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## UNITED STATES PUBLIC HEALTH SERVICE

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# LIST OF MAPS, CHARTS, AND ILLUSTRATIONS.

# REPORT OF INVESTIGATION OF COASTAL WATERS IN THE VICINITY OF GULFPORT AND BILOXI, MISS., WITH SPECIAL REFERENCE TO THE POLLUTION OF SHELL FISH.[1]

By R. H. CREEL, Passed Assistant Surgeon.

After conference with the Chief of the Bureau of Chemistry, Department of Agriculture, I proceeded, on January 24, to Gulfport, Miss. On January 26, in company with Mr. W. H. Hartigan, representing the Bureau of Chemistry, a sanitary survey was made of the oyster beds and their surroundings.

The oyster grounds were mapped out and the different points from which samples of water and oysters should be selected were decided upon. Mr. Hartigan attended to the subsequent collecting and shipping of the samples to the laboratory at Mobile. Upon the completion of the survey at Gulfport I went to Mobile, where the samples were received. The laboratory equipment of the marine hospital in Mobile was utilized, supplemented by additional supply of materials from the Hygienic Laboratory.

On January 30 a sanitary survey was made of the oyster grounds in the vicinity of Biloxi, Miss., and arrangement completed whereby Mr. Hartigan would follow a similar plan of collecting and shipping the samples as used at Gulfport.

At Gulfport the samples were secured in the morning, sent to Mobile on a midday train, and examined the same afternoon. At Biloxi the collection was made in the afternoon, shipment made the same night, and examination performed on the following morning. All samples were expressed in hermetically sealed tin cans, surrounded by crushed ice, in a box container. Without exception all the oyster samples arrived at Mobile in good condition, well chilled, and with tightly closed shells.

## SANITARY SURVEY OF GULFPORT.

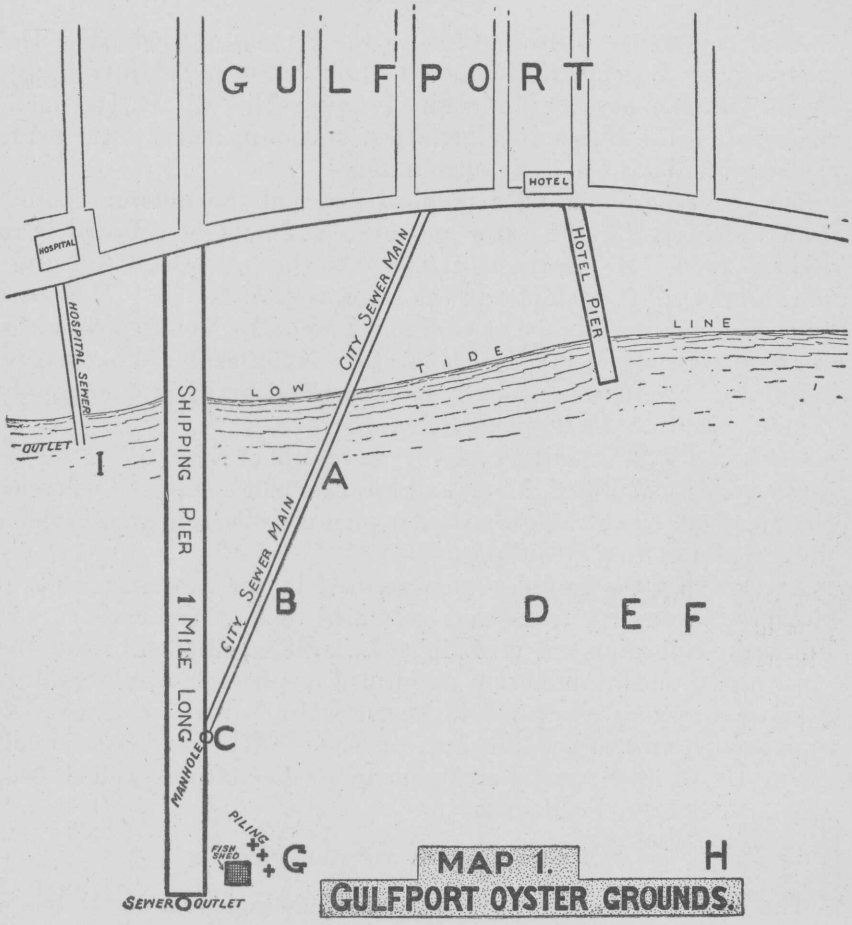
The city of Gulfport is located on Mississippi Sound. It has a population of some 7,000 people, there being one or two thousand more in winter than in summer, as the place is a winter resort for northern people.

The community has a modern sewer system, and the refuse is discharged through a large iron pipe into the sound about 1 mile off shore.

[1] Manuscript submitted for publication May 15, 1914.

The shore line at Gulfport is without indentation, but a causeway 1 mile in length serves to deflect the natural currents of the harbor and constitutes a catch basin for the discharged refuse. There is a fairly strong current parallel to the coast line at the rise and fall of the tide. This current sets from the east to the west or the reverse, according to the state of the tide.

As the sewer-pipe outlet is located at the end of the pier, a certain amount of the sewage is spread along the beach and tends to concentrate along the length of the causeway.




MAP 1.
GULFPORT OYSTER GROUNDS.

The oyster growth at Gulfport is comparatively small and of recent development. The oyster beds extend from the causeway eastward for a mile or possibly a greater distance, and are chiefly confined to an artificial ledge made of stone ballast discharged in past years from incoming ships.

To the west of the pier is one small bed from which sample "I" was taken. This latter lies just below the outlet of a hospital sewer and has been abandoned by order of Dr. Capps, the city health officer.

Samples of oysters and water were examined from nine different points on the Gulfport waterfront. Map No. 1 gives location of each sample and Table I gives result of oyster and water examination with *B. coli* rating of each for Gulfport.

TABLE I.—*Pollution and rating of oysters and water at Gulfport, Miss.*

| Oyster sample. | Date. | Location. | Gas. | | | Confirmation on Endo plate. | | | Rating. |
|---|---|---|---|---|---|---|---|---|---|
| | | | 1 c. c. | 0.1 c. c. | 0.01 c. c. | 1 c. c. | 0.1 c. c. | 0.01 c. c. | |
| A 1 | Jan. 27 | Attached to sewer pipe 200 feet from shore; not covered by water except at high tide. | − | − | − | − | − | ..... | 1 |
| A 2 | ...do.... | | + | + | − | + | − (Anaerobe.) | ..... | 1 |
| A 3 | ...do.... | | + | − | − | + | ..... | ..... | 1 |
| A 4 | ..... | | + | + | − | + | + | ..... | 1 |
| A 5 | ..... | | + | − | − | + | ..... | ..... | 1 |
| | | | | | | | | | 5 |
| B 1 | ...do.... | Attached to sewer pipe about ½ mile from shore line, covered by 6 inches of water at low tide. | + | + | + | ..... | ..... | + | 100 |
| B 2 | ...do.... | | + | + | − | ..... | + | ..... | 10 |
| B 3 | ...do.... | | + | + | + | ..... | ..... | + | 100 |
| B 4 | ...do.... | | + | + | + | ..... | ..... | + | 100 |
| B 5 | ...do.... | | + | + | + | ..... | ..... | + | 100 |
| | | | | | | | | | 410 |
| C 1 | ...do.... | On sewer pipe ¾ mile from shore line; barely covered by water at low tide. | + | + | − | ..... | + | ..... | 10 |
| C 2 | ...do.... | | + | + | − | ..... | + | ..... | 10 |
| C 3 | ...do.... | | + | + | − | ..... | + | ..... | 10 |
| C 4 | ...do.... | | + | + | + | ..... | ..... | + | 100 |
| C 5 | ...do.... | | + | + | + | ..... | ..... | + | 100 |
| | | | | | | | | | 230 |
| D 1 | ...do.... | ½ mile east of pier; 6 feet of water at low tide. | + | + | − | ..... | + | ..... | 10 |
| D 2 | ...do.... | | + | + | − | + | − | ..... | 10 |
| D 3 | ...do.... | | + | + | + | ..... | ..... | + | 10 |
| D 4 | ...do.... | | + | + | + | ..... | ..... | + | 10 |
| D 5 | ...do.... | | + | − | − | + | ..... | ..... | 10 |
| | | | | | | | | | 50 |
| E 1 | ...do.... | 1,000 feet east of "D," covered by 5 feet of water at low tide. | + | − | − | + | ..... | ..... | 1 |
| E 2 | ...do.... | | + | + | − | ..... | + | ..... | 10 |
| E 3 | ...do.... | | + | + | − | ..... | + | ..... | 10 |
| E 4 | ...do.... | | + | + | − | ..... | + | ..... | 10 |
| E 5 | ...do.... | | + | + | − | ..... | + | ..... | 10 |
| | | | | | | | | | 41 |
| F 1 | ...do.... | ¾ mile east of pier covered by 8 feet of water at low tide. | + | + | + | ..... | ..... | + | 10 |
| F 2 | ...do.... | | + | − | − | + | ..... | ..... | 10 |
| F 3 | ...do.... | | − | − | − | − | − | − | 1 |
| F 4 | ...do.... | | + | + | − | ..... | + | ..... | 1 |
| F 5 | ...do.... | | + | + | − | ..... | + | ..... | 10 |
| | | | | | | | | | 32 |
| G 1 | Jan. 28 | Near end of pier covered by 8 feet of water at high tide. | + | + | + | ..... | ..... | + | 100 |
| G 2 | ...do.... | | + | + | − | ..... | + | ..... | 10 |
| G 3 | ...do.... | | + | + | + | ..... | ..... | + | 100 |
| G 4 | ...do.... | | + | + | + | ..... | ..... | + | 100 |
| G 5 | ...do.... | | + | + | − | ..... | + | ..... | 10 |
| | | | | | | | | | 320 |

NOTE.—Under column "Confirmatian on Endo" anerobe in parentheses indicates that the fermentation was due to an anerobic bacillus and not to B. Coli.

TABLE I.—*Polluting and rating of oysters and water at Gulfport, Miss.*—Continued.

| Oyster sample. | Date. | Location. | Gas. | | | Confirmation on Endo plate. | | | Rating. |
|---|---|---|---|---|---|---|---|---|---|
| | | | 1 c.c. | 0.1 c. c. | 0.01 c. c. | 1 c.c. | 0.1 c. c. | 0.01 c.c. | |
| H[1] | ...do... | ¾ mile east from end of pier in 8 feet of water at high tide. | + | + | − | ..... | + | ..... | 10 |
| H[2] | ...do... | | + | + | − | ..... | + | ..... | 10 |
| H[3] | ...do... | | + | − | − | + | ..... | ..... | 1 |
| H[4] | ...do... | | + | + | − | ..... | + | ..... | 1 |
| H[5] | ...do... | | − | − | − | − | ..... | ..... | 1 |
| | | | | | | | | | 23 |
| I[1] | ...do... | Off shore line near small hospital sewer, covered by water only at high tide. | + | + | − | ..... | + | ..... | 10 |
| I[2] | ...do... | | + | + | − | ..... | + | ..... | 10 |
| I[3] | ...do... | | + | + | − | ..... | + | ..... | 10 |
| I[4] | ...do... | | + | − | − | + | ..... | ..... | 10 |
| I[5] | ...do... | | + | + | + | ..... | ..... | + | 10 |
| | | | | | | | | | 50 |

| Water sample. | Date. | Location. | Gas. | | | Confirmation on Endo plate. | | | B. coli per c. c. of water. |
|---|---|---|---|---|---|---|---|---|---|
| | | | 1 c.c. | 0.1 c. c. | 0.01 c. c. | 1 c.c. | 0.1 c. c. | 0.01 c. c. | |
| A | Jan. 27 | Same as oyster samples, 2 feet below surface water. | + | + | ..... | + | − (Anerobe.) | ..... | 1 |
| B | ...do... | | + | + | ..... | ..... | + | ..... | 10 |
| C | ...do... | | + | + | + | ..... | ..... | + | 100 |
| D | ...do... | | + | + | ..... | ..... | + | ..... | 10 |
| E | ...do... | | + | + | + | ..... | ..... | + | 100 |
| F | ...do... | | + | ..... | ..... | + | ..... | ..... | 1 |
| G | Jan. 28 | | + | + | + | ..... | ..... | + | 100 |
| H | ...do... | | + | − | ..... | + | ..... | ..... | 1 |

NOTE.—Under column "Confirmation on Endo" anerobe in parentheses indicates that the fermentation was due to an anerobic bacillus and not to B. coli.

Dr. H. S. Capps reports that there have been within the past four months, since the oyster season opened, 10 cases of typhoid fever. Prior to this period there had been only 6 cases from the first of the year. Most of the cases had a history of having eaten raw oysters, but, as oysters were not an unusual article of diet in the town, it is difficult to definitely ascribe the infection to this source. However, the marked increase in number of cases coincident with the oyster season is strikingly significant.

