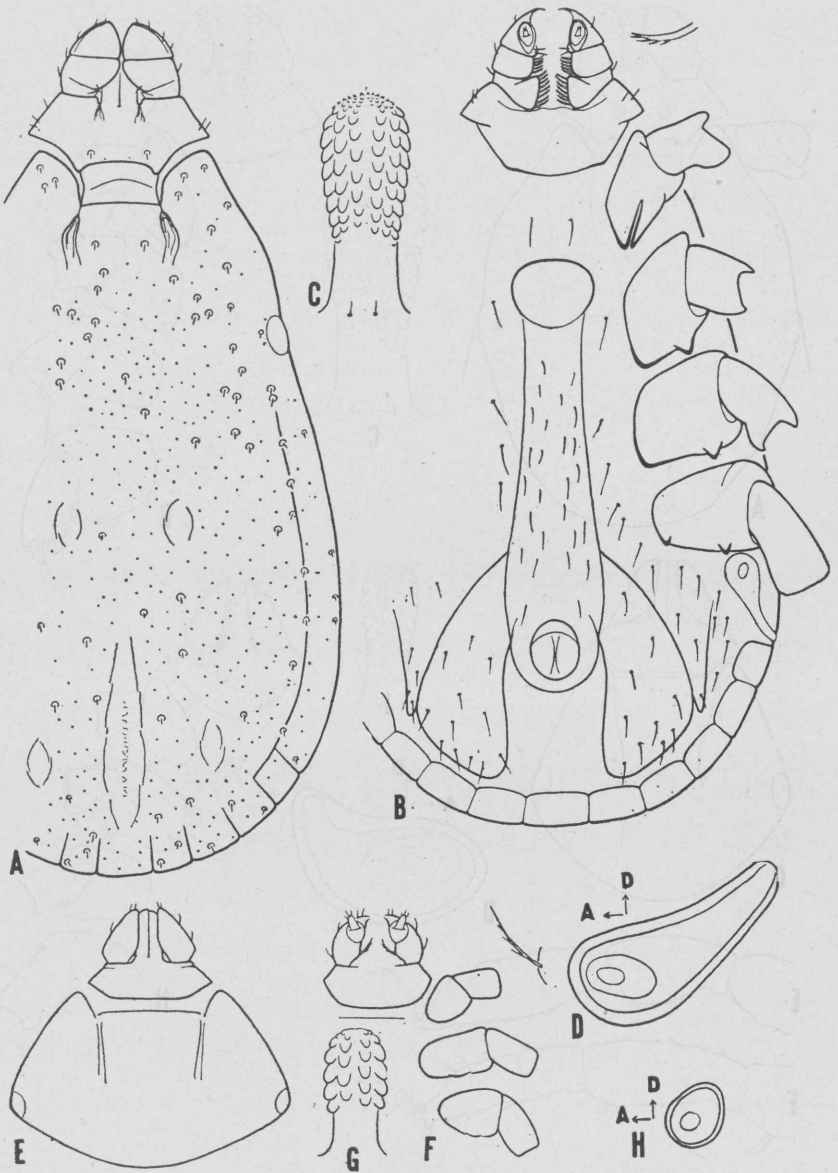


FIGURE 7. *Rhipicephalus sanguineus* (Latreille). A. Male capitulum and scutum, dorsum. B. Male capitulum, coxae, and shields, venter, and palpal hair enlarged. C. Male hypostome. D. Male spiracular plate. E. Larva capitulum and scutum, dorsum. F. Larva capitulum and coxae, venter, and palpal hair enlarged. G. Larva hypostome. H. Nymph spiracular plate.

and posterior areas. Short, fine hairs arising from the punctations in moderate numbers.

**Legs.** Long, moderately thin. Tarsi II, III, and IV, each with a terminal and a subterminal ventral spur, the latter shorter than in the male. Length of tarsus I, 0.60; metatarsus, 0.42. Length of tarsus IV, 0.54; metatarsus, 0.51.

**Coxae.** Coxa I deeply cleft, the two spurs close together, internal spur wider, Coxae II, III, and IV with short, rounded, external spurs, diminishing in size posteriorly; internal spurs on II, III, and IV as broad, short, saliences. Hairs on coxae few, long.

**Spiracular plate.** Short, comma-shaped.

**Genital aperture.** Situated between coxae II.

#### MALE

**Body.** Length, from tips of palpi to posterior margin, 2.28 to 3.18; width, 1.11 to 1.68. Oval, long, widest back of the middle.

**Capitulum.** Length, tips of palpi to posterolateral corners, 0.48 to 0.60; width of basis, 0.49 to 0.69. Posterior margin convex, salient, more convex than in the female. Palpal article 1, in dorsal view, with a small sharp point on the inner side as in the female. In ventral view, palpal article 1 with a distinct plate as in the female. Inner edges of 1, 2, and 3 with hairs as in the female.

**Hypostome.** Much as in the female but smaller. Seven or eight denticles in each file. Length about 0.30.

**Scutum.** Length, 1.86 to 2.70; width, 0.84 to 1.68. Scapulae long, blunt. Surface convex and increasingly declivitous on lateral and posterior marginal areas. Cervical grooves short, deep. Lateral grooves starting at the eyes and terminating after crossing two festoons. Festoons about as long as wide. Eyes oval, mildly convex. Punctations small, intermingled with large pits, each with a fine short hair.

**Legs.** Progressively larger (heavier) posteriorly, otherwise essentially as in the female. Tarsi II, III, and IV with a terminal ventral spur and a subterminal ventral spur, the latter longer than in the female. Length of tarsus I, 0.57; metatarsus, 0.39. Length of tarsus IV, 0.51; metatarsus, 0.54.

**Coxae.** As in the female, excepting that II and III have salient inner corners, and IV a small, short, inner spur.

**Spiracular plate.** Elongate, comma-shaped.

**Genital aperture.** Situated between the anterior portions of coxae II.

#### NYMPH

**Body.** Unengorged. Length, tip of palpi to posterior margin, from 1.14 to 1.30; width, from 0.57 to 0.66. Oval, widest at about the middle.

Scutum occupying half the length of the body. Lateral profile lines of postscutal area diverging at the eyes. Fестоons present. Engorged nymphs broad oval and measure 3.5 by 2.15.

**Capitulum.** Length, tip of palpi to posterior margin from 0.20 to 0.23; width of basis, from 0.28 to 0.30. Surfaces of basis and palpi smooth, impunctate; hairs absent. In dorsal view, palpal article 1 not visible; in ventral view visible as a rounded lobe on the inner side which bears two long hairs, branched on one side. Article 2 with one similar branched hair.

**Hypostome.** Clavate, small. Dentition 2/2 with six or seven teeth in each file. Length, 0.125.

**Scutum.** Length, 0.42 to 0.51; width, 0.45 to 0.48. Short and broad, anterolateral sides nearly straight, posterior side broadly rounded. Eyes large, oval, mildly convex. Cervical grooves deep, short. Surface faintly crazed, impunctate; hairs very few, short.

**Legs.** Much as in the adults but lacking ventral spurs on all tarsi.

**Coxae.** Coxa I with two short spurs widely separated, the external spur larger. II and III with very short, rounded, external spurs. IV with no spurs.

**Spiracular plate.** Oval, short; greatest length, 0.01.

#### LARVA

**Body.** Total length from tips of palpi to posterior margin, 0.54; width, 0.39. Broad oval, wider behind. Scutum occupying about one-third the length of body. With festoons as in nymphs and adults. When fully fed, larvae may measure at least 1.20 by 0.78.

**Capitulum.** Length from tips of palpi to posterior margin of basis, 0.11; width of basis, 0.14. Basis pointed at the sides. Cornua absent. Palpal article 2 (venter) with one long hair which is branched on one side.

Hypostome with dentition 2/2, four or five denticles in each file. Length, about 0.048.

**Scutum.** Length, 0.21; width, 0.33. Eyes on the lateral corners, large. Cervical grooves shallow, moderately long, nearly parallel. A very few faint punctations present; hairs absent.

#### HOSTS AND DISTRIBUTION

*Rhipicephalus sanguineus*, probably the most widely distributed species of tick in the world, was described by Latreille from "Gallia," and early authors record it from the Mediterranean area. It has been dispersed widely and is now present in all, or nearly all, countries lying between about 40° N. and 40° S. Dogs are the principal hosts. Since the tick was present before the coming of modern man, it seems probable that the host in primitive time was some animal of canine lineage.

In the New World, this tick is present in South America, Central America, and Mexico and is spreading rapidly in the United States. So far as we know, it does not occur in Canada. The spot map given by Bishopp and Trembley (1945, p. 47) shows it in the following States: South Carolina, Georgia, Florida, Mississippi, Louisiana, Texas, Minnesota, Wisconsin, Michigan, Illinois, Indiana, Ohio, Missouri, Maine, Vermont, Massachusetts, Connecticut, New York, New Jersey, Pennsylvania, Delaware, Maryland, West Virginia, Virginia, North Carolina, Kentucky, Tennessee, Nebraska, Colorado, Kansas, Oklahoma, Washington, California and Arizona.

The Rocky Mountain Laboratory has records from many of the same States, which in some cases show the ticks to be somewhat more prevalent than is indicated on the spot map. The records show clearly that since the first mention of this tick in the United States by Banks in 1908, the tick has been found in the greater part of the United States, and it may be assumed that it will become more prevalent in future years.

Humans are infrequently attacked by *R. sanguineus*; but when dogs and their bedding places are in close association with man, bites become more frequent. In recent years, specimens of this tick from houses and apartments have been sent to the Rocky Mountain Laboratory for identification.

Neumann (1911, p. 36) in summarizing the known hosts gives a list, not including man and dogs, as follows:

*Canis vulpes* L., *C. aureus* L., *C. anthus* F. Cuv., *C. variegatus* Rüpp., *C. megalotis* Desm., *C. persicus* Blanford, *Viverra genetia* L., *Lynx* sp.?, *Felis domestica* Briss., *F. leo* L., *Lepus timidus* L., *L. aethiopicus* H. E., *Erinaceus europaeus* L., *E. niger* Blanford, *E. pictus* Stol., *E. collaris* Gray, *Equus caballus* L., *Camelus dromedarius* L., *C. bactrianus* L., *Gazella dorcas* (L.), *Colus ellipsiprymnus* Ogilb. & H. Sm., *Ovis aries* L., *Capra hircus* L., *Bos taurus* L., *Buffelus indicus* Rüt., *Manis* sp., *Struthio massaicus* Neum., *Strix ascalaphus* Sav., *Theristicus leucocephalus* (Gm.) [*Ibis hagedash*], *Testudo mauritanica* D. B.

Bedford (1932, p. 298) gives a list from Howard (1908) which adds to the foregoing as follows: lion, genet, hare, pangolin, and waterbuck, and adds apparently from his own records, *Raphiceros campestris* and *Lepus campestris*.

Bishopp and Trembley (1945, p. 47) add ass and rabbit as hosts.

From the Rocky Mountain Laboratory are to be added as hosts: deer (Texas and La Labor, Sinaloa, Mexico), mule (Colombia, South America), jack rabbit (Texas), *Rombomys opimus* (Russia), and javelina (Los Pozos, Mexico).



## GENUS *HAEMAPHYSALIS* KOCH, 1844

1844. *Haemaphysalis* Koch, p. 237. Original description.  
 1844. *Rhipistoma* Koch, p. 239. (Neumann 1911, p. 105, makes *Rhipistoma* a synonym.)  
 1882. *Pseudixodes* Haller, p. 311. Not this genus as stated by Canestrini 1890, pp. 485-526.  
 1888. *Gonixodes* Alf. Dugès, p. 129.  
 1890. *Herpetobia* Canestrini, pp. 486, 493, 527.  
 1897<sup>(1)</sup> *Opisthodon* Canestrini, p. 468.  
 1897<sup>(2)</sup> *Prospodon* Canestrini, p. 417.  
 1911. *Haemaphysalis* Koch: Neumann, p. 105. Description and synonymy.  
 1911. *Haemaphysalis* Koch: Nuttall and Warburton, p. 119.  
 1915. *Haemaphysalis* Koch: Nuttall and Warburton, p. 349, extended synonymy.  
 1945. *Haemaphysalis* Koch: Mackie et al., p. 536.

Inornate, without eyes, but with festoons. Anal grooves present. Usually of small size. Sexual dimorphism slight. Palpi short, usually conical with article 2 extending laterally beyond the basis capituli. Basis subrectangular. Male with no ventral plates or shields. Coxa I never bifid or deeply divided on posterior side. Spiracular plates usually oval or comma-shaped in the male; oval or rounded in the female.

**Genotype:** *Haemaphysalis concinna* Koch.

### KEY TO FEMALES AND MALES

1. Palpal article 3 with a very short retrograde ventral spur..... 2  
    Palpal article 3 with a long retrograde ventral spur..... 3
2. Ventral cornua present..... *leporis-palustris*  
    Ventral cornua absent..... *chordeilis*
3. Hypostome with dentition 5/5..... *kochi*  
    Hypostome with dentition 4/4..... *juxtakochi*

### KEY TO NYMPHS

Nymphs of *kochi* and *juxtakochi* are unknown.

- Basis not pointed at the sides..... *leporis-palustris*  
 Basis pointed at the sides..... *chordeilis*

## HAEMAPHYSALIS LEPORIS-PALUSTRIS (PACKARD), 1869

### FIGURES 8 AND 9

1869. *Ixodes leporis-palustris* Packard, p. 67. Original description. (Not *Ixodes chordeilis* Packard 1869 as stated by Neumann, 1897, p. 343.)
1896. *Rhipistoma leporis* Osborn, p. 261.
1888. *Gonixodes rostralis* Dugès, p. 129. (See also Nuttall and Warburton, 1915, p. 387.)
1897. *Haemaphysalis leporis* (Packard): Neumann, p. 343.
1901. *Haemaphysalis leporis* (Packard): Neumann, p. 262.
1905. *Haemaphysalis leporis* (Packard): Lahille, p. 45.
1907. *Haemaphysalis leporis-palustris* (Packard): Hunter and Hooker, pp. 53, 54. Distribution, biology, hosts.
1908. *Haemaphysalis leporis-palustris* (Packard): Banks, pp. 33-54. Redescription, distribution.
1908. *Haemaphysalis leporis-palustris* (Packard): Hooker, pp. 47-48. Biology.
1909. *Haemaphysalis leporis-palustris* (Packard): Hooker, p. 423. Geographical distribution.
1909. *Haemaphysalis proxima* Aragão, pp. 100-101. *Nomen nudum*. (Not to be confused with *Haemaphysalis proxima* Warburton and Nuttall which = *H. corniger* Neumann, 1897.)
1911. *Haemaphysalis leporis-palustris* (Packard): Hunter and Bishopp, pp. 228-229. Distribution, hosts, biology.
1911. *Haemaphysalis leporis* var. *proxima* Aragão, p. 167. Synonym.
1912. *Haemaphysalis leporis-palustris* (Packard): Hooker et al., pp. 89-96. Distribution, biology, rearing.
1913. *Haemaphysalis leporis-palustris* (Packard): Hadwen, p. 97.
1915. *Haemaphysalis leporis-palustris* (Packard): Nuttall and Warburton, pp. 387-394. Descriptions, synonymy, hosts, and distribution.
1938. *Haemaphysalis leporis-palustris* (Packard): Hearle, p. 100.
1941. *Haemaphysalis proxima* Aragão: Osorno-Mesa, p. 428.
1943. *Haemaphysalis leporis-palustris* (Packard): Fairchild, p. 586.

### FEMALE

**Body.** Length from tips of palpi to posterior margin from 1.71 to 2.58; width from 0.90 to 1.50. Oval, widest back of the middle. Scutum occupying half the length of the body. Marginal groove deep, and continuous across all festoons. Hairs absent on postscutal areas. Fully engorged females become as large as 10.00 by 6.90.