The Gulfport oysters are prohibited from sale locally, but they are gathered in small quantities by different people for their own consumption.

The prohibition of the sale of Gulfport oysters in the local markets does not prevent the gathering of the oysters and the shipping of them to outside points. This proceeds uninterrupted.

## SURVEY OF BILOXI OYSTER BEDS.

The city of Biloxi has a winter population of some 9,000 people. In the summer there is a decrease of two or three thousand, owing to the departure of winter tourists.

The community has no sewage-collection system. There are a large number of privies and some cesspools. A certain proportion of residences have private sewer pipes discharging directly into the Gulf and several have sewer connection with the city storm sewers. Through the activities of the city health officer, Dr. T. O. Hunter, the number of people having this sewer connection has been materially lessened, but in so far as the effluent from these so-called storm sewers have *B. coli* in 0.001 c. c., it is evident that there is considerable contamination poured into the Mississippi Sound through these channels.

It is apparent that Biloxi sewage, either directly or indirectly by surface drainage, is discharged into the water immediately surrounding Biloxi, wherein are located the oyster beds.

In addition to the Biloxi pollution, the oyster beds in the lower part of Back Bay and Biloxi Bay are subjected to the pollution arising from Ocean Springs, a town of about 2,000 population.

The Back Bay of Biloxi is exposed to pollution from various sources. The part of Biloxi adjoining this body of water is not so thickly populated as the south side of the town, and the pollution chiefly arises from surface drainage. In addition to this drainage from near-by privies, an open sewer in the nature of a small stream discharges into Back Bay. This stream contains the sewage of the railroad station and the two large commercial hotels in Biloxi.

Several rivers empty into Back Bay—the Biloxi River, the Tchula River, and several smaller streams. These tributaries carry pollution from a large rural population and several small hamlets. It is also probable that a certain amount of the contamination on the south side of Biloxi and that from Ocean Springs is swept well up into Biloxi Bay by a rising time. Typhoid prevails in and around Biloxi, but I have been unable to secure definite statement as to the extent of its prevalence. From hearsay evidence the disease occurs in about the same proportion as in Gulfport.

Map II and Table II show the locations from which the Biloxi oysters and water samples were taken and the amount of pollution in each.

MAP 2:—BILOXI OYSTER GROUNDS.

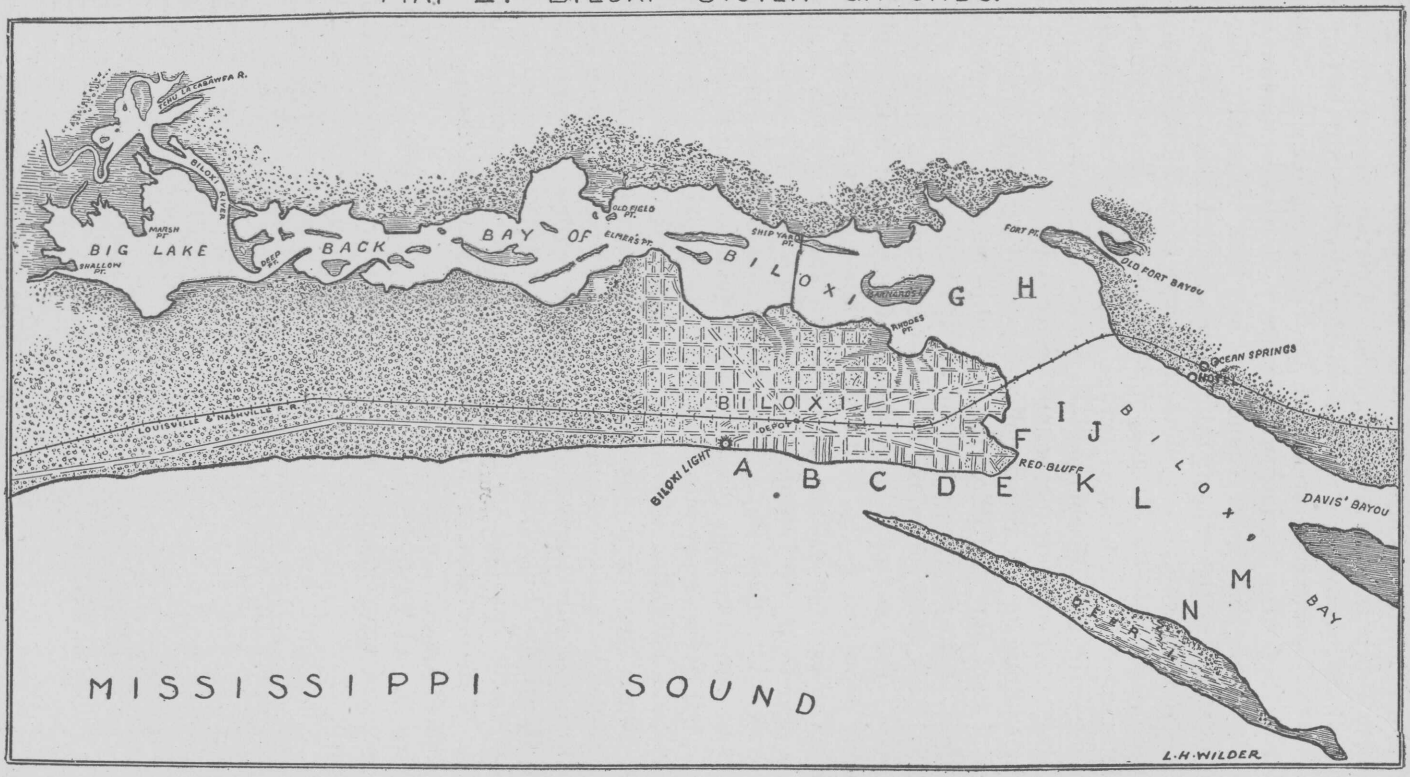


TABLE II.—*Pollution and rating of oyster and water at Biloxi, Miss.*

| Oyster sample. | Date. | Location. | Gas. | | | Confirmation on Endo plate. | | | Rating. |
|---|---|---|---|---|---|---|---|---|---|
| | | | 1 c. c. | 0.1 c. c. | 0.01 c. c. | 1 c. c. | 0.1 c. c. | 0.01 c. c. | |
| $A^1$ | Jan. 30 | Off shore 100 yards, 3 feet of water at low tide. | + | + | + | ..... | ..... | + | 100 |
| $A^2$ | ...do..... | | + | + | + | ..... | ..... | + | 10 |
| $A^3$ | ...do..... | | − | − | − | ..... | ..... | ..... | 10 |
| $A^4$ | ...do..... | | + | + | + | ..... | ..... | + | 10 |
| $A^5$ | ...do..... | | + | + | − | ..... | + | ..... | 10 |
| | | | | | | | | | 140 |
| $B^1$ | ...do..... | 50 feet off shore, 3½ feet of water at low tide. | + | + | − | ..... | + | ..... | 10 |
| $B^2$ | ...do..... | | + | + | − | ..... | + | ..... | 10 |
| $B^3$ | ...do..... | | + | − | − | + | ..... | ..... | 10 |
| $B^4$ | ...do..... | | + | + | + | ..... | ..... | + | 10 |
| $B^5$ | ...do..... | | + | + | + | ..... | ..... | + | 100 |
| | | | | | | | | | 140 |
| $C^1$ | ...do..... | 150 yards off shore, 3 feet of water at low tide. Oyster bed near outlet of several alleged storm sewers. | + | + | − | ..... | + | ..... | 10 |
| $C^2$ | ...do..... | | + | + | + | ..... | ..... | + | 100 |
| $C^3$ | ...do..... | | + | + | − | ..... | + | ..... | 10 |
| $C^4$ | ...do..... | | + | + | + | ..... | ..... | + | 100 |
| $C^5$ | ...do..... | | + | + | − | ..... | + | ..... | 10 |
| | | | | | | | | | 230 |
| $D^1$ | ...do..... | 200 yards off shore in 2 feet of water at low tide. | + | + | − | ..... | + | ..... | 10 |
| $D^2$ | ...do..... | | + | + | − | ..... | + | ..... | 10 |
| $D^3$ | ...do..... | | + | + | + | ..... | ..... | + | 100 |
| $D^4$ | ...do..... | | + | + | − | ..... | + | ..... | 10 |
| $D^5$ | ...do..... | | + | + | + | ..... | ..... | + | 100 |
| | | | | | | | | | 230 |
| $E^1$ | ...do..... | ....do..... | + | + | + | ..... | ..... | + | 100 |
| $E^2$ | ...do..... | | + | + | − | ..... | + | ..... | 10 |
| $E^3$ | ...do..... | | + | + | − | ..... | + | ..... | 10 |
| $E^4$ | ...do..... | | + | + | − | ..... | + | ..... | 10 |
| $E^5$ | ...do..... | | + | + | − | ..... | + | ..... | 10 |
| | | | | | | | | | 140 |
| $F^1$ | ...do..... | Off Point Cadet, 200 yards from shore, in 2 feet of water at low tide. | + | − | − | + | ..... | ..... | 1 |
| $F^2$ | ...do..... | | + | − | − | + | ..... | ..... | 1 |
| $F^3$ | ...do..... | | + | − | − | + | ..... | ..... | 1 |
| $F^4$ | ...do..... | | + | − | − | + | ..... | ..... | 1 |
| $F^5$ | ...do..... | | + | + | − | + | − | ..... | 1 |
| | | | | | | | (Anaerobe.) | | 5 |
| $G^1$ | Jan. 31 | Back Bay, off shore ½ mile in 5 feet of water. Between L. & N. Bridge and Kennedy's factory. | + | + | + | ..... | ..... | + | 10 |
| $G^2$ | ...do..... | | + | + | − | + | − | ..... | 10 |
| | | | | | | | (Anaerobe.) | | |
| $G^3$ | ...do..... | | + | + | + | ..... | + | − | 10 |
| | | | | | | | | (Anaerobe.) | |
| $G^4$ | ...do..... | | + | + | − | ..... | + | ..... | 10 |
| $G^5$ | ...do..... | | + | + | − | ..... | + | ..... | 10 |
| | | | | | | | | | 50 |
| $H^1$ | Jan. 31 | North of L. & N. Bridge, off shore ½ mile in 6 feet of water. | + | + | + | ..... | ..... | + | 10 |
| $H^2$ | ...do..... | | + | − | − | + | ..... | ..... | 10 |
| $H^3$ | ...do..... | | + | + | + | ..... | ..... | + | 100 |
| $H^4$ | ...do..... | | + | + | − | ..... | + | ..... | 10 |
| $H^5$ | ...do..... | | + | + | + | ..... | ..... | + | 100 |
| | | | | | | | | | 230 |
| $I^1$ | ...do..... | ½ mile off shore in 6 feet of water; Ott Bros. and McCaleb reef between L. & N. and L bridge and Point Cadet. | + | + | − | ..... | + | ..... | 10 |
| $I^2$ | ...do..... | | + | − | − | + | ..... | ..... | 10 |
| $I^3$ | ...do..... | | + | + | + | ..... | ..... | + | 10 |
| $I^4$ | ...do..... | | + | + | − | ..... | + | ..... | 10 |
| $I^5$ | ...do..... | | + | + | − | ..... | − | ..... | 10 |
| | | | | | | | | | 50 |

TABLE II.—*Pollution and rating of oyster and water at Biloxi, Miss.*—Continued.