**Capitulum.** Length from tips of palpi to posterior margin from 0.48 to 0.57; length of basis 0.18; width of basis from 0.42 to 0.58. Sides of basis convex, mildly converging posteriorly. Cornua present or absent; when well-developed, rounded or bluntly pointed and about as long as the width at the base. Posterior margin nearly straight, salient. Surface flat or faintly concave. Porose areas oval, placed far apart, mildly depressed,

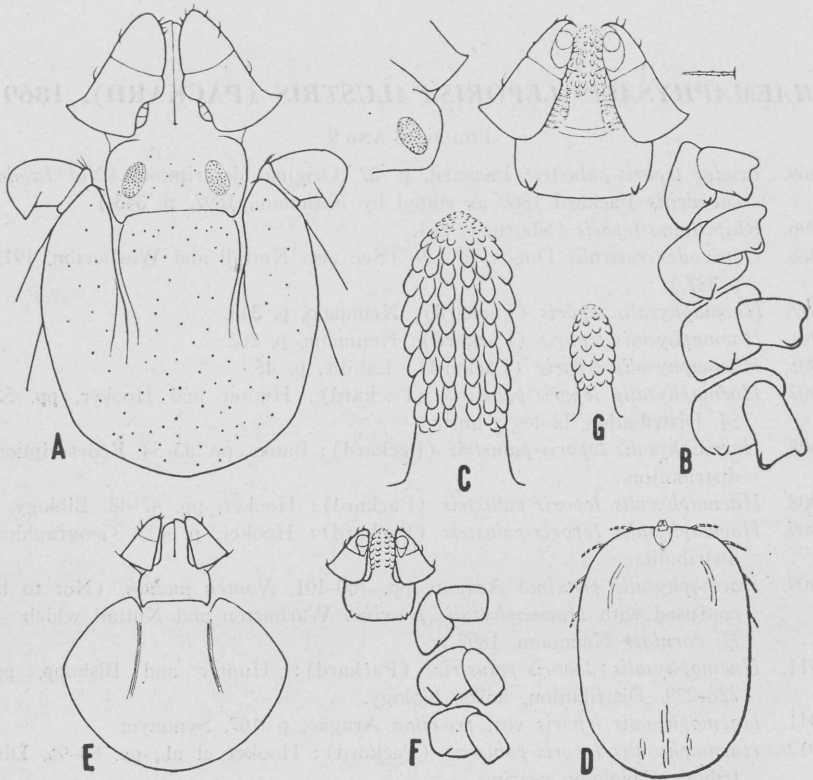


FIGURE 8. *Haemaphysalis leporis-palustris* (Packard). A. Female capitulum and scutum, dorsum. B. Female capitulum and coxae, venter, and palpal hair enlarged. C. Female hypostome. D. Engorged female. E. Larva capitulum and scutum, dorsum. F. Larva capitulum and coxae, venter. G. Larva hypostome.

their longer axes converging anteriorly. Surface of palpi smooth, shining, impunctate. Posterior margin of article 2 more reflexed than in *chordeilis*.

In ventral view, basis with small cornua on the broadly rounded posterior margin. Palpal article 2 fringed on the inner side with parallel, flat, mildly barbed hairs, which are directed toward the hypostome. Article 3, with a short retrograde spur and one or two tapering hairs on the inner edge.

**Hypostome.** Clavate, with a small corona. Dentition 3/3. Denticles about equal in size and eight in number in each file. Length, about 0.30.

**Scutum.** Length, 0.78 to 0.84; width, 0.66 to 0.75. Oval, widest in front of the middle. Scapulae large, rounded. Cervical grooves conspicuous, deep, long. Lateral carinae absent. Punctations numerous, evenly distributed.

**Legs.** Moderate in length and size. Trochanter I with a large, pointed dorsal spur; absent on II, III, and IV. All trochanters with very small

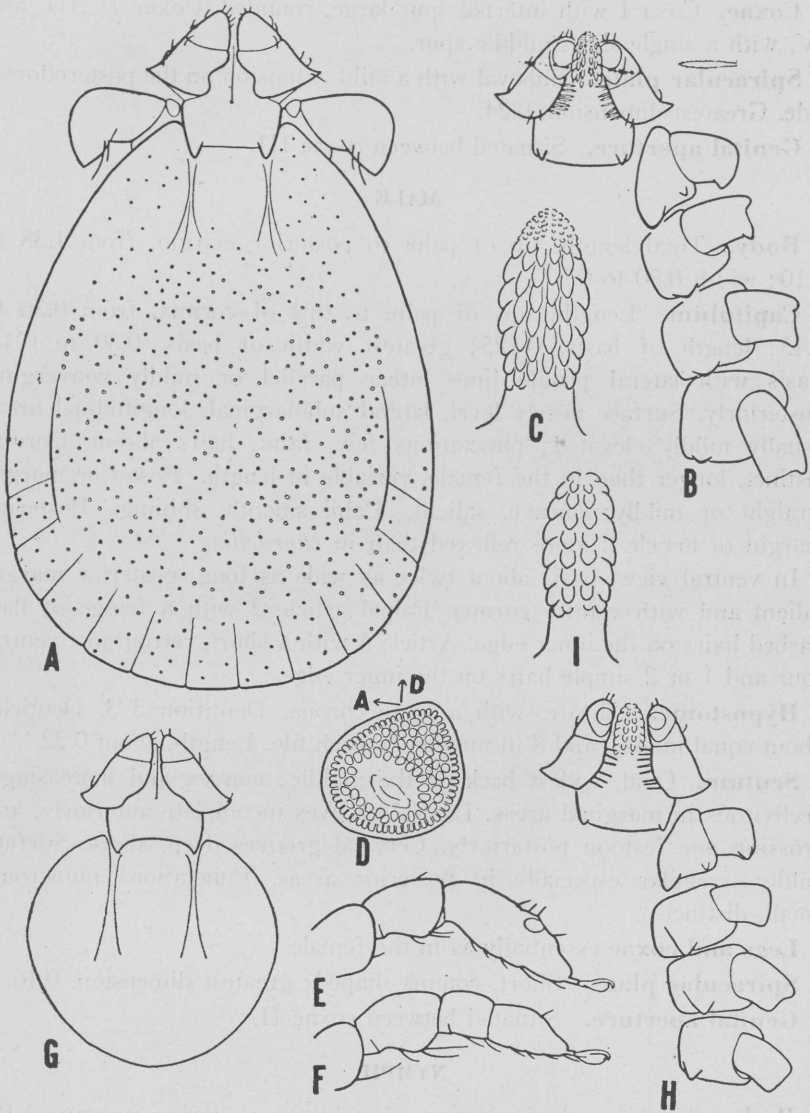


FIGURE 9. *Haemaphysalis leporis-palustris* (Packard). A. Male capitulum and scutum, dorsum. B. Capitulum and coxae, venter, and palpal hair enlarged. C. Male hypostome. D. Male spiracular plate. E. Male metatarsus and tarsus, leg I. F. Male metatarsus and tarsus, leg IV. G. Nymph capitulum and scutum, dorsum. H. Nymph capitulum and coxae, venter. I. Nymph hypostome.

ventral spurs. Terminal ventral spurs absent on all tarsi. Length of tarsus I, 0.39; metatarsus, 0.27. Length of tarsus IV, 0.36; metatarsus, 0.27.

**Coxae.** Coxa I with internal spur large, rounded. Coxae II, III, and IV, with a single short middle spur.

**Spiracular plate.** Suboval with a mild extension on the posterodorsal side. Greatest dimension, 0.24.

**Genital aperture.** Situated between coxae III.

#### MALE

**Body.** Total length, tip of palpi to posterior margin, from 1.38 to 2.10; width 0.80 to 0.90.

**Capitulum.** Length, tips of palpi to tips of cornua, from 0.36 to 0.45; length of basis, 0.125; greatest width of basis, 0.30 to 0.45. Basis with lateral profile lines either parallel or mildly converging posteriorly. Surface nearly level, lateral submarginal, longitudinal areas usually mildly elevated; punctations few, faint, hairs absent. Cornua distinct, longer than in the female, variable in length. Posterior margin straight or mildly concave, salient. Palpi smooth, shining. Posterior margin of article 2 more reflexed than in *chordeilis*.

In ventral view, basis about twice as wide as long, posterior margin salient and with ventral cornua. Palpal article 2 with a fringe of flat-barbed hairs on the inner edge. Article 3 with a short, retrograde, ventral spur and 1 or 2 simple hairs on the inner edge.

**Hypostome.** Clavate, with a large corona. Dentition 3/3. Denticles about equal in size, and 8 in number in each file. Length, about 0.22.

**Scutum.** Oval, widest back of the middle; convex and increasingly declivitous in marginal areas. Lateral grooves incomplete anteriorly, and crossing one festoon posteriorly. Cervical grooves deep, short. Surface mildly irregular especially in posterior areas. Punctations numerous, small, distinct.