| Oyster sample. | Date. | Location. | Gas. | | | Confirmation on Endo plate. | | | Rating. |
|---|---|---|---|---|---|---|---|---|---|
| | | | 1 c. c. | 0.1 c. c. | 0.01 c. c. | 1 c. c. | 0.1 c. c. | 0.01 c. c. | |
| J[1] | ...do... | On State Reef opposite Point Cadet, 1 mile off shore, in 6 feet of water. | + | + | ..... | ..... | + | ..... | 10 |
| J[2] | ...do... | | + | + | + | ..... | ..... | + | 100 |
| J[3] | ...do... | | + | + | + | ..... | ..... | + | 100 |
| J[4] | ...do... | | + | + | + | ..... | ..... | + | 100 |
| J[5] | ...do... | | + | + | ..... | ..... | + | ..... | 10 |
| | | | | | | | | | 320 |
| K[1] | Feb. 1 | On Lewis Reef, midway between Point Cadet and east end of Deer Island, in 5 feet of water. | + | − | − | + | ..... | ..... | 1 |
| K[2] | ...do... | | + | − | − | + | ..... | ..... | 10 |
| K[3] | ...do... | | + | + | − | ..... | + | ..... | 10 |
| K[4] | ...do... | | + | + | − | ..... | + | ..... | 10 |
| K[5] | ...do... | | + | + | + | ..... | ..... | + | 10 |
| | | | | | | | | | 41 |
| L[1] | ...do... | On State Reef, opposite east end of Deer Island, 5-foot depth. | + | − | − | + | ..... | ..... | 10 |
| L[2] | ...do... | | + | + | − | ..... | + | ..... | 10 |
| L[3] | ...do... | | + | + | − | ..... | + | ..... | 10 |
| L[4] | ...do... | | + | + | + | ..... | ..... | + | 10 |
| L[5] | ...do... | | + | + | − | ..... | + | ..... | 10 |
| | | | | | | | | | 50 |
| M[1] | ...do... | On Akins Reef, near east end of Deer Island, ¼ mile off shore, in 5 feet of water. | + | + | + | ..... | ..... | + | 100 |
| M[2] | ...do... | | + | + | − | ..... | + | ..... | 10 |
| M[3] | ...do... | | + | + | − | ..... | + | ..... | 10 |
| M[4] | ...do... | | + | + | + | ..... | ..... | + | 100 |
| M[5] | ...do... | | + | + | + | ..... | ..... | + | 100 |
| | | | | | | | | | 320 |
| N[1] | ...do... | ½ mile off Deer Island, opposite Point Cadet, in 6 feet of water. | + | + | + | ..... | ..... | + | 100 |
| N[2] | ...do... | | + | + | + | ..... | ..... | + | 100 |
| N[3] | ...do... | | + | + | + | ..... | ..... | + | 10 |
| N[4] | ...do... | | + | − | − | + | ..... | ..... | 10 |
| N[5] | ...do... | | + | + | − | ..... | + | ..... | 10 |
| | | | | | | | | | 230 |

| Water sample. | Date and location. | Gas. | | | Confirmation on Endo plate. | | | B. coli per c. c. |
|---|---|---|---|---|---|---|---|---|
| | | 1 c. c. | 0.1 c. c. | 0.01 c. c. | 1 c. c. | 0.1 c. c. | 0.01 c. c. | |
| A | Date and location correspond to the oyster samples—water taken 2 feet below surface. | + | + | − | ..... | + | ..... | 10. |
| B | | + | + | − | ..... | + | ..... | 10. |
| C | | + | − | − | + | ..... | ..... | 1. |
| D | | + | + | − | ..... | + | ..... | 10. |
| E | | + | + | − | + | − | ..... | 1. |
| | | | | | | (Anaerobe.) | | |
| F | | + | − | − | ..... | ..... | ..... | 1. |
| G | | − | − | − | ..... | ..... | ..... | 0. |
| H | | − | − | − | ..... | ..... | ..... | 0. |
| I | | + | − | − | ..... | ..... | ..... | 1. |
| J | | − | − | − | ..... | ..... | ..... | 0. |
| K | | + | − | − | ..... | ..... | ..... | 1. |
| L | | − | − | − | ..... | ..... | ..... | 0. |
| M | | + | + | − | ..... | ..... | ..... | 10. |
| N | | + | − | − | ..... | ..... | ..... | 1. |

Table III gives the result of examination of shucked stock in Biloxi. Samples of shucked oysters were examined from seven different packing houses.

TABLE III.—*Examination of shucked stock at Biloxi.*

| Sample. | Gas. | | | Confirmation on Endo plate. | | | Total bacterial count.[1] |
|---|---|---|---|---|---|---|---|
| | 1 c. c. | 0.1 c. c. | 0.01 c. c. | 1 c. c. | 0.1 c. c. | 0.01 c. c. | |
| Ax | + | + | ..... | ..... | + | ..... | 16,180 per c. c. |
| Bx | + | + | + | ..... | ..... | + | 31,240 per c. c. |
| Cx | + | + | + | ..... | ..... | + | 25,300 per c. c. |
| Dx | + | + | + | ..... | ..... | + | Plates discarded "spreading colonies." |
| Ex | + | + | + | ..... | ..... | + | 4,780 per c. c. |
| Fx | + | − | − | + | ..... | ..... | 19,450 per c. c. |
| Gx | + | − | − | + | ..... | ..... | 16,400 per c. c. |

[1] The total bacterial count did not correspond to standard methods. As a 20° C. incubator was not available, the cultivation of plates was accomplished at 37° for 24 hours. The above bacterial count is not accurate, therefore, but is merely suggestive.

NOTE.—Each sample was taken from a separate packing house and represented the shucked stock before being washed and ready for shipment. Some of the samples were from Biloxi beds, others were reputed to be from Louisiana marsh.

## METHOD OF EXAMINATION.

Water samples were secured in sterile glass bottles. Each water sample was taken below surface and corresponded to the oyster sample marked by same symbol.

The water was examined in 1 c. c., 0.1 c. c., and 0.01 c. c. quantities planted in standard lactose broth. No presumptive test in the way of gas production as indicative of presence of *B. coli* was relied upon. Confirmation by plating out on Endo medium was performed in each sample and in each dilution marked positive for the presence of *B. coli*.

On several instances the fermentation proved to be due to an anaerobic bacillus.

In the examination of the oysters, standard methods were observed. The shell stock was first thoroughly cleaned with a brush and water, then dried and the edge of each shell well flamed before being opened. The oyster shells were opened with an oyster knife previously sterilized by flame.

Five oysters from each sample were tested, using 1 c. c., 0.1 c. c., and 0.01 c. c. quantities of the shell liquor in lactose broth tubes. All tubes showing fermentation after 48 hours were plated out on Endo plates. Most of the samples showing gas formation showed *B. coli* on Endo plates, but a few samples in the higher dilutions proved negative for *B. coli*.

In these gas-producing tubes that were negative to *B. coli* an anaerobic bacillus was demonstrated to be the cause of the fermentation. Agar plates were made for each sample of oysters, but the large number of "spreaders" so affected an accurate count of total bacteria that the plates were discarded except for the shucked stock.

## SUMMARY.

In Gulfport the sewage arising from a town of 7,000 people is discharged into Mississippi Sound in the immediate vicinity of oyster beds. Both oyster and water samples show *B. coli* in 0.01 c. c. in the

specimens examined. The pollution seems more concentrated near the causeway, the samples farthest away from the mouth of the sewer showing less amount of pollution. While the typhoid rate in Gulfport is not excessive when compared to other cities the increase in the disease coincident with the oyster season, suggests that raw oysters play a rôle in causing typhoid at Gulfport.

In Biloxi the examination of water and oysters showed *B. coli* in high dilutions. While the water on the south side of Biloxi is more heavily polluted than that of Back Bay, the oysters from these two different localities evidenced no material difference in the degree of pollution.

The oysters in Mississippi Sound off the south coast of Biloxi gave positive *B. coli* test in 0.01 c. c. shell liquor; the water from some places having *B. coli* in 0.1 c. c.

In Back Bay the oysters had *B. coli* in 0.01 c. c. shell liquor; the water in 1 c. c.

These results are not very surprising when consideration is given to the fact that these oyster beds are exposed to the sewage pollution from 9,000 people.

For the abatement of these conditions, there is a choice of two remedies—either the transfer of the oyster growth from the present beds to an uncontaminated location; or, the elimination of the pollution of the oyster grounds as they exist at present.

In Gulfport the oyster cultivation is of such small extent that it seems unjustifiable to make any change in the present disposal of sewage. The fact that samples "H. & E." located three-quarters of a mile from sewer outlet, have satisfactory ratings, 23 and 41, respectively, suggests as the most practical solution of the Gulfport situation that oyster cultivation or the gathering of oysters be prohibited within 1 mile or 1½ miles of the sewer outlet.

The condition at Biloxi is more complicated. To eliminate the present pollution of the Biloxi beds would necessitate the construction of a city sewage system and the discharge of its sewage not less than 1 mile from the nearest oyster grounds or an efficient disinfectant treatment of the sewage. Possibly 2 miles would be a safer distance and the direction of the currents, if present, would have to be studied so that the refuse would not be swept back to Biloxi. The sewage disposal at Ocean Springs would have to be treated the same as for Biloxi. Even with these precautions, it might prove necessary later on to prevent the pollution of the several streams entering Back Bay by the several small hamlets located thereon.

The alternative to this method of correction would be the abolishment of oyster culture on the present beds, and the restriction of culture or gathering of oysters to such grounds in the vicinity of Biloxi as can be demonstrated to be uncontaminated.

# A COMPARISON OF METHODS FOR THE DETERMINATION OF OXYGEN IN WATERS IN PRESENCE OF NITRITE.[1]

By ELIAS ELVOVE, *Technical Assistant.*

It has been pointed out by Phelps [2] that the study of a stream pollution problem, from the chemical point of view, resolves itself essentially into a study of the oxygen economy between that stream and the sewage which is discharged into it. That is, we need only to determine the power of the given sewage to absorb oxygen, under conditions as nearly as practicable to the natural conditions, and balance this oxygen requirement against the available oxygen resources of the stream into which the sewage is discharged. In following out such a plan, in connection with an investigation [3] of the polluting effect of the Washington sewage on the Potomac River, the writer has recently had occasion to compare several methods for determining dissolved oxygen which have been proposed by various authors, the results of which may be of interest in connection with the proposed change of the official method of the American Public Health Association, as described in their "Standard Methods for Water Analysis."

In the last edition (1912) of this treatise, the Winkler [4] method is recommended as the standard method. In the report of the committee, however, it is pointed out that the Winkler method gives unreliable results when it is applied to polluted waters containing nitrites or considerable organic matter. The committee recommends, therefore, pending investigation, that when polluted waters are analyzed for dissolved oxygen that corrections by means of a blank be made or that the Levy [5] method be used. According to this report the Levy method, "though more time consuming than the Winkler method, gives results in close accordance with the truth and it eliminates the errors caused by nitrogen compounds and organic matter." Reference is had to the modification of the Levy method used by the Metropolitan Sewerage Commission [6] of New York, which is recommended, and presumably the results recorded in the latter are largely relied upon for the statement quoted above,

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[1] Manuscript submitted for publication May 15, 1914.

[2] "The Chemical Measure of Stream Pollution and Specifications for Sewage Effluents," By Earle B. Phelps, Amer. Journal Pub. Health, **3**, 524–34 (1913).

[3] It is expected that the results of this investigation will shortly be published as a bulletin of the Hygienic Laboratory.

[4] Ber., **21**, 2843–54 (1888).

[5] Ann. de L'Observatoire de Mont-Souris, 1883 et seq.

[6] Report for 1910, p. 401.

which is to the effect that the Levy method eliminates the errors caused by nitrogen compounds such as nitrites.

From the report [1] of the Metropolitan Sewerage Commission of New York it is seen, however, that the original basis of their method was that modification of the Levy method which was employed by Letts and Adeney in the work published in the Fifth Report of the Royal Commission on Sewage Disposal. And an examination of the latter shows that Letts and Blake [2] had actually tried the effect of the presence of nitrite and had found that its presence would cause erroneous results, the extent of the deviation from the true figure for dissolved oxygen depending on the amount of nitrite present. Thus in the presence of 1.43 parts per million of nitrogen as nitrite the result for dissolved oxygen was 7.82 c. c. per liter, while in the absence of the nitrite the result obtained was only 7.47 c. c., a plus error of about 4.7 per cent; and in the presence of 14.3 parts per million of nitrogen as nitrite the result was 9.96 c. c. against 7.46 c. c. in the absence of the nitrite, a plus error of over 33 per cent. Letts and Blake explain the abnormal results in the presence of nitrite on the assumption that the nitrite acts as an oxidizing agent and oxidizes some of the ferrous hydroxide in addition to that which is oxidized by the dissolved oxygen, thus giving higher results, when in the calculation it is assumed that the entire oxidation of the ferrous hydroxide was brought about by the dissolved oxygen only. Furthermore, we see from the Report [3] of the Metropolitan Sewerage Commission of New York that when their method was compared with the Winkler and the gasometric methods, using samples of sea water from Boston and New York Harbors, the results obtained were in very close agreement and the very slight differences from the results obtained by the absolute or gasometric method were even in favor of the Winkler method.

An examination of the literature on the subject also shows that the Levy method has not given satisfactory results in the hands of some authors even in the absence of interferences from nitrite. Thus, Chlopin [4] obtained much lower results by the Levy method than by the Winkler even in the case of distilled water, after he had proven the accuracy of the latter method by direct comparisons with the results obtained gasometrically. Working with distilled water and duplicating each determination, Chlopin found a difference of 19.9 per cent in the average results of 11 sets of determinations, the Levy

[1] Ibid., p. 399.

[2] Fifth Report of the Royal Commission on Sewage Disposal, Appendix I, p. 616.