**Legs and coxae** essentially as in the female.

**Spiracular plate.** Short, comma-shaped; greatest dimension, 0.16.

**Genital aperture.** Situated between coxae II.

#### NYMPH

**Body.** Total length from tips of palpi to posterior margin, 1.18; width, 0.84. When unfed, oval, widest back of the middle. Scutum occupying less than half the length. On the dorsum with festoons, lateral, median, and posterolateral grooves, and on the venter with anal, median, postanal and genital grooves resembling the same in the adults. Engorged specimens may reach a total length of 2.25.

**Capitulum.** Length, tips of palpi to tips of cornua, 0.29; width of basis, 0.21. Basis quadrate, sides mildly convergent posteriorly, posterior margin straight, salient. Cornua distinct, triangular, about as long as wide. Surface flattened, impunctate, hairs absent. Palpi much as in adults



on both surfaces but on the venter, article 2 with four to eight simple hairs on the inner edge. Basis (venter) with distinct cornua on the posterior corners as in adults.

**Hypostome.** Clavate, dentition 2/2. Lateral and median denticles about equal, seven to eight in each file. Length, about 0.014.

**Scutum.** Length, 0.45; width, 0.48. Nearly circular, slightly wider than long. Cervical grooves distinct, moderately deep, nearly parallel. Impunctate, hairs absent.

**Legs.** Trochanter I with dorsal and ventral spurs as in adults.

**Coxae.** Essentially as in the female.

**Spiracular plates.** Essentially as in the adults. Greatest dimension, 0.16.

### LARVA

**Body.** Length, tip of palpi to posterior margin, 0.66; width, 0.45. Broad, oval. Scutum occupying about two-fifths of the length of the body. Festoons and dorsal spurs on trochanters present as in adults and nymphs.

**Capitulum.** Length, tip of palpi to posterior margin, 0.168; width of basis, 0.134. Capitulum throughout much as in the nymph.

**Hypostome.** Dentition 2/2, with six teeth in each file; length about 0.075.

**Scutum.** Length, 0.25; width, 0.30. Cervical grooves long, moderately deep. Impunctate, hairs absent.

**Coxae.** Coxa I with a salient lobe on the inner side; II and III each with a relatively large spur.

*H. leporis-palustris* may be separated from *H. chordeilis* as follows: the former is usually found on rabbits, while the latter is found mostly on birds. Adults, nymphs, and larvae of *leporis-palustris* have ventral cornua which are lacking in *chordeilis*. Males have a short internal spur on coxa IV in *leporis-palustris*, while this spur is very long in *chordeilis*. The nymph of *leporis-palustris* has the basis capituli quadrangular in dorsal view, while in *chordeilis* it is hexagonal.

### HOSTS AND DISTRIBUTION

*Haemaphysalis leporis-palustris* was described by Packard in 1869 from *Lepus palustris*, Fort Macon, N. C. The type is in the Museum of Comparative Zoology, Harvard University, Cambridge, Mass. This tick has been recorded from Alaska, Canada, all the United States, and from Mexico. Dunn (1923) reported it on domestic rabbits and *Dasyprocta* in Panama, and Aragão (1938, p. 321) records it (as *H. leporis*) on *Sylvilagus* sp. in Argentina. Valadez (1923, p. 204) records it from Tampico and Vera Cruz, Mexico. Published records do not show the extent of distribution of this tick in Mexico.

*H. leporis-palustris* is common on the various species and subspecies

of *Lepus* and *Sylvilagus* as they occur throughout the United States and is also found on a wide variety of hosts, including birds and small mammals. It is probable that the other rabbits, small mammals, and birds not yet recorded as hosts may be used when available.

Hosts other than rabbits are as follows: Neumann (1911, p. 111) lists *Equus caballus* and *Paradoxurus* sp. Bishopp and Trembley (1945) in bringing the records of the Bureau of Entomology up to date add many new birds and small mammals, a total of 85 records, which need not be repeated here; 65 of these records are from birds.

From Canada, Hadwen (1912, 1914) gives the following localities for this species: Awame, Manitoba; Prince Albert, Saskatchewan; Mount Lehman, Agassiz, Nelson, and Peardonville, British Columbia. Hewitt (1915, p. 230) reported the tick from Awame, Manitoba, and Jeffrey Corner, Kings Co., New Brunswick.

The Rocky Mountain Laboratory has accumulated over 300 records, many of which conform to those already published. The following new records may be added: white-throated sparrow (Arkansas), Gambel's quail (Arizona), ruffed grouse (Montana), magpie (Idaho), sage hen (Idaho), and eagle (Colorado).

## HAEMAPHYSALIS CHORDEILIS (PACKARD), 1869

FIGURES 10 AND 11

1869. *Ixodes chordeilis* Packard, p. 67. Original description.  
1905. *Haemaphysalis punctata* var. *cinnabarina* Koch: Neumann, p. 237.  
1908. *Haemaphysalis chordeilis* Packard: Banks, p. 34.  
1911. *Haemaphysalis punctata cinnabarina* Koch: Neumann, p. 108.  
1912. *Haemaphysalis chordeilis* (Packard): Hooker et al., p. 97.  
1915. *Haemaphysalis cinnabarina* Koch: Nuttall and Warburton, p. 372 (in part).

### FEMALE

**Body.** Unengorged females are not available. Engorged specimens become as large as 9.5 by 8.25 and are broad oval, and equally thick at both ends.

**Capitulum.** Length, tips of palpi to posterior margin, from 0.60 to 0.66; length of basis, from 0.24 to 0.27; width of basis from 0.48 to 0.54. Sides of basis convex. Surface nearly flat, with a few punctations. Cornua faint or absent; posterior margin nearly straight or faintly sinuous, salient.

In ventral view, basis is quadrate, convex; ventral cornua absent. Porose areas large, depressed, oval, with their longer axes parallel or faintly converging posteriorly. Palpal article 2 with posterior edge less reflexed than in *leporis-palustris* and with a row of flat, mildly barbed hairs on the inner edge, overlapping the hypostome; article 3 with a short, ventral, retrograde spur and two or three fine, tapering hairs on the inner edge.

**Hypostome.** Clavate, with a small corona. Dentition 5/5, with principal denticles progressively smaller from laterals to the middle files and with 12 to 14 teeth in each file. Length, about 0.36.

**Scutum.** Length, 1.26 to 1.32; width, 1.11 to 1.14. Oval, widest in front of the middle, with some tendency to have posterolateral corners. Scapulae large, bluntly pointed. Cervical grooves conspicuous, deep, long. Lateral carinae absent. Punctations numerous, evenly distributed.

**Legs.** Moderate in length and size. Trochanteral dorsal spur large, pointed on I; faint or absent on II, III, and IV. Ventral trochanteral spurs absent on all legs. Small terminal ventral spurs present on all tarsi. Length of tarsus I, 0.60; metatarsus, 0.39. Length of tarsus IV, 0.48; metatarsus, 0.39.

**Coxae.** Coxa I with internal spur moderately long. Coxae II and III with distinct short spurs placed near the middle of the posterior side; IV with a longer spur placed near the inner side.

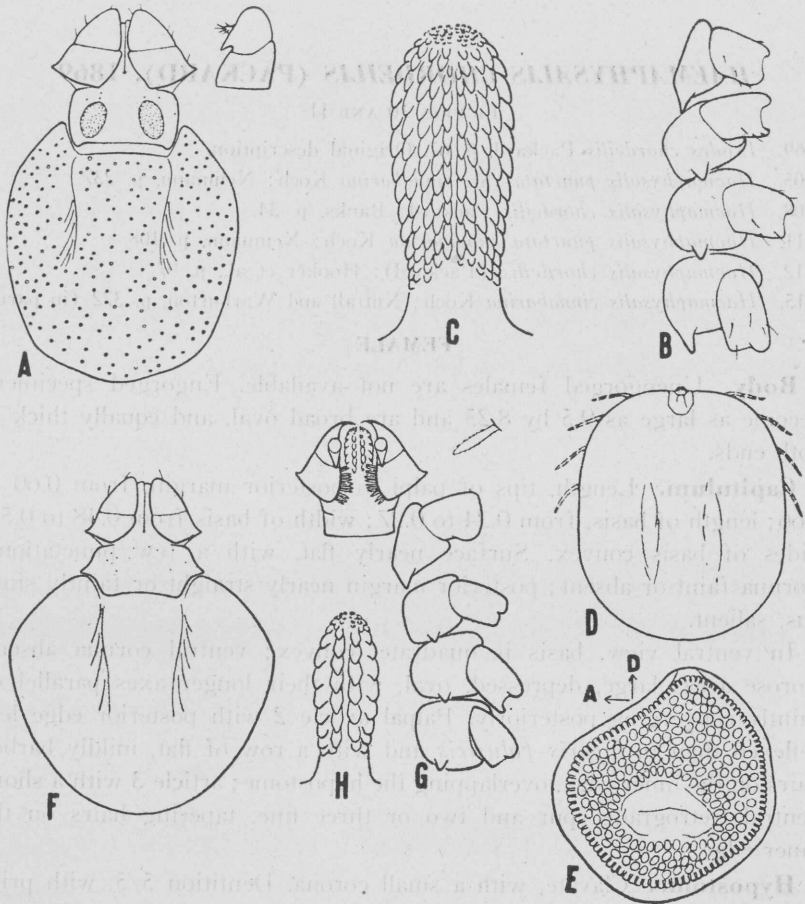


FIGURE 10. *Haemaphysalis chordeilis* (Packard). A. Female capitulum and scutum, dorsum, and side view of palpus. B. Female coxae, venter. C. Female hypostome. D. Engorged female. E. Female spiracular plate. F. Nymph capitulum and scutum, dorsum. G. Nymph capitulum and coxae, venter, and palpal hair enlarged. H. Nymph hypostome.

**Spiracular plate.** Suboval with a mild, blunt extension on the posterodorsal side. Greatest dimension, 0.36.

**Genital aperture.** Opposite the intervals between coxae I and II.

#### MALE

**Body.** Total length, tips of palpi to posterior margin, 2.20 to 2.80; width, 1.26 to 1.50. Oval, widest back of the middle.

**Capitulum.** Length, tip of palpi to tips of cornua, from 0.42 to 0.49; length of basis, from 0.20 to 0.21; width of basis, from 0.30 to 0.36. Sides of basis nearly straight. Surface nearly flat, with a few puncta-

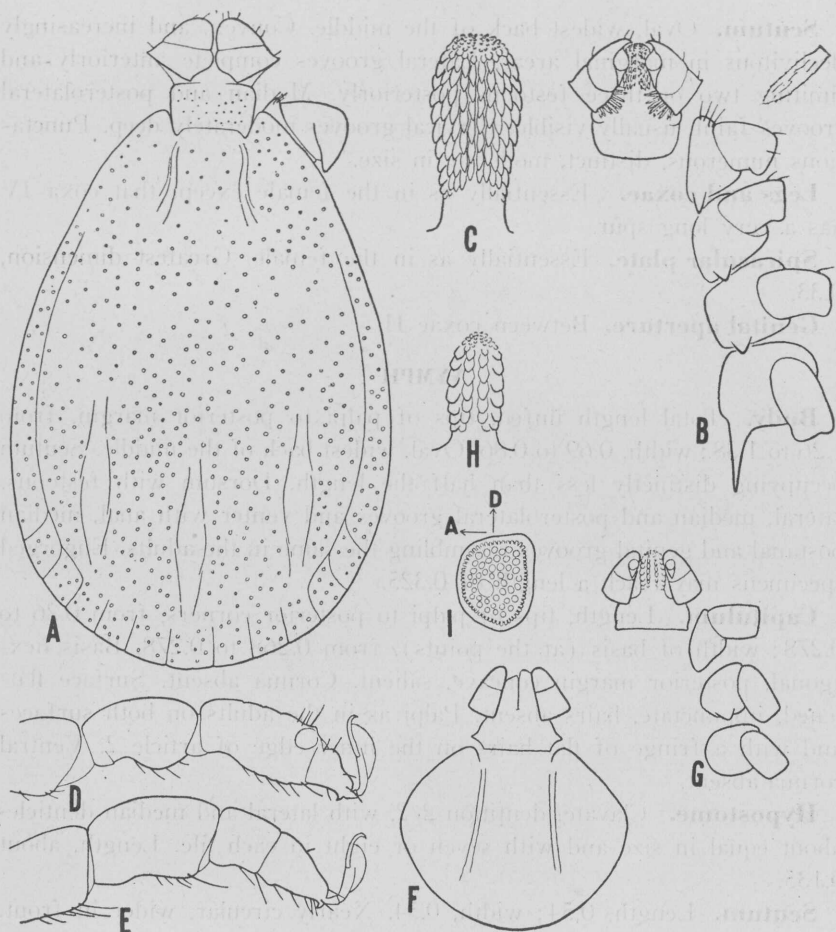


FIGURE 11. *Haemaphysalis chordeilis* (Packard). A. Male capitulum and scutum, dorsum. B. Male capitulum and coxae, venter, and palpal hair enlarged. C. Male hypostome. D. Male metatarsus and tarsus, leg I. E. Male metatarsus and tarsus, leg IV. F. Larva capitulum and scutum, dorsum. G. Larva capitulum and coxae, venter. H. Larva hypostome. I. Nymph spiracular plate.

tions. Cornua distinct, longer than in the female. Posterior margin concave, salient. Posterior edge of palpal article 2 less reflexed than in *leporis-palustris*.

In ventral view, basis about twice as wide as long, nearly flat, posterior edge salient. Palpal article 2 with a fringe of mildly barbed, flat hairs which overlap the hypostome. Article 3 with a retrograde ventral spur and one or two smooth tapering hairs on the inner edge.

**Hypostome.** Clavate, with a small corona. Denticles 5/5. Principal denticles all of about equal size and with 8 to 10 in each file. Length, about 0.24.



**Scutum.** Oval, widest back of the middle. Convex, and increasingly declivitous in marginal areas. Lateral grooves complete anteriorly and limiting two or three festoons posteriorly. Median and posterolateral grooves faint, usually visible. Cervical grooves moderately deep. Punctations numerous, distinct, moderate in size.

**Legs and coxae.** Essentially as in the female except that coxa IV has a very long spur.

**Spiracular plate.** Essentially as in the female. Greatest dimension, 0.33.

**Genital aperture.** Between coxae II.

#### NYMPH

**Body.** Total length unfed, tips of palpi to posterior margin, from 1.26 to 1.38; width, 0.69 to 0.86. Oval, widest back of the middle. Scutum occupying distinctly less than half the length. Dorsum with festoons, lateral, median and posterolateral grooves and venter with anal, median postanal and genital grooves resembling the same in the adults. Engorged specimens may reach a length of 0.325.

**Capitulum.** Length, tips of palpi to posterior corners, from 0.26 to 0.278; width of basis (at the points), from 0.268 to 0.278. Basis hexagonal, posterior margin concave, salient. Cornua absent. Surface flattened, impunctate, hairs absent. Palpi as in the adults on both surfaces and with a fringe of flat hairs on the inner edge of article 2. Ventral cornua absent.

**Hypostome.** Clavate, dentition 2/2, with lateral and median denticles about equal in size and with seven or eight in each file. Length, about 0.135.

**Scutum.** Length, 0.54; width, 0.54. Nearly circular, wider in front. Cervical grooves distinct, moderately deep, long. Surface crazed, impunctate, and without hairs.

**Legs.** Trochanter I with a distinct dorsal spur as in the adults. Ventral trochantal spurs absent.

**Coxae.** Essentially as in the female.

**Spiracular plate.** Suboval with longer axis transverse. Greatest dimension, 0.16.

#### LARVA

**Body.** Total length, tips of palpi to posterior margin, 0.63; width, 0.42. Broad oval. Scutum occupying one-third the length of the body. Faint festoons and dorsal trochantal spur present.

**Capitulum.** Length, tip of palpi to posterior margin, 0.12; width of basis, 0.16. Capitulum throughout much as in the nymph.

**Hypostome.** Dentition 2/2, with six or seven denticles in each file. Length, about 0.072.

**Scutum.** Length, 0.21; width, 0.33. Cervical grooves distinct, long, nearly parallel. Surface crazed, impunctate; hairs absent.

**Coxae.** Coxa I with an internal lobe in place of a definite spur. Coxa II with one short, rounded spur. Coxa III with no spurs.

For a comparison of *chordeilis* and *leporis-palustris*, see page 35.