[3] Report for 1912, p. 302. The following results are reported, the figures representing cc. 0 per liter:

| METHOD. | | | |
|---|---|---|---|
| Metropolitan Sewerage Commission | 5.84 | 6.00 | 6.87 |
| Winkler | 5.88 | 6.04 | 6.56 |
| Gasometric | 5.91 | 6.08 | 6.70 |

[4] Arch. f. Hygiene, 32, 294-309 (1898).

method giving the lower result. With drinking water the differences in the results were still greater. The average result of 15 determinations carried out by both methods was 26.9 per cent lower by the Levy than by the Winkler method. According to Chlopin, the results obtained by the Levy method, with the same sample of water, will vary with the time which is allowed for the oxidation of the ferrous hydroxide by the dissolved oxygen. Thus, a sample of distilled water, which by the Winkler method was found to contain 6.17 c. c. of oxygen per liter, showed only 4 c. c. by the Levy method when the time allowed for the oxidation of the ferrous hydroxide was 1½ minutes, 4.6 c. c. after 3 minutes, 4.69 c. c. after 5 minutes, 4.72 c. c. after 15 minutes, 4.80 c. c. after 20 minutes, and 5.48 c. c. after 60 minutes. Thus, even when allowing an hour for the oxidation of the ferrous hydroxide by the dissolved oxygen, the result obtained was still considerably lower by the Levy method. Chlopin also investigated the effect of temperature and found that with the same sample of water, varying results are obtained if the oxidation of the ferrous hydroxide by the dissolved oxygen is allowed to take place at different temperatures. Thus, a sample of river water was found to contain 3.14 c. c. of oxygen per liter when the oxidation of the ferrous hydroxide took place for 10 minutes at a temperature of 5° C. The same water treated the same way but conducting the oxidation at 28° C. gave 5.69 c. c. With a sample of well water similar results were obtained. Allowing the oxidation to take place at 5° C., the result was 3.68 c. c., while at 30° C. the same water showed the presence of 4.46 c. c. of oxygen per liter. Chlopin, therefore, concludes that the Levy method gives inaccurate results and cannot be recommended.

That the method for determining dissolved oxygen through the oxidation of ferrous hydroxide gives low results even when allowing 30 minutes for the oxidation has also been the experience of Tiemann and Preusse.[1] These authors also record results which are in harmony with those obtained by Chlopin as to the effect of temperature on the oxidation of the ferrous hydroxide by the dissolved oxygen. Thus, when working with a water which actually contained 6 c. c. of oxygen per liter, only 3.6 c. c. were found by the ferrous hydroxide method when the oxidation was allowed to take place at 10° C. The same water, treated in the same way, but allowing the oxidation to take place at 37° C. gave a result of 4.92 c. c. Tiemann and Preusse also obtained similar results with other waters. With one water the result was 0.89 c. c. at 8° C., and 2 c. c. at 37.5° C. With the other water, the result was 1.12 c. c. at 3° C. and 3.20 c. c. at 37.5° C.

[1] Ber., 12, pp. 1784-5.

In replying to the work of Chlopin, Levy and Marboutin [1] point out that Chlopin erred in assuming that carbonate of potassium is used in the Levy method. The reagent which should be employed in the Levy method is the hydroxide of potassium. This is of interest in view of the fact that in the modification of the Levy method used by the Metropolitan Sewerage Commission of New York, to which reference has already been made, sodium carbonate is used in place of the hydroxide.

On the other hand, the Winkler method when used for determining the oxygen dissolved in distilled water or a natural water which is comparatively free from nitrite or organic matter, seems to have given highly satisfactory results in the hands of all who have investigated it. Winkler [2] himself proved its accuracy by comparison with very refined gasometric determinations. Later, Kisch [3] made a study of the Winkler method, comparing it with the Bunsen-Tiemann gasometric method and with the volumetric methods of Schützenberger and also that of Mohr, and reached the conclusion that none of the methods he investigated are so easy of application and so reliable as the Winkler method. Chlopin [4] also reaches similar conclusions with regard to the Winkler method. Chlopin compared the Winkler method with Bunsen's gasometric method, carrying out the latter in its classic form and observing all the precautions which are required for determining very small quantities of oxygen. His results showed that on the average there was only about 0.1 per cent difference between the results obtained gasometrically and those obtained by the Winkler method. He also determined gasometrically and by the Winkler method the dissolved oxygen in various natural waters which contained as low at 1 c. c. and as high as 9.98 c. c. of oxygen per liter, and although the amount of oxygen in the total gas varied between 4.65 and 35 per cent and one of the waters also contained a comparatively large amount of nitrite, the average difference in the results was only about 0.21 per cent. Likewise, Spitta [5] decided that the Winkler method is preferable to the Levy method. Korschun [6] compared the Winkler method with the method of Ramsay [7] and with the use of the "Tenax" apparatus of Müller, but concluded that neither of these methods can replace the Winkler method. Birge and Juday [8] compared the Winkler method with a gasometric procedure and obtained a generally satisfactory

[1] Bull. Soc. Chim., **19**, (3), 149-51 (1898).
[2] Loc. cit.
[3] Zeit. Angew. Chem., 1891, 105-8.
[4] Arch. f. Hygiene, **27**, 18-33 (1896).
[5] Arch. f. Hygiene, **38**, p. 220 (1900).
[6] Arch. f. Hygiene, **61**, 324-35 (1907).
[7] Jour. Soc. Chem. Ind., **20**, 1071-5 (1901).
[8] Wisconsin Survey Bulletin, No. 22, pp. 11-12 (1911)

agreement. According to Cronheim [1], the Winkler method is the most convenient method we have.

An examination of Winkler's paper, in which his method was first described, shows that he appreciated the effect of the presence of nitrite and proposed a modification for use in such cases. This modification is based upon the destruction of the nitrite through oxidation to nitric acid, in acid solution, by means of manganic chloride. The latter also oxidizes any strongly reducing organic matter which may be present, and thus safeguards against loss of iodine through its interaction with such organic matter. Since manganic chloride is formed in the course of the test, to apply this modification one need only keep the alkaline hydroxide and potassium iodide solutions separate and add the iodide solution last, after the hydroxide precipitate has been redissolved by the hydrochloric acid, doubling the amount of the latter and allowing a certain time for the oxidation of the nitrous acid to take place. According to Winkler this time should be two to three minutes. The amount of manganic chloride which is consumed in the oxidation of the nitrite is determined by a control experiment in which 100 c. c. of the water under investigation and 100 c. c. of distilled water are each treated with a definite amount (100 c. c.) of a specially prepared manganic chloride solution. The amount of the latter which remains after the mixtures have stood as long as in the first case (two to three minutes) is determined iodometrically, using the same thiosulphate solution which is used in the dissolved oxygen determination. The difference between the amount of the thiosulphate solution required for the mixture, in which distilled water was used and that in which the water under investigation was used, represents the amount of manganic chloride used for oxidation purposes by 100 c. c. of the water. This is then calculated for the amount of the water used in the dissolved oxygen determination, thus obtaining a correction in terms of the standard thiosulphate in use. Adding this to the amount of thiosulphate required in the first direct titration gives the corrected thiosulphate value. Using a water which contained 6.14 c. c. of oxygen per liter and which also contained about 1 part per million of $N_2O_3$ and added albumin, Winkler obtained by his modified procedure 6.08, 6.11, 6.16, and 6.11, or an average of 6.12 c. c.

Another modification of the Winkler method which renders it applicable in the presence of nitrite and organic matter has been proposed by Rideal and Stewart.[2] This modification is based on the same principle as Winkler's modification, namely, on the destruction of the nitrite through oxidation to nitric acid in acid solution and the simultaneous oxidation of any strongly reducing organic

[1] Zeit. Angew. Chem., 20, 1939–42 (1907).

[2] Analyst, 26, 141–8 (1901).

matter which may be present; but, instead of utilizing for this purpose the manganic chloride which is produced in the course of the dissolved oxygen determination, Rideal and Stewart use potassium permanganate to effect the oxidation. They titrate 50 c. c. of the sample with N/10 $KMnO_4$ after acidifying with 1 c. c. of concentrated $H_2SO_4$ till a faint pink color persists after 10 minutes. Knowing the volume of the bottle in which the dissolved oxygen determination is to be made, the amount of N/10 $KMnO_4$ required for that volume is calculated. The calculated amount plus an additional 0.1 c. c. is then placed in the bottle, together with 1 c. c. of sulphuric acid the bottle is filled with the liquid to be examined, mixed by rotation, and allowed to stand 5 or 10 minutes. The excess of permanganate is then destroyed by the addition of 0.5 c. c. of a 2 per cent solution of potassium oxalate. From this point the ordinary Winkler procedure is followed, but the amount of alkali added is increased so as to neutralize the sulphuric acid added. The tests which they carried out showed that this modification gives accurate results [1] even in the presence of comparatively large amounts (2 parts per 100,000) of nitrite and considerable organic matter.

This modification of the Winkler method has been investigated by the British Royal Commission on Sewage Disposal.[2] They compared the results obtained by the modified Winkler method with gasometric determinations on the same samples. These comparative tests were made on mixtures of 1 per cent sewage, some of which were prepared with tap water and others with sea water, and the comparisons between the results by the two methods were made on both incubated and non-incubated samples. In general, the agreements in the results obtained by the two methods were very satisfactory.

Recently Hale and Melia [3] have described another modification of the Winkler method for rendering it applicable in the presence of nitrite. In this modification the effect of the nitrite is counteracted by the use of potassium acetate solution, which is added after the

[1] In the paper of Rideal and Stewart reference is made to the methods of Schützenberger, Thresh, Romijn, Mohr, Lalieu, Blarez, Linossier, Levy, Letts and Blake, Ramsay, MacKay and Middleton, but they conclude that none of these methods is "so portable, rapid, and generally reliable" as the modified Winkler.

[2] Eighth Report, Vol. II, Appendix, pp. 93–102 (1913). The following results are reported, the figures representing c. c. of oxygen per liter:

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Tap-water mixture: | | | | | | | | | | | |
| Gasometrically | 6.2 | 6.0 | 5.4 | 5.0 | 4.1 | 3.0 | 4.2 | 4.2 | 0.9 | 2.9 | 0.2 |
| By Winkler | 6.6 | 5.9 | 5.5 | 5.0 | 4.0 | 3.0 | 4.0 | 4.2 | 0.9 | 2.9 | 0.1 |
| Sea-water mixture: | | | | | | | | | | | |
| Gasometrically | 5.5 | 4.7 | 3.9 | 3.5 | 2.7 | 3.3 | 3.2 | 1.6 | 2.7 | 2.5 | 0.2 |
| By Winkler | 5.7 | 5.0 | 4.0 | 3.6 | 2.8 | 3.2 | 3.1 | 1.5 | 2.5 | 2.3 | 0.1 |

[3] Jour. Ind. Eng. Chem., 5, 976–980 (1913). Referring to the interference of nitrite, Hale and Melia state that "Winkler, and also Max Müller, tried to correct the error by adding acid and iodide and making a separate titration and correction, but this could not accomplish the result since the reaction is catalytic and varies with the time of exposure to the air." This statement, however, appears to be erroneous, since Winkler (Ber. 21, 2843–54, 1888) and Max Müller (J. Soc. Chem. Ind., 8, 923, 1889; from Chem..Zeit.,

precipitate of the manganese hydroxides have been entirely dissolved by the hydrochloric acid and before the solution has been exposed to the air.

Having thus three modifications of the Winkler method it was thought desirable to carry out some tests and find out how the results compared. Since, however, the modification proposed by Winkler himself and that proposed by Rideal and Stewart are based on the same principle, it was thought sufficient for the present purpose to compare only one of these modifications with the acetate modification of Hale and Melia. In the case of the latter modification, it was thought desirable also to carry out a few experiments with the object of finding out more definitely the true explanation of the counteracting action of the potassium acetate on the nitrite interference. Hale and Melia apparently explain this counteracting effect of the acetate on the assumption that its function is "to neutralize the hydrochloric acid and render the solution acid with acetic acid." According to these authors, "after the solution contains only free acetic acid it may be exposed to the air without any danger of nitrite interference." And their reason for introducing the acetic acid indirectly through the acetate, instead of simply substituting acetic acid for the hydrochloric acid, is because they had found that acetic acid "would not readily entirely dissolve" the precipitate. It was thought desirable to test this explanation experimentally. It may be recalled that the nitrite interference is due primarily to the reaction between nitrous and hydriodic acids in the sense of the equation

$$HNO_2 + HI = H_2O + NO + I$$

according to which each molecule of nitrous acid liberates one atom of iodine, thus increasing the amount of iodine proportionately to the amount of nitrite present; and, secondly, the nitric oxide (NO) being readily oxidizable in the presence of oxygen, acts as a catalytic agent and continues indefinitely the liberation of iodine when in contact with atmospheric oxygen. Now, if the above explanation

13, 1188–90, 1889) do not correct for nitrite in the same way. As far as Winkler is concerned, the criticism of Hale and Melia does not apply, since Winkler does not correct, as does Max Müller, by determining the amount of nitrite iodometrically in a separate titration and subtracting the amount of iodine thus found due to the nitrite from the total iodine found in the direct determination of the dissolved oxygen. Winkler *destroys* the nitrite by allowing time for the manganic chloride in the solution to oxidize it to nitric acid, as has been shown. Hale and Melia also state that "as nitrite may be oxidized and quantitatively determined by permanganate by the method of Kinnicutt and Nef it was hoped that by allowing for a certain length of time contact in alkaline condition before acidifying, the manganese oxyhydrate could be made to destroy the nitrite and introduce thereby a known error." This procedure, however, failed completely, as the errors remained high and irregular. It would seem that Hale and Melia have also erred in their application of Kinnicutt and Nef's procedure (Sutton: "Volumetric Analysis," 10th ed., p. 288), since the latter carry out the oxidation in *acid* solution. Had they tried the oxidation in acid solution they probably would have hit upon the modification of Rideal and Stewart, to which they refer, but which apparently they have not tried.

of the counteracting effect of the potassium acetate is correct, namely, that it is due to the replacement of the hydrochloric acid by acetic acid, we should certainly find this counteracting effect where acetic acid is added in the first place. The following experiments were, therefore, carried out:

An aqueous solution of sodium nitrite and potassium iodide was prepared, containing 5 parts per million of nitrogen as nitrite and 0.7 gm. KI [1] per liter. Six glass-stoppered bottles, each of about 270 c. c. capacity, designated Nos. 1, 2, 3, 4, 5, and 6, respectively, were completely filled with this nitrite-iodide solution. To each of Nos. 1 and 2 there was added 1 c. c. of acetic acid, near the bottom, by means of a pipette, the stoppers replaced, and the contents mixed. To each of Nos. 3 and 4 there were similarly added 2 c. c. of the acetic acid; while to each of Nos. 5 and 6 there were similarly added 3 c. c. of the acetic acid. In every case a distinct iodine color was imparted to the solution immediately after mixing with the acetic acid. After the closed bottles had stood at room temperature (about 21° C.) for an hour, these iodine colors were compared with the color of an iodine solution which contained an amount (45.3 p. p. m.) of iodine equivalent to the amount of nitrite in the solution as calculated from the above equation of the reaction between nitrous and hydriodic acids. All showed a much deeper iodine color than this standard iodine solution, showing some catalytic action. The amount of iodine in each was also determined by titrating 200 c. c. of each solution with N/40 $Na_2S_2O_3$. To each of Nos. 2, 4, and 6, however, there were added, just before the sample was taken for titration, 2 c. c. of a concentrated (100 gms. to 100 c. c.) potassium acetate solution. The results obtained are given in Table I.

TABLE I.—*Showing ineffectiveness of an acetic acid medium for preventing the return of the color of the starch indicator and the corresponding effectiveness of potassium acetate.*

| No. of experiment. | Amount of acetic acid used (c. c.). | Potassium acetate. | N/40 $Na_2S_2O_3$ required for 200 c.c. of solution (c.c.). | Character of end-reaction. | Additional N/40 $Na_2S_2O_3$ required to discharge color 10 minutes after: | | |
|---|---|---|---|---|---|---|---|
| | | | | | First discharge (c. c.). | Second discharge (c. c.). | Third discharge (c. c.). |
| 1 | 1 | Absent | 6.78 | Color returned | 1.10 | 0.82 | 0.75 |
| 2 | 1 | Present | 6.65 | Sharp | None. | ..... | ..... |
| 3 | 2 | Absent | 9.97 | Color returned | 1.80 | 1.50 | 1.20 |
| 4 | 2 | Present | 9.60 | Sharp | None. | ..... | ..... |
| 5 | 3 | Absent | 10.25 | Color returned | 3.07 | 2.28 | 1.55 |
| 6 | 3 | Present | 10.00 | Sharp | None. | ..... | ..... |

[1] Approximately this amount of KI is added per liter of the water in applying the Winkler method according to Hale and Melia.

It is seen from Table I that the amount of N/40 $Na_2S_2O_3$ required was in all cases much in excess of 2.85 c. c. which should have been required if the free iodine in the solutions had been only the product of the action of the nitrous acid originally present. Moreover, the excess is greater the greater the acid concentration. These results show, therefore, that not only is an acetic-acid medium incapable of preventing the primary reaction between the nitrous and hydriodic acids, but even the secondary catalytic reaction, between the nitric oxide and the free oxygen in the water or the atmosphere, takes place where acetic acid is the only acid added. On the other hand, potassium acetate effectively prevents the return of the blue color of the starch indicator.

A brief consideration of the facts in the light of physical chemistry makes it evident that acetic acid differs from hydrochloric in possessing a smaller dissociation coefficient, and that to this fact alone is due its lessened influence upon the nitrite-iodide reaction. But even acetic acid dissociates sufficiently to exert a disturbing influence of too great magnitude to be ignored. In order to reduce errors due to this source it is necessary to still further depress the dissociation and in consequence the free hydrogen ion concentration by the presence of a considerable excess of the acetate. It is of great importance, therefore, in the working of the method that a correct balance be always maintained between the strengths of the hydroxide and acid solutions used and the amount of acetate finally added. It is not merely necessary to "neutralize" the hydrochloric acid so that the solution contains only free acetic acid; but it is necessary, furthermore, to add a sufficient excess of acetate to reduce the hydrogen ion concentration to a safe value.

Hale and Melia also allowed some solutions, after they were ready for titration to stand overnight in contact with air before they were titrated. All gave considerably lower results, from which they draw the conclusion that "solutions acidified ready for titration should not stand in contact with air for several hours before titrating." It is difficult to see, however, why exposure to the air should give *lower* results. On the contrary, if the air has any effect at all on the results, it would seem reasonable to expect that this effect would manifest itself by giving higher results, since we would expect that the oxidizing action of the atmosphere would liberate more iodine. It seemed probable that the true explanation of the low results of Hale and Melia referred to above is not that the solutions had been exposed to the air but simply that the potassium acetate itself had rendered the iodine solution less stable. In order to find out if this assumption has a basis in fact, the following experiments were carried out:

Distilled water was shaken in the presence of air so as to nearly saturate it with oxygen at the temperature of the room (about 20°

C.). A nitrite solution, containing 1,000 parts per million of nitrogen as nitrite [1] which had likewise been shaken so as to nearly saturate it with oxygen at the room temperature, was then mixed with a portion of the distilled water in the ratio of 1 part of the former to 199 parts of the latter, thus giving a solution which contained 5 parts per million of nitrogen as nitrite. The remaining distilled water was used for determining, by the ordinary Winkler method, the amount of dissolved oxygen present. A number of glass-stoppered bottles, each of about 270 c. c. capacity, were then carefully filled with the diluted nitrite solution, by means of a long-stemmed funnel, and the dissolved oxygen determined by the Winkler method as modified by Hale and Melia. The time interval, however, between the mixing with the potassium acetate and the titration was varied from zero to 48 hours. After the addition of the 2 c. c. of the potassium acetate solution (100 gms. to 100 c. c.), the stopper was quickly replaced and the contents mixed by inverting the bottle about a dozen times. A 200 c. c. sample from one bottle was then at once pipetted out and titrated with N/40 $Na_2S_2O_3$. The other bottles were similarly treated after the *closed* bottles had stood for the stated intervals of time. During these intervals, the bottles were kept in a dark room, the temperature of which was fairly constant (about 19° C.). The results obtained are given in Table II.

TABLE II.—*Effect of varying the time interval between the mixing with the potassium acetate and the titration.*

Dissolved oxygen in the water.....8.95 p. p. m.
Added nitrogen as nitrite.....5.00 p. p. m.

| No. of experiment. | Interval between the mixing with the potassium acetate and the titration. | N/40 $Na_2S_2O_3$ required for 200 c. c. of solution [2] (c. c.). | Results for dissolved oxygen expressed in percentage of amount actually present. | Degree of error expressed in percentage. |
|---|---|---|---|---|
| 1 | None | 10.56 | 118.0 | +18.0 |
| 2 | 5 minutes | 9.53 | 106.5 | + 6.5 |
| 3 | 15 minutes | 8.95 | 100.0 | None. |
| 4 | 30 minutes | 8.75 | 97.8 | − 2.2 |
| 5 | 60 minutes | 8.57 | 95.8 | − 4.2 |
| 6 | 120 minutes | 8.25 | 92.2 | − 7.8 |
| 7 | 180 minutes | 7.89 | 88.1 | −11.9 |
| 8 | 240 minutes | 7.72 | 86.2 | −13.8 |
| 9 | 24 hours | 5.81 | 64.9 | −35.1 |
| 10 | 48 hours | 4.25 | 47.5 | −52.5 |

The results given in Table II show that low results were obtained even when the solutions were kept in closed bottles which had been completely filled with these solutions. Therefore, Hale and Melia's

[1] Used $NaNO_2$.

[2] As control experiments, similar bottles were filled with an iodine solution approximately equivalent in strength to the dissolved oxygen in the water used in the experiments, and these were allowed to stand under the same conditions as the others. 200 c. c. of this iodine solution, when titrated immediately after the bottles were filled, required 9.89 c. c. N/40 $Na_2S_2O_3$; after having stood for 48 hours, the result was 9.80 c. c., showing that the change in the iodine solution which is not due to the potassium acetate is practically negligible.

admonition "that solutions acidified ready for titration should not stand *in contact with air* [1] for several hours before titrating" must be regarded as not sufficient warning to guard against error, and it may be misleading. Furthermore, these results show that not only should such solutions not be kept for a period of several hours before titrating, but even a period of only one hour was sufficient to cause low results. When such solutions were kept for 2 hours before titrating, the result obtained was 25.8 per cent lower than that obtained with an exactly similar solution, but which was titrated immediately after the mixing with the potassium acetate. Again, the results show that not only does the acetate modification of the Winkler method give low results when the solutions mixed with the acetate are kept longer than a certain period, but, in the presence of nitrite, the results may also be too high if there is not a sufficiently long period intervening between the addition of the potassium acetate and the titration of the iodine. Thus, when the titration was carried out immediately after the mixing with the acetate, the result obtained was 18 per cent too high. When an interval of 5 minutes was allowed to intervene between the mixing with the acetate and the titration, the result was still 6.5 per cent too high. When the titration was carried out after an interval of 15 minutes, however, the result obtained was in agreement with the amount of dissolved oxygen actually present. In order to prove conclusively that the low results which are obtained with the acetate modification of the Winkler method, when the solutions with the acetate in them are allowed to stand longer than a certain interval, are due simply to the interaction between the potassium acetate and the iodine, another series of similar experiments was carried out, in which the nitrite and all the reagents required in the Winkler method were entirely omitted. The procedure was as follows: A dilute solution of iodine was prepared by diluting the ordinary N/10 iodine with distilled water. Glass-stoppered bottles similar to those used in the first series were then similarly filled with this dilute iodine solution. The contents of each bottle was then similarly mixed with 2 c. c. of the potassium acetate solution and allowed to stand for varying intervals, under similar conditions as those of the first series. At the end of the stated intervals, 200 c. c. were pipetted out and titrated with N/40 $Na_2S_2O_3$. The results obtained are given in Table III.

[1] The original is not in *italics*.

TABLE III.—*Effect of the potassium acetate*[1] *on the stability of the iodine solution.*

| No. of experiment. | Interval between the mixing with the potassium acetate and the titration. | N/40 $Na_2S_2O_3$ required for 200 c. c. of solution[2] (c. c.). | Amount of I found expressed in percentage of that originally present. |
|---|---|---|---|
| 1 | None | 9.60 | 100.0 |
| 2 | 5 minutes | 9.55 | 99.5 |
| 3 | 15 minutes | 9.43 | 98.2 |
| 4 | 30 minutes | 9.34 | 97.3 |
| 5 | 60 minutes | 9.03 | 94.1 |
| 6 | 120 minutes | 8.48 | 88.3 |
| 7 | 180 minutes | 7.95 | 82.8 |
| 8 | 240 minutes | 7.46 | 77.7 |
| 9 | 24 hours | 4.32 | 45.0 |
| 10 | 48 hours | 2.57 | 26.8 |

The results given in Table III show conclusively that there is quite a marked interaction between the potassium acetate and the iodine which results in the loss of free iodine. As compared with the results of the first series (Table II) there is noticeable a slightly greater proportion of loss of free iodine. This may be explained as being due to the fact that in these experiments, although each bottle received 2 c. c. of the potassium acetate solution as in the first series, the actual concentration of potassium acetate in these solutions was slightly greater than in the first series, since in the latter case some of the potassium acetate was used up in its reaction with the free HCl present. We must conclude, therefore, that in order to obtain accurate results by the acetate modification of Hale and Melia, in the presence of nitrite, the titration should be carried out immediately after a definite interval has elapsed since the mixing with the potassium acetate. This interval should be long enough to permit all the excess iodine, which is due to the interaction between the nitrous and hydriodic acids, to recombine but not too long to cause a considerable loss of iodine through the continued action of the potassium acetate on the free iodine in the solution. Under the present conditions (5 p. p. m. of nitrite and temperature about 20° C.), an interval of 15 minutes was found to be the proper time to allow between the mixing with the potassium acetate and the titration.