Banks (1908, p. 34) accepted the name *chordeilis* Packard for this *Haemaphysalis* found on birds in the United States, after he had examined the specimens labeled as types, which consist of two engorged females taken in Massachusetts.

The writer has had the opportunity to examine the two types in the Museum of Comparative Zoology in Harvard University, mentioned by previous writers, and it is evident that they represent the common tick on birds that has been called both *chordeilis* and *cinnabarina* in this country. However, Packard, in describing the species in 1869, mentions only one. He says, "From the nighthawk (*Chordeilis popetue*), Mass. (Coll. F. G. Sanborn)." The "types" are in two vials, and one contains two labels reading, "*Ixodes chordeilis* Pack." (probably in Packard's handwriting), and "from *Chordeilis popetue*, F.G.S., Mass." The second vial contains a label in different handwriting reading, "*Ixodes chordeilis* Pack. E. A. Samuels, Milton, Mass." Note that "E. A. Samuels" is not "F. G. Sanborn" mentioned in describing the species. Thus, there is only one true type, and it came from Massachusetts and from the night-hawk. "Milton, Mass." should not be considered to be the exact type locality.

Nuttall and Warburton (1915, pp. 372 and 378), without seeing the type of *chordeilis*, adopted *cinnabarina* as the name for this tick. Nuttall studied and made drawings in Berlin of Koch's unique type of *cinnabarina* which came from Brazil (no host given). The authors state, "\*\*\* and after consultation with the late Professor Dönitz concluded that it was closely allied if not identical with *H. punctata* Canestrini and Fanzago. We have since been able, however, to identify it with the American form known as *H. chordeilis* (Packard) which therefore falls into synonymy." The authors had for study some North American specimens of this *Haemaphysalis* as is shown by their listings, but the evidence is not convincing that the North American form is identical with Koch's *cinnabarina* from Brazil.

Since the time *cinnabarina* was published by Koch in 1844, this name has not appeared in listings of ticks from South America and Central America. See Banks (1908), Dunn (1923), Bequaert (1938), Osorno-Mesa (1941), and Fairchild (1943). Aragão (1911, p. 157), does include *cinnabarina* as a species occurring in Brazil, but this probably referred only to the Koch type. This Laboratory has in the past received for identification many ticks from northern South America, Panama, and

Central America, and the author has studied the collections of many of the various institutions of the United States but has never seen specimens of either *cinnabarina* or *chordeilis* from these areas.

For these reasons the name *chordeilis* is being used for the common *Haemaphysalis* on birds in North America.

Bishopp and Trembley (1945, p. 25) indicate that because of the existing confusion regarding *cinnabarina* and *chordeilis*, it is possible that two forms are present in the United States. The present writer is assuming that only one, *chordeilis*, is found in this country.

### HOSTS AND DISTRIBUTION

Packard described *chordeilis* from a nighthawk taken in Massachusetts, and the type, 1 fed female, is in the Museum of Comparative Zoology, Harvard University, Cambridge, Mass.

Banks (1908, p. 34) records a female from turkey, Taftsville, Vt., and a nymph which might be this species from killdeer, Fort Collins, Colo.

Miscellaneous writers have reported this tick in various States as follows: Hadley (1909, p. 306) from Norwich, Vt.; Hooker *et al.* (1912, p. 98) from Victoria, Refugio, and Medina counties, Tex.; and larvae and nymphs thought to be of this species from Grand Cave, La., and from Hawthorne and Quincy, Fla. Hooker (1908, p. 403) stated that the tick occurs in the State of New York, and Nuttall and Warburton (1915, p. 377) reported the species (as "*cinnabarina*") from the Catskill Mountains in the State of New York.

Bishopp and Trembley (1945, p. 25) state that the species occurs in eastern and southern United States and eastern Canada. The States specified are: New Hampshire, Vermont, New York, Pennsylvania, South Carolina, Georgia, Florida, Texas, and Michigan. They state further, Nuttall *et al.* (1915) regard *Haemaphysalis chordeilis* Packard as a synonym of *H. cinnabarina* Koch. We are inclined to think these forms distinct and the records presented herein are based on the restricted application of the name\*\*\*. If the broader interpretation of the species as mentioned above is applied, the western states and western Canada would also be included.

Hewitt (1915, p. 229) reports this tick (as *H. cinnabarina*) at Winnipeg and Awame, Manitoba, on *Pediaecetes phasianellus campestris*, and on rabbit at Kleena Kleena, Tatla Lake, British Columbia.

Hearle (1938, p. 99) records this tick (as *H. cinnabarina*) from Manitoba, Alberta, Saskatchewan, and British Columbia.

The Rocky Mountain Laboratory has one record from Wisconsin on greater prairie chicken (*Tympanuchus cupido americanus*).

The preferred hosts of *H. chordeilis* are various ground birds, but a

few mammals including man have been reported. Hosts have been recorded as follows: Banks (1908, p. 34), turkey; Hewitt (1915, p. 229), *Pediacetes phasianallus campestris*; Nuttall and Warburton (1915, p. 367), cattle, man, meadow lark, jackdaw, red-winged blackbird, marsh hawk, quail, ruffed grouse; Bishopp and Trembley (1945, p. 26), cow, horse, sheep, Florida grasshopper sparrow, Savannah sparrow, Wakulla seaside sparrow, towhee, Florida wren.

Travis (1941, p. 466) reports this tick on the southern meadow lark (*Sturnella magna argutula*) and white-eyed towhee (*Pipilo erythrophthalmus allenii*).

The Rocky Mountain Laboratory has records from Montana on sage hen (*Centrocercus urophasianus*), ruffed grouse (*Bonasa umbellus*), and pocket gopher (*Thomomys sp.*).

This tick has been reported as killing turkeys in various places in the United States.

## HAEMAPHYSALIS KOCHI ARAGÃO, 1908

### FIGURE 12

1908. *Haemaphysalis kochi* Aragão, p. 3-6 (reprint).

1911. *Haemaphysalis kochi* Aragão, pp. 178-181.

1915. *Haemaphysalis kochi* Aragão: Nuttall and Warburton, 413-415.

### FEMALE

**Body.** Total length in unfed specimens from tips of palpi to posterior margin, 2.30 to 2.88; width, 1.68 to 2.04. Oval, widest at about the middle. Scutum occupying about two-fifths the length of the body. Marginal grooves distinct extending from near the scutum to the third festoon. Festoons about as wide as long. Engorged females about as broad as long and may be as large as 6.0 by 6.0.

**Capitulum.** Length from tips of palpi to tips of cornua, 0.72 to 0.80; length of basis from base of palpi to tips of cornua, 0.27 to 0.33; width of basis, 0.57 to 0.60. Lateral profile lines of basis convex, posterior margin nearly straight, salient. Lateral submarginal areas with longitudinal elevations. Cornua distinct, rounded or pointed, about as broad as long. Porose areas mildly depressed, oval, placed close against the lateral elevations. Surface smooth, shining, impunctate, and without hairs. Palpi longer than the basis, smooth, and with few hairs.

In ventral view, basis with faint posterolateral salient corners; surface faintly crazed. Palpal article 2 with five to six simple parallel hairs, directed toward the hypostome. Article 3 with a conspicuous, long, retrograde horn, and on its inner side three simple hairs directed toward the hypostome.

**Hypostome.** Clavate and with a distinct corona. Dentition 5/5, denticles progressively smaller in the successive files from the laterals to the median file, and 10 denticles in each file. Length, about 0.39.

**Scutum.** Length, from 1.08 to 1.26; width, 1.23 to 1.44. Slightly pointed posteriorly. Scapulae, short, bluntly rounded. Cervical grooves, moderately deep, long. Surface smooth, shining, faintly crazed; punctations fine, evenly distributed; hairs few, very fine, often visible only with reflected light, sometimes absent.

**Legs.** Long and slim. A large dorsal trochantal spur on I; absent on II, III, and IV. Small, rounded ventral trochantal spurs on I to IV. Ventral terminal spurs on tarsi absent. Hairs numerous, fine, and long; those on the inner side of femur IV long, parallel and directed toward the spiracular plate. Length of tarsus I, 0.66; metatarsus, 0.36. Length of tarsus IV, 0.60; metatarsus, 0.45.