In brief, the satisfactory results reported by Hale and Melia seem to be due to a fortunate counterbalancing of two errors of opposite tendencies. For it must be obvious that the nitrite-iodide reaction does take place upon acidification and is complete before the addition of the acetate. The acetate can not, therefore, prevent this reaction. The counteracting tendency seems to be due to one or more reactions by which the iodine is reduced from the excessive initial value, through the correct value, and on continuously to values that are below the

[1] The potassium acetate used bore the manufacturer's "C. P." label and it was also found to comply with the purity requirements of the U. S. Pharmacopœia.

[2] For controls see foot of Table II.

true value. The counteracting is, therefore, a time function. The nature of this secondary reducing reaction has not yet been determined, and is now being made the subject of a new investigation. It may be noted, however, that it varies with various brands of acetate that have been tried.

In order to compare the results obtained by the permanganate modification of the Winkler method under conditions similar to those under which the acetate modification was tried, a nitrite solution was prepared in the same way as in the latter case. And just as was done in the case of the experiments with the acetate modification, so in this case the dissolved oxygen was determined by the ordinary Winkler method in the absence of the nitrite and by the permanganate modification after the nitrite had been added. The distilled water used was shaken so as to nearly saturate it with atmospheric oxygen at the room temperature (about 19° C.) and then was mixed with the strong nitrite solution (1,000 p. p. m.), in the ratio of 1 part of the latter to 199 parts of the former. Glass-stoppered bottles of about 270 c. c. capacity, similar to those used in the experiments with the acetate modification, were similarly filled with this diluted nitrite solution and the dissolved oxygen was determined by the permanganate modification of the Winkler method, carried out [1] as follows: The liquid in the bottle received, under the surface, 0.7 c. c. of $H_2SO_4$ (sp. gr., 1.84) and 1 c. c. N/5 $KMnO_4$ and, after carefully replacing the stopper so as to exclude all air, the contents were mixed by rotating the bottle several times. After having stood for 20 minutes the color of the excess permanganate was discharged by the addition of 1 c. c. of a 2 per cent potassium oxalate solution. When the color had entirely disappeared there were added 1 c. c. of manganous sulphate solution (48 gms. to 100 c. c.) and 3 c. c. of an alkaline KI solution which contained 70 grams KOH and 10 grams KI in 100 c. c. From this point the ordinary Winkler technique was followed, using 2 c. c. of concentrated HCl (sp. gr., 1.18) to redissolve the precipitated manganese hydroxides. All the reagents were added [2] in the usual way, under the surface of the liquid. The results obtained are given in Table IV.

[1] The procedure used in carrying out the permanganate modification is essentially that employed by the Royal Commission on Sewage Disposal (8th Report, Vol. II, Appendix, p. 97), except that it was deemed advantageous to use here a somewhat stronger permanganate solution (N/5 instead of N/8). It also differs slightly from the latter in that the manganous sulphate solution, which is called for in the detailed procedure of the acetate modification of Hale and Melia, was used in place of the manganous chloride solution used by the Royal Commission.

[2] It was found that considerable time can be saved when there are a comparatively large number of determinations to carry out, by using burettes for adding the reagents, instead of the usual pipettes. This has also the advantage that it avoids contamination of the reagents by what adheres to the pipette from the different samples into which it is immersed.

TABLE IV.—*Showing results by the permanganate modification in presence of nitrite.*

Dissolved oxygen in the water ..... 9.07 p. p. m.
Added nitrogen as nitrite ..... 5.00 p. p. m.
Amount of potassium oxalate added to each bottle ..... 1 c. c. of 2 per cent sol.
Amount of KI added to each bottle ..... 0.3 gm.

| Number of experiment. | N/40 $Na_2S_2O_3$ required for 200 c. c. of solution (c. c.). | Dissolved oxygen found [1] (p. p. m.). | Error (p. p. m.). |
|---|---|---|---|
| 1 | 8.85 | 8.92 | —0.15 |
| 2 | 8.83 | 8.90 | — .17 |
| 3 | 8.85 | 8.92 | — .15 |
| 4 | 8.80 | 8.87 | — .20 |
| 5 | 8.82 | 8.89 | — .18 |
| 6 | 8.83 | 8.90 | — .17 |
| Average | 8.83 | 8.90 | — .17 |

The results given in Table IV show that concordant results were obtained by the permanganate modification even in the presence of a comparatively large amount of nitrite and that the average result (8.90 p. p. m.) thus obtained, although differing slightly from the actual amount (9.07 p. p. m.) of dissolved oxygen in the water, was nevertheless quite close to it. Since such a slight difference seemed to persist in all the experiments, however, it was thought desirable to try to find out the cause of this. It seemed probable that the slight excess of oxalic acid which remains in the solutions, by virtue of its power to reduce manganic salts to the manganous condition, might be the cause of these slight differences from the true figure for dissolved oxygen. Accordingly a series of experiments was carried out on the effect of the presence of varying amounts of oxalic acid on the results for dissolved oxygen by the ordinary Winkler method in the absence of nitrite. In carrying out the latter method, the same $MnSO_4$ and alkaline KI solutions were employed as in the case of the permanganate modification, but the volume of the alkaline KI solution used was the same as that of the $MnSO_4$ solution,[2] namely, 1 c. c. These experiments, like the preceding, were carried out with distilled water which had been shaken so as to nearly saturate it with atmospheric oxygen at the room temperature (19.5° C.). Glass-stoppered bottles similar to those used in the previous experiments, were similarly filled with this distilled water and each bottle then received 1 c. c. of an oxalic acid solution, the strength of which varied in the different experiments, the limits being N/100 and N/1 oxalic acid. The results obtained are given in Table V.

[1] According to the Royal Commission on Sewage Disposal (8th Report, Vol. II, Appendix, p. 97), we may assume that the concentrated KOH reagent contains practically no oxygen in solution. Allowance of 0.07 p. p. m. was therefore made for the extra 2 c. c. of the alkaline KI used in this modification.

[2] According to Winkler (Ber., 21 p. 2844) 1 c. c. of each of the reagents is ordinarily sufficient, except in cases where the water contains considerable carbonate or much dissolved oxygen.

TABLE V.—*Effect of the presence of varying amounts of oxalic acid on the results for dissolved oxygen by the Winkler method.*

Dissolved oxygen in the water ..... 9.08 p. p. m.
Amount of KI added to each bottle ..... 0.1 gm.
Time interval between the addition of the HCl and the titration ..... 10 minutes.

| No. of experiment. | Amount of oxalic acid added. | N/40 $Na_2S_2O_3$ required for 200 c. c. of solution [1] (c. c.). | Dissolved oxygen found (p. p. m.). | Error (p. p. m.). |
|---|---|---|---|---|
| 1 | 1 c. c. N/100 | 9.04 | 9.04 | −0.04 |
| 2 | 1 c. c. N/50 | 9.06 | 9.06 | − .02 |
| 3 | 1 c. c. N/10 | 8.75 | 8.75 | − .33 |
| 4 | 1 c. c. N/5 | 8.48 | 8.48 | − .60 |
| 5 | 1 c. c. N/2 | 7.77 | 7.77 | −1.31 |
| 6 | 1 c. c. N/1 | 7.11 | 7.11 | −1.97 |

From the results given in Table V, we see that while very small amounts of oxalic acid did not interfere appreciably with the results for dissolved oxygen, there was a considerable lowering of the results when the amounts of oxalic acid present were greater. Comparing the lowering obtained here when 1 c. c. of N/5 oxalic acid was present (Experiment No. 4), which is about the amount added in the preceding experiments, we note that the effect of the oxalic acid was here much greater than the previous error. This may be partly explained by the fact that in the preceding experiments, although the amount of oxalate added (1 c. c. of a 2 per cent solution) is even slightly more [2] than the equivalent of the 1 c. c. of N/5 oxalic acid, still the actual amount of oxalic acid remaining in the solution was probably less, since a portion of the oxalate was used up in reducing the slight excess of permanganate remaining in the solution. Since, however, Experiment No. 3, where the amount of oxalic acid used was only 1 c. c. of N/10 also shows a greater lowering than that found in the preceding experiments, it indicated that this greater effect of the oxalic acid here is probably due to some other difference in the conditions of the two series of experiments. It was thought that this difference might be due to the different amounts of KI present in the two cases. A series of experiments was therefore carried out to determine the effect of a comparatively larger amount of KI on the oxalic acid interference. These experiments were an exact repetition of the experiments of Table V except that each bottle received 2 c. c. of a 30 per cent KI solution before the addition of the oxalic acid and

[1] According to Winkler (Ber. **21**, p. 2845), the error which may be introduced by disregarding in the calculations the volume of the reagents added is neglible even when 2 c. c. of each of the reagents are added. Similar views are held by the Royal Commission on Sewage Disposal (8th Report, Vol. II, Appendix, p. 97). And since these views are especially applicable to the present case, where only 1 c. c. of each of the reagents was used, no correction was therefore introduced for the volume of the reagents added. Furthermore, by pipetting out 200 c. c. of the solution in the bottle, after it had been acidified and the equivalent amount of iodine liberated, and titrating this portion of the solution with N/40 $Na_2S_2O_3$, the burrette readings in c. c. give directly the dissolved oxygen in p. p. m., thus saving the time required for the calculations by the formula given in the "Standard Methods for Water Analysis," A. P. H. A. (1912). It also becomes unnecessary to calibrate each of the bottles in which the samples are collected, since by working as thus outlined it is unnecessary to know the exact capacity of each bottle.

[2] 1 c. c. of the 2 per cent potassium oxalate solution is equivalent to about 1.1 c. c. N/5 oxalic acid.

the reagents for the determination of the dissolved oxygen. As in the previous experiments, the distilled water used was shaken so as to nearly saturate it at the room temperature (18.6° C.). The results obtained are given in Table VI.

TABLE VI.—*Showing counteracting effect of KI on the oxalic acid interference.*

Dissolved oxygen in the water ..... 9.32 p. p. m.
Amount of KI added to each bottle ..... 0.7 gram.
Time interval between the addition of the HCl and the titration ..... 10 minutes.

| No. of experiment. | Amount of oxalic acid added. | N/40 $Na_2S_2O_3$ required for 200 c.c. of solution (c. c.). | Dissolved oxygen found (p. p. m.). | Error (p. p. m.). |
|---|---|---|---|---|
| 1 | 1 c. c. N/100 | 9.26 | 9.26 | −0.06 |
| 2 | 1 c. c. N/50 | 9.26 | 9.26 | − .06 |
| 3 | 1 c. c. N/10 | 9.27 | 9.27 | − .05 |
| 4 | 1 c. c. N/5 | 9.31 | 9.31 | − .01 |
| 5 | 1 c. c. N/2 | 9.22 | 9.22 | − .10 |
| 6 | 1 c. c. N/1 | 9.17 | 9.17 | − .15 |

The results given in Table VI show that potassium iodide has the power of counteracting the oxalic acid interference. Thus, when we compare the results of experiments Nos. 3 to 6 of Table V with the results of the corresponding experiments of Table VI, we see that the increasing of the KI in the solution from 0.1 gram to 0.7 gram has served to counteract the harmful effect of the oxalic acid on the results for dissolved oxygen, giving normal results in experiments Nos. 3 and 4, and almost normal results in experiments Nos. 5 and 6. The series of experiments of Table IV were, therefore, repeated with the use of more KI and with less oxalate. The alkaline KI solution used was made up to contain 15 grams KI per 100 c. c., thus giving 0.45 grams KI in the 3 c. c. of the reagent which were used in each determination; while the potassium oxalate solution used was reduced in strength from 2 to 1 per cent but the same volume of it was used as in the previous experiments, namely, 1 c. c. The results obtained are given in Table VII.

TABLE VII.—*Showing results by the permanganate modification in presence of nitrite when using an increased amount of KI and less oxalate.*

Dissolved oxygen in the water ..... 8.87 p. p. m.
Added nitrogen as nitrite ..... 5.00 p. p. m.
Amount of potassium oxalate added to each bottle ..... 1 c. c. of 1 per cent solution.
Amount of KI added to each bottle ..... 0.45 gram.

| No. of experiment. | N/40 $Na_2S_2O_3$ required for 200 c. c. of solution (c. c.). | Dissolved oxygen found [1] (p. p. m.). | Error (p. p. m.). |
|---|---|---|---|
| 1 | 8.75 | 8.82 | −0.05 |
| 2 | 8.71 | 8.78 | − .09 |
| 3 | 8.75 | 8.82 | − .05 |
| 4 | 8.76 | 8.83 | − .04 |
| 5 | 8.72 | 8.79 | − .08 |
| 6 | 8.74 | 8.81 | − .06 |
| Average | 8.74 | 8.81 | − .06 |

[1] See corresponding footnote to Table IV.

As compared with the results of Table IV, the results given in Table VII show that by increasing the amount of KI in each bottle from 0.1 gram to 0.45 gram and decreasing the amount of potassium oxalate added from 1 c. c. of the 2 per cent solution to 1 c. c. of the 1 per cent solution, the error in the results for dissolved oxygen was decreased from −0.17 p. p. m. to −0.06 p. p. m., which latter may be regarded as within the limit of the experimental error of the ordinary Winkler method. In carrying out the permanganate modification of the Winkler method in bottles of about 270 c. c. capacity, it is well, therefore, to have at least 0.45 gram KI present and of excess potassium oxalate not more than 1 c. c. of the 1 per cent solution. By using 3 c. c. of the alkaline KI which contains 15 grams KI per 100 c. c., this amount of KI will be present in the solution. For most ordinary river waters and largely diluted sewage effluents, 1 c. c. of N/10 $KMnO_4$ to about 270 c. c. of the water or diluted effluent would ordinarily be sufficient to leave a slight excess of permanganate at the end of the 20-minute period of oxidation. In these cases, therefore, the permanganate modification can be carried out by adding 1 c. c. of N/10 $KMnO_4$ after acidifying with 0.7 c. c. of concentrated $H_2SO_4$, allowing to stand 20 minntes and then destroying the excess permanganate by the addition of 1 c. c. of 1 per cent potassium oxalate. Should the permanganate color disappear entirely before the expiration of the 20 minutes, 1 more c. c. of the N/10 $KMnO_4$ can be added, and so on until there is a slight excess of permanganate 20 minutes after the last addition. The excess of permanganate can then be destroyed by the addition of 1 c. c. of the 1 per cent potassium oxalate solution, and a suitable correction introduced for the extra volume of the $KMnO_4$ solution added. But even when dealing with strongly reducing liquids, one can ordinarily avoid the necessity for introducing a correction factor by using stronger solutions and adjusting the strength of the permanganate so that 1 c. c. of it will leave a slight excess at the end of 20 minutes, which will be destroyed by 1 c. c. of the 1 per cent potassium oxalate solution. And having chosen such a permanganate solution, if it should occasionally happen that 1 c. c. of it does not leave a slight excess at the end of the 20 minutes, one can add another c. c. of the N/10 $KMnO_4$, the excess of which will then be reducible by 1 c. c. of the 1 per cent potassium oxalate solution. On the other hand, should the exceptional case be of the kind where the excess of $KMnO_4$ remaining is not entirely reducible by 1 c. c. of the 1 per cent potassium oxalate, which can often be judged by the fact that the remaining permanganate color will be deeper than that produced by 1 c. c. of N/10 $KMnO_4$ in an equal volume of distilled water, this will be an indication that it is necessary to use 1 c. c. of the stronger (2 per cent) potassium oxalate solution. Should, however, the case be so exceptional that none of these changes in the procedure is appli-

cable the determination would have to be repeated with a permanganate solution of more suitable strength, or with the N/10 $KMnO_4$ and correcting for the extra volume if more than 2 c. c. of it is required.

Having found that in order to obtain accurate results for dissolved oxygen by the Winkler method the amount of oxalic acid in the solution should not exceed a certain maximum, it was thought desirable to also find out the effect of oxalic acid on the stability of the iodine solution which is produced in the course of the Winkler method. For according to the "Standard Methods for Water Analysis" of the American Public Health Association [1] regarding the Winkler method, after the acid has been added and the equivalent amount of iodine liberated, "there is no further change, and the rest of the operation may be conducted at leisure." Now, if the oxalic acid remaining in the solution when the permanganate modification of the Winkler method is used should appreciably affect the stability of the iodine solution, the advantage above referred to regarding the keeping qualities of the iodine solution would be lost. A series of experiments was, therefore, carried out as follows: A dilute solution of iodine was prepared by diluting the ordinary N/10 iodine with distilled water so as to obtain an iodine solution of about the same strength as that produced in the course of the Winkler method when applied to water which is saturated with oxygen at room temperature (about 18° C.). Fourteen glass-stoppered bottles, each of about 270 c. c. capacity, were filled with this diluted iodine solution, and to each of 12 of these bottles there was added, by displacement, 1 c. c. of an oxalic acid solution, the stoppers replaced, and the contents mixed, while the remaining 2 bottles constituted the controls. Three different strengths of oxalic acid were used, and each was allowed to remain in contact with the iodine for four different periods. The details and results are given in Table VIII.

TABLE VIII.—*Effect of oxalic acid on the stability of the iodine solution.*

| No. of experiment. | Amount of oxalic acid added. | Time bottles were kept before titrating (hours). | N/40 $Na_2S_2O_3$ required for 200 c. c. of solution (c. c.). | Error expressed in c. c. of N/40 $Na_2S_2O_3$. |
|---|---|---|---|---|
| 1 | None | 1 | 9.49 | ..... |
| 2 | 1 c. c. N/10 | 1 | 9.40 | −0.09 |
| 3 | 1 c. c. N/5 | 1 | 9.38 | − .11 |
| 4 | 1 c. c. N/1 | 1 | 9.54 | + .05 |
| 5 | 1 c. c. N/10 | 4 | 9.48 | − .01 |
| 6 | 1 c. c. N/5 | 4 | 9.51 | + .02 |
| 7 | 1 c. c. N/1 | 4 | 9.50 | + .01 |
| 8 | 1 c. c. N/10 | 24 | 9.55 | + .06 |
| 9 | 1 c. c. N/5 | 24 | 9.53 | + .04 |
| 10 | 1 c. c. N/1 | 24 | 9.53 | + .04 |
| 11 | 1 c. c. N/10 | 48 | 9.56 | + .07 |
| 12 | 1 c. c. N/5 | 48 | 9.61 | + .12 |
| 13 | 1 c. c. N/1 | 48 | 9.40 | − .09 |
| 14 | None | 48 | 9.58 | + .09 |
| Average | | ..... | 9.50 | + .01 |

[1] 1912 ed., p. 61.

The results given in Table VIII show that the amounts of free iodine in the solutions were not appreciably reduced by the oxalic acid even after standing 48 hours and even when the amount of oxalic acid in the solution was about ten times as much as the maximum which can remain in the solution when 1 c. c. of 1 per cent potassium oxalate is used in the permanganate modification of the Winkler method. Therefore, so far as the very slight excess of oxalic acid is concerned, the keeping qualities of the iodine solution remain in the case of the permanganate modification just as in the case of the ordinary Winkler method. These results also show that the oxalic acid interference, which was found in the case of the experiments of Table V, was not due to the reducing action of the oxalic acid on the iodine, but probably to its reducing action on the manganic chloride formed in the course of the test, which then manifested itself through the liberation of a proportionately less amount of iodine and consequently indicated a proportionately lower result for dissolved oxygen.

We are now in a position to briefly discuss the relative merits of the several methods for determining dissolved oxygen referred to above. We have seen that, according to Letts and Blake,[1] in the presence of considerable amounts of nitrite, the Levy method does not give accurate results. We have also seen that, according to Chlopin,[1] the Levy method gives inaccurate results even in the absence of nitrite. Gill [2] has also pointed out that the "blank" determination required in the Levy method is open to the objection that if there are pieces of solid, easily putrescible matter, in the sample in which the oxygen was determined and not in the blank, these would use up the permanganate and give rise to low results for dissolved oxygen. Then there is the objection to the Levy method that it calls for a specially constructed pipette and therefore lacks the simplicity and convenience of the Winkler method whereby the determination can be carried out in an ordinary glass-stoppered bottle. Furthermore, according to Letts [3] and also according to Levy [4] himself, potassium bichromate should be substituted for potassium permanganate when dealing with sea water or other liquid which contains much chloride; while the difficulties attending the use of the bichromate on small boats and in rough weather are almost insurmountable.[5] Finally, there is the objection to the Levy method that each determination requires a second determination or the so-called "blank" test, thus practically *doubling* the work and the amount of time consumed. We

[1] Loc. cit.

[2] Report of the Metropolitan Sewerage Commisssion of New York (1910), pp. 400-401.

[3] Fifth Report Royal Commission on Sewage Disposal, Appendix VI, pp. 221-226.

[4] See Mason: "Examination of Water," 4th ed., p. 107 (footnote).

[5] See report of the Metropolitan Sewerage Commission of New York (1912), p. 300.

must conclude therefore that, in comparison with the Winkler method, the Levy method is decidedly disadvantageous.

Of the permanganate and acetate modifications of the Winkler method, both of which have for their object the extension of the usefulness of the Winkler method, the permanganate modification appears to be more advantageous than the acetate modification, since the latter does not counteract the harmful effects of organic matter or other reducing substances which may be present along with the nitrite. That such interfering substances may be present has been shown by Hefelmann and Barth.[1] Likewise, Cronheim[2] found a very active reduction of the iodine by the organic matter in the waters which he investigated. Thus the liberated iodine in the case of a water, which when titrated immediately was found to be equivalent to 9.53 c. c. N/100 $Na_2S_2O_3$, showed an equivalent of only 8.75 c. c. of the same solution after the iodine had been allowed to act on the organic matter of the water for 1 minute; and in similar terms, 8.15 c. c. after 2 minutes, 7.82 c. c. after 3 minutes, and 7.79 c. c. after 4 minutes. According to Hale and Melia, organic matter has no effect on the results for dissolved oxygen by the Winkler method. In the experiments,[3] however, which they cite in support of this conclusion, the only sewage mixture which gave a sharp end reaction, showed 6.91 p. p. m. of oxygen against the theoretical amount of 7.22 p. p. m., a lowering of 0.31 p. p. m.,[4] or about 4.3 per cent. This experiment of Hale and Melia, therefore, can hardly be regarded as conclusively "showing lack of effect of organic matter." Besides, the term "organic matter" is so general and the composition of sewages and sewage effluents is so much subject to variation that even if we should find that a certain sewage from a certain locality has absolutely no effect on the results, it would not necessarily mean that all the different sewages from that locality would behave in exactly the same way; and certainly we would have no valid reason for assuming that sewages even from entirely different sources would likewise be free from strongly reducing organic matter or other reducing substances which might interfere with the results for dissolved oxygen by the Winkler method. It is clear, therefore, that in order to render the Winkler method applicable even in such cases, another modification is necessary. It is reasonable, therefore, that we should prefer as a general method the permanganate modification which renders the Winkler method applicable in the presence of nitrite and organic matter, rather than the acetate modification which is designed only to counteract the interfering effects of nitrite.

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[1] Chem. Zeitung, **13**, 1337 (1889).

[2] Zeit. Angew. Chem. **20**, 1939–42 (1907).

[3] Jour. Ind. Eng. Chem., **5**, p. 979 (Table VI).

[4] According to these same authors, the Winkler method in routine work ordinarily checks "within 0.1 p. p. m. oxygen."

## SUMMARY.

In comparison with the Winkler method, the Levy method is decidedly disadvantageous. Regarding the Winkler method as modified by Hale and Melia, it is pointed out that their warning "that solutions acidified ready for titration should not stand *in contact with air*[1] for several hours before titrating" may be misleading, since low results were obtained even when the solutions were kept in closed bottles which had been completely filled with these solutions. Furthermore, even a period of only one hour was sufficient to cause low results. In the presence of considerable nitrite, the results may also be too high if there is not a sufficiently long period intervening between the addition of the potassium acetate and the titration of the iodine. In order to obtain accurate results by the acetate modification of the Winkler method, the titration should therefore be carried out immediately after a certain interval has elapsed since the mixing with the potassium acetate. Under the present conditions (5 p. p. m. nitrite and temperature about 20° C.), an interval of 15 minutes was found to be the proper time to allow for the potassium acetate to remain in contact with the iodine before the latter is titrated. Hale and Melia's apparent explanation of the counteracting effect of the acetate on the nitrite interference on the assumption that its function is "to neutralize the hydrochloric acid and render the solution acid with acetic acid" does not seem to be a sufficiently complete explanation, since an acetic acid medium was found not to prevent these interferences. The power of potassium acetate to counteract the nitrite interference is probably due to its further depression of the dissociation of the acid; an excess of the acetate is therefore essential. The permanganate modification of the Winkler method, since it is applicable in the presence of nitrite and organic matter, is preferable to the acetate modification which counteracts only the interference from nitrite. In carrying out the permanganate modification in bottles of about 270 c. c. capacity, each bottle should contain not less than 0.45 grams KI and the amount of excess of potassium oxalate should be not more than 1 c. c. of the 1 per cent solution.