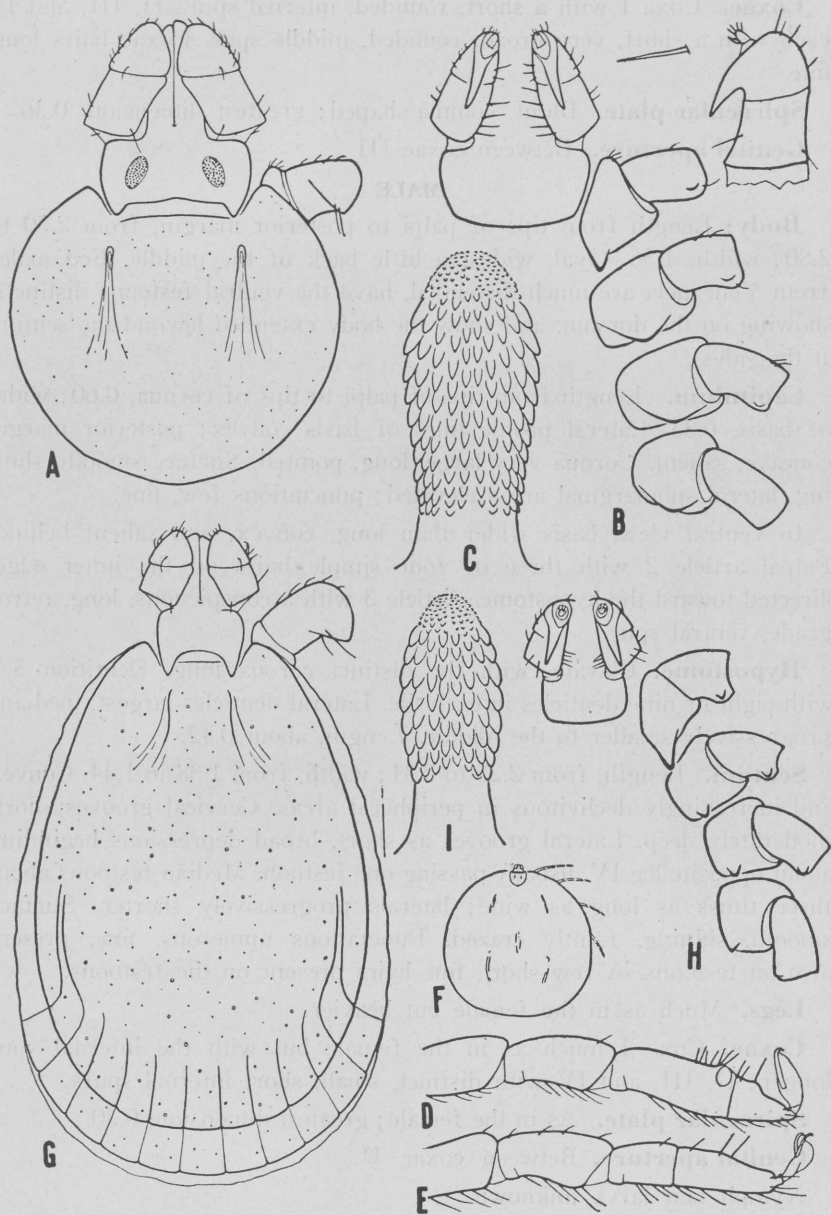


FIGURE 12. *Haemaphysalis kochi* Aragão. A. Female capitulum and scutum, dorsum. B. Female capitulum and coxae, venter, and side view of palpus, and palpal hair enlarged. C. Female hypostome. D. Female metatarsus and tarsus, leg I. E. Female metatarsus and tarsus, leg IV. F. Engorged female. G. Male capitulum and scutum, dorsum. H. Male capitulum and coxae, venter. I. Male hypostome.

**Coxae.** Coxa I with a short, rounded, internal spur; II, III, and IV each with a short, very broad, rounded, middle spur. Coxae hairs long, fine.

**Spiracular plate.** Blunt, comma-shaped; greatest dimension, 0.36.

**Genital aperture.** Between coxae III.

#### MALE

**Body:** Length from tips of palpi to posterior margin, from 2.70 to 2.80; width, 1.56. Oval, widest a little back of the middle. Fed males from Venezuela are much distended, have the ventral festoons distinctly showing on the dorsum, and show the body extended beyond the scutum at the sides.

**Capitulum.** Length from tips of palpi to tips of cornua, 0.60; width of basis, 0.33. Lateral profile lines of basis convex; posterior margin concave, salient. Cornua very large, long, pointed. Surface smooth, shining, lateral submarginal areas elevated; punctations few, fine.

In ventral view, basis wider than long, convex, and salient behind. Palpal article 2 with three or four simple hairs on the inner edge, directed toward the hypostome. Article 3 with a conspicuous, long, retrograde, ventral spur.

**Hypostome.** Clavate, with the distinct corona long. Dentition 5/5 with eight or nine denticles in each file. Lateral denticles largest, medians progressively smaller to the middle. Length, about 0.42.

**Scutum.** Length, from 2.25 to 2.31; width, from 1.38 to 1.44. Convex and increasingly declivitous in peripheral areas. Cervical grooves short, moderately deep. Lateral grooves as short, broad depressions beginning about opposite leg IV, usually passing one festoon. Median festoons about three times as long as wide; laterals progressively shorter. Surface smooth, shining, faintly crazed. Punctations numerous, fine, present also on festoons. A few short, fine hairs present on the festoons.

**Legs.** Much as in the female but heavier.

**Coxae.** Coxa I much as in the female but with the internal spur longer; II, III, and IV with distinct, small, short internal spurs.

**Spiracular plate.** As in the female; greatest dimension, 0.30.

**Genital aperture.** Between coxae II.

Nymph and larva unknown.

In *kochi*, the dentition is 5/5 while in *juxtakochi*, it is 4/4. Coxae II, III, IV of the females of *kochi* have very large, broad, rounded, middle spurs, while coxae II and III of *juxtakochi* have middle spurs similar to those on *kochi* but more pointed, and IV has a small, rounded, inner spur.

#### HOSTS AND DISTRIBUTION

*H. kochi* was originally described from 1 male and 2 females taken

from *Cervus campestris*, Bauru, State of São Paulo, and 1 male taken from *Cervus rufus*, Jacutinga, State of São Paulo. Dr. Aragão has recently sent us 2 females and 1 male taken from *Mazama* sp., 1929, Pernambuco, Brazil. From Dr. Charles H. Ballou, Estacion Experimental de Agricultura, El Valle, D. F., Venezuela, we received 9 females and 7 males taken from tapir, February 14, 1938, Selva, Gran Sabana, Venezuela.

## **HAEMAPHYSALIS JUXTAKOCHI, N. SP.**

FIGURE 13

### **FEMALE**

**Body.** Length (unengorged) from tips of palpi to posterior margin, 2.30 to 2.58; width, 1.41 to 1.45. Oval, widest at about the middle. Scutum occupying half the length of the body. Marginal grooves distinct, extending from the edge of the scutum posteriorly to the third festoon. Fully fed females may be as large as 7.00 by 5.75 and are short-oval, wider posteriorly, and about equally thick at both ends.

**Capitulum.** Length from tips of palpi to the tips of the cornua, 0.54 to 0.63; length of basis from base of palpi to tips of cornua, 0.20 to 0.25; width of basis, 0.48 to 0.51. Lateral profile lines convex, posterior margin nearly straight, salient. Cornua as long as wide, rounded or pointed. Lateral submarginal areas with longitudinal elevations. Porose areas faint, superficial, crowded against the submarginal elevations but sometimes a little depressed and in an oval pattern. Surface smooth, shining, impunctate; hairs absent. Palpi smooth, shining; hairs few.

In ventral view, basis broadly rounded behind, convex, impunctate, and without hairs. Palpal article 2 with four or five simple hairs on the inner edge directed toward the hypostome. Article 3 with a conspicuous, long, retrograde horn, and on its inner edge three hairs.

**Hypostome.** Clavate, with a large corona. Denticles 4/4, with 9 or 10 in each file. Denticles decreasing in size from laterals to the medians. Length, about 0.33.

**Scutum.** Length, 0.96 to 1.02; width, 0.96 to 1.08. Nearly circular, often with mild posterolateral and posterior corners. Scapulae rounded, much separated with the interval moderately deep. Cervical grooves distinct, long. Punctations fine, numerous, evenly distributed. Hairs absent.

**Legs.** Long, moderately heavy, I somewhat the heaviest. Trochanter I with a distinct dorsal trochanteral spur; absent on II, III, and IV. Very mild ventral trochanteral spurs present on I, II, and III. Ventral spurs on tarsi absent. Length of tarsus I, 0.66; metatarsus, 0.36. Length of tarsus IV, 0.54; metatarsus, 0.36.

**Coxae.** Coxa I with short, rounded internal spur; II and III with broad, salient middle spurs which are bluntly pointed (more pointed than in *kochi*); IV with a faint internal spur.

**Genital aperture.** Situated just posterior to the level of the intervals between coxae II and III.

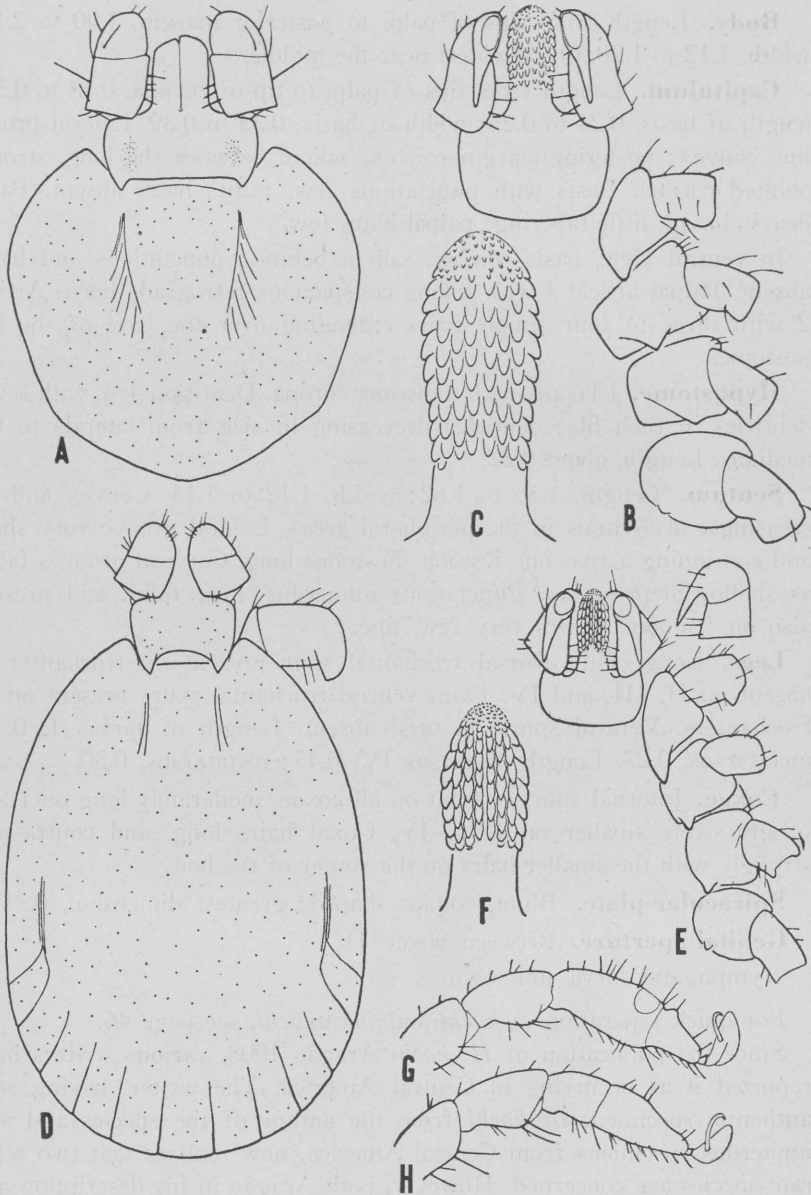


FIGURE 13. *Haemaphysalis juxtakochi*, n. sp. A. Female capitulum and scutum, dorsum. B. Female capitulum and coxae, venter. C. Female hypostome. D. Male capitulum and scutum, dorsum. E. Male capitulum and coxae, venter, and side view of palpus. F. Male hypostome. G. Male metatarsus and tarsus, leg I. H. Male metatarsus and tarsus, leg IV.



## MALE

**Body.** Length from tips of palpi to posterior margin, 1.90 to 2.16; width, 1.12 to 1.20. Oval, widest near the middle.

**Capitulum.** Length from tips of palpi to tip of cornua, 0.48 to 0.51; length of basis, 0.24 to 0.28; width of basis, 0.33 to 0.39. Lateral profile line convex; posterior margin convex, salient between the long, strong, pointed cornua. Basis with punctations few, faint; hairs absent. Palpi heavy, broad, little tapering; palpal hairs few.

In ventral view, basis convex, salient behind; punctations and hairs absent. Palpal article 3 with a long conspicuous retrograde horn. Article 2 with three to four simple hairs extending over the base of the hypostome.

**Hypostome.** Clavate, with a distinct corona. Dentition 4/4, with seven denticles in each file; denticles decreasing in size from laterals to the medians. Length, about 0.22.

**Scutum.** Length, 1.56 to 1.62; width, 1.12 to 1.14. Convex and increasingly declivitous in the peripheral areas. Lateral groove very short and continuing across one festoon. Festoons long. Cervical grooves faint, as shallow depressions. Punctations numerous, fine, faint, and present also on festoons. Hairs very few, fine.

**Legs.** Long, slim. Dorsal trochanteral spur present on trochanter I; absent on II, III, and IV. Faint ventral trochanteral spurs present on all trochanters. Ventral spurs on tarsi absent. Length of tarsus I, 0.48; metatarsus, 0.27. Length of tarsus IV, 0.45; metatarsus, 0.30.

**Coxae.** Internal spurs present on all coxae, moderately long on I and progressively smaller on II to IV. Coxal hairs long, and contrasting strongly with the smaller hairs on the venter of the body.

**Spiracular plate.** Blunt, comma-shaped; greatest dimension, 0.21.

**Genital aperture.** Between coxae II.

Nymph and larva unknown.

For quick separation of *kochi* and *juxtakochi*, see page 46.

Since the publication of *H. kochi* Aragão 1908, various writers have reported it as occurring in Central America. The writer, having seen authentic specimens of *kochi* from the author of the species and also numerous specimens from Central America, now realizes that two separate species are concerned. However, both Aragão in his description and Nuttall (1915, p. 414), state that *kochi* has dentition 4/4 on the hypostome. The true pattern of the dentition of these ticks is obscure when the hypostome is viewed *in situ*, but very clear in specimens mounted in balsam, and I believe that the types of *kochi* would show a 5/5 pattern on the hypostome. In all specimens of both sexes of *H. kochi* and *H. juxtakochi* that have been examined there are several other definite

correlating characters which justify two species as pointed out in the descriptions.

The descriptions and figures of *Haemaphysalis juxtakochi* were made from the following: 19855, 3 males from deer, April 6, 1942, Alajuela, Panama; 21585, 4 females, 11 males, Panama; 21572, 1 male, 1 female, vegetation, March 3, 1943, Corozal, Canal Zone; 21574, 1 female, from *Odocoileus*, July 1942, Guayabalito, Panama; 21571, 1 female, 2 males, from *Odocoileus*, August 1942, Juan Mina, Canal Zone.

Holotype: female from 21585.

Allotype: male from 21585.

Paratypes: females and males from 21585, 21574, 21572, 21571, and 19855.

Types are deposited as follows: Holotype, allotype, and paratypes in the Rocky Mountain Laboratory, Hamilton, Mont.; 1 paratype female, 2 paratype males (21571), United States National Museum, Washington, D. C.; 1 paratype female (21574), 1 paratype male (21572), Museum of Comparative Zoology, Harvard University, Cambridge, Mass.; 1 paratype female, 1 paratype male (21585), Department of Entomology, University Farm, St. Paul, Minn.

Dunn (1923) states that *kochi* is common on deer in Panama and records three lots of specimens of 37, 3 and 2, respectively, all from tapir (*Tapirella beardi*). Fairchild (1943) reports *kochi* from deer in Panama, and from "brush" near Corozal, Canal Zone. All the specimens which I have seen from Panama are *juxtakochi*. In his paper, Osorno-Mesa (1941, p. 428) seems to imply that *kochi* is present in Colombia but gives no definite records. If such a *Haemaphysalis* is present in Colombia, it is probable that it is *kochi*.

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