[1] The original is not in *italics*.

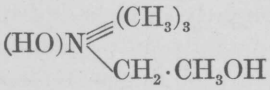
# SOME NEW COMPOUNDS OF THE CHOLINE TYPE (III), INCLUDING PREPARATION OF MONOACETATE OF α, β DIOXY-β-METHYL-BUTANE.[1]

By G. A. MENGE, *Technical Assistant.*

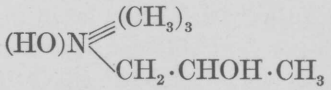
As implied in the above title, two papers on the same subject have been previously published [2] from this laboratory by the same author. Obviously a very considerable hiatus exists between the publication of the second paper and the third now submitted, the protracted delay having been due to the fact that the pressing demands of other work of the laboratory not only interrupted the further development of choline compounds but also denied opportunity for assembling, in a form suitable for publication, the incomplete results already accomplished. The delayed appearance of the new work here submitted, the fact that it appears in a different publication, and the improbability, at least for the present, of the further development of choline compounds in this laboratory will perhaps justify a brief résumé here of the two papers previously published.

As noted in the preliminary paper, previous to the inception of the work therein reported the synthetic development of compounds of the choline type had not apparently extended beyond the preparation of choline, formocholine, the so-called homocholines (β- and γ-), and some acyl- and certain other derivatives of these, reference to practically all of which is made in the work of Hunt and Taveau on the toxicity of such compounds and their effects on the blood pressure.[3]

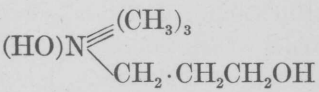
The structural relation between choline and the homocholins referred to above is shown in the following formulas:



Choline.



β-Homocholine.



γ-Homocholine.

the distinguishing nomenclature for the two latter being obviously based upon the position of the hydroxyl in the carbon chain with reference to the nitrogen.

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[1] Manuscript submitted for publication May 15, 1914.

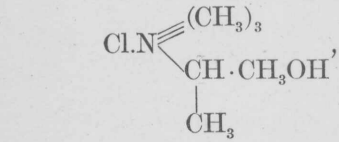
[2] Some New Compounds of the Choline Type (preliminary paper), J. Bio. Chem., 10, 399–406, Dec., 1911; second paper, J. Bio. Chem., 13, Oct., 1912.

[3] Hunt, R., and Taveau, R. de M., Hygienic Laboratory Bull. No. 73, U. S. Public Health Service, 1911; British Med. Jour., 1906, II, p. 1788; Jour. Pharm. and Exp. Therapeutics, I, 303, 1909.

The physiological activity of these compounds and their derivatives as determined by the work reported in Bulletin 73 indicates in a general way that the greatest activity and least toxicity is obtained in those compounds which contain the trimethylamine nucleus and in which the alcoholic hydroxyl is not further removed from the nitrogen than the $\beta$-position; that is, the substitution of the $\alpha$- and $\beta$- hydrogen of the carbon chain by alkyl and other groups rather than the direct extension of the chain was indicated as the most promising development from the standpoint of physiological activity.

Probably the simplest method of nomenclature for such a class of compounds would be to consider them as substituted cholines, with the further designation of $\alpha$- or $\beta$- according as the substituted group is linked to the first or second carbon. From this point of view the $\beta$-homocholine given above becomes $\beta$- methylcholine.

The first new compound obtained in line with the suggested development was $\alpha$-methylcholine in the form of its chloride,[1]



, which was obtained in the following manner:

Allyl chloride was converted, by the method of Oppenheim,[2] into the chlorhydrine, $CH_2CHOH \cdot CH_2Cl$. The chlorine of this compound was replaced by an acetate group, according to the method of Henry,[3] yielding the acetate, $CH_3COOCH_2 \cdot CHOH \cdot CH_3$, which in turn was treated with hydrochloric acid,[4] yielding the chlor-acetate, $CH_3COO \cdot CH_2 \cdot CHCl \cdot CH_3$, and this compound, on saponification with dry hydrochloric acid in absolute methyl alcohol,[5] yielded the desired chlorhydrine, $CH_3 \cdot CHCl \cdot CH_2OH$. This chlorhydrine was mixed in a Carius tube with a calculated quantity (slightly in excess of theory) of a 33 per cent solution of trimethylamine in absolute alcohol. The tube was sealed and the mixture heated in a boiling water bath for three hours. The reaction product was poured into a distilling bulb, the alcohol distilled off in vacuo, and the residue washed several times with methyl alcohol (each wash portion being distilled off in vacuo) to remove all excess of trimethylamine. The final product thus obtained was identified as $\alpha$-methylcholine by means of the platinum and gold salts. The pure dry chloride is a white crystalline solid,

[1] Practically all choline compounds and their derivatives prepared in this laboratory have been obtained and used pharmacologically in the form of chlorides. The preparation of $\alpha$-methycholine was originally briefly described, also its physiological action and that of the acetyl derivative, in a footnote on page 33 of Hygienic Laboratory Bulletin 73. Mention is also made there of Morley's supposed preparation of it and his subsequent correction.

[2] Oppenheim: Ann. d. Chem. u. d. Pharm., 1867–1868, p. 367, et seq.

[3] Henry: Bull. de l'Academie Royal de Belgique, Classe de Sciences, 1903, p. 407.

[4] Henry: Loc. cit.

[5] Henry: Ibid., 1906, p. 734.

extremely hygroscopic and very soluble in absolute alcohol. The platinum salt is a well crystallized compound, practically insoluble in absolute alcohol, but quite soluble in hot water, from which it readily crystallizes. It decomposes vigorously at about 252° to 253° (corr.).[1] The gold salt, precipitated from an aqueous solution of the α-methylcholine chloride, was at first more or less oily, but became definitely crystalline on rubbing with a glass rod. It sinters gradually above 179° and slowly melts at 197° to 198.5° (corr.).

A number of acyl derivatives of α-methylcholine and their platinum and gold salts have also been prepared. These include the acetyl-, benzoyl-, phenylacetyl-, propionyl-, valeryl-, monobromisocapronyl-, and palmityl derivatives, all of which, together with other compounds, are described in the second paper (cited above). In all cases, except that of the acetyl derivative, the method of preparation was, in general, the same, and consisted in heating the α-methylcholine chloride with a considerable excess of the acyl chloride in a flask with reflux condenser. On pouring the reaction products into dry ether the acyl derivatives separate as oils or oily solids, which are more or less difficult to purify according as the acylation has proceeded smoothly and completely or not. In all cases their preparation in only small quantities was attempted, primarily for pharmacological investigation, and any study of their general properties was accordingly restricted. In the case of the acetyl derivative the procedure was the same except that acetic anhydride, instead of acetyl chloride, was used. In all cases the products obtained were identified and analyzed by means of the platinum and gold salts.

For the purpose of comparison both physiologically and chemically, with the phenylacetyl derivative of α-methylcholine, the corresponding derivative both of "β-homocholine" and "γ-homocholine" were also prepared by the same general method, and are described in the second paper.

The physiological activity[2] of α-methylcholine and certain of its acyl derivatives—more especially the acetyl derivative—has justified the promise offered in the development of this class of compounds and stimulated effort to further extend it. As stated in the preliminary paper (previously cited) a detail of primary importance to such

[1] All "melting points" here reported were determined by the usual capillary-tube method in a bath of sulphuric acid, using an Anschutz thermometer, for which a correction for emergent stem was not considered necessary. The rate of heating in all cases was regulated so as to closely approximate 5° a minute within 20° to 30° of the melting point. As pointed out in Hygienic Laboratory Bulletin 70, and as corroborated in this and other work, the decomposition point may vary quite widely with comparatively slight variation in manipulation. Results obtained on the above platinum salt varied with varying procedure from 248.5°–249.5° to 254°–255° (uncorr.), but practically identical results were obtained with similar procedure.

[2] All pharmacological investigation to which these and other choline compounds have been applied has been made by Dr. Reid Hunt, formerly professor of pharmacology in this laboratory, under whose able, generous, and helpful leadership in this and other work it was the writer's honored privilege and great pleasure to serve.

development is the preparation of suitable chlorhydrines, by a method which leaves the least doubt as to structure. Consideration of several possible means to that end led to the application to monochloracetone of Grignard's reaction for the synthesis of tertiary alcohols, which various investigators have shown is readily adaptable for the preparation of chlorhydrines of similar type, and concerning the structure of which there seems to be no doubt.

In this work the Grignard method has been successfully applied to the preparation of the chlorhydrines of asymmetrical dimethyl glycol, $CH_2Cl.COH$ ($CH_3$)($CH_3$), of asymmetrical methylethyl glycol, $CH_2Cl.COH$ ($CH_3$)($C_2H_5$), and of asymmetrical methylphenyl glycol, $CH_2Cl.COH$ ($CH_3$)($C_6H_5$).[1] These three chlorhydrines have been successfully combined with trimethylamine, forming in each case the chloride of the corresponding choline compound:

I. $\beta$-dimethylcholine chloride ($\alpha$-dimethylethoxytrimethylammonium chloride), $Cl.N(CH_3)_3$ $CH_2.COH$ ($CH_3$)($CH_3$).

II. $\beta$, $\beta$-methylethylcholine ($\alpha$,$\alpha$-methylethylethoxytrimethylammonium chloride), $Cl.N(CH_3)_3$ $CH_2.COH$ ($CH_3$)($C_2H_5$).

III. $\beta$, $\beta$-methylphenylcholine chloride ($\alpha$, $\alpha$-methylphenylethoxytrimethylammonium chloride), $Cl.N(CH_3)_3$ $CH_2.COH$ ($CH_3$)($C_6H_5$).

The preparation of $\beta$-dimethylcholine chloride and of $\beta$, $\beta$-methylethylcholine chloride, (I and II above), also their platinum salts, was described in the preliminary paper. At that time, however, the $\beta$, $\beta$-methylethyl compound (II) had been obtained only in crude condition. The presence of the compound in the crude product was determined by the preparation, purification, and analysis of the platinum salt. The crude product itself was much more contaminated by "schmiere" than that obtained in the preparation of the $\beta$-dimethylcholine (I), doubtless due in large measure to variation in one detail of procedure in the two preparations. $\beta$-dimethylcholine chloride (I)

[1] All three chlorhydrines have been previously prepared by Tiffeneau: Compt. rend. acad. d. sci., CXXXIV, p. 775, and by other investigators.

was prepared by heating the corresponding dimethylglycolchlorhydrine with a slight excess of a 33 per cent solution of trimethylamine in absolute alcohol, in a sealed tube, to 100° (water bath) for about four hours. The procedure for the β, β-methylethylcholine chloride differed from this only in that the reaction mixture, of methylethylglycolchlorhydrine and trimethylamine, in a sealed tube, was heated in a tube furnace to 150°–160° for about three hours.

Although there has been no opportunity for repeating the experiment just described, the writer is convinced, in the light of both previous and subsequent experience, that the higher temperature applied in this reaction served only, or at least largely, to induce a greater degree of decomposition and consequent contamination of the reaction product, even though it be admitted that the preparation of the higher homologues and other more complex derivatives of this type of choline compounds would probably offer progressively increasing difficulty in obtaining smooth reactions and clean products.

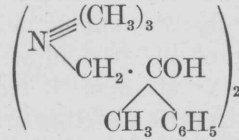
Pure β, β-methylethylcholine chloride was obtained from the crude reaction product by indirect means. The latter, evaporated to dryness in vacuo and washed free of trimethylamine, was dissolved in absolute alcohol and completely precipitated with an alcoholic solution of chlorplatinic acid. The platinum salt thus obtained was purified by recrystallizing from dilute alcohol. The pure salt was dissolved in hot water and completely decomposed by a current of hydrogen sulphide. The filtrate from this decomposition was evaporated to dryness in vacuo, with the aid of repeated additions of alcohol and of gentle heat, yielding a white crystalline, though more or less oily, solid. On converting a small portion of this product into the platinum salt and analyzing the dry salt for platinum by ignition the following duplicate results were obtained:

I. 0.134 gram salt gave ..... 0.0375 gram Pt.
II. 0.1251 gram salt gave ..... 0.0347 gram Pt.

| | Calculated for $C_{16}H_{40}O_2N_2Cl_6Pt$. | Found | |
|---|---|---|---|
| | | I. | II. |
| Pt.... | 27.84 per cent. | 27.93 per cent. | 27.74 per cent. |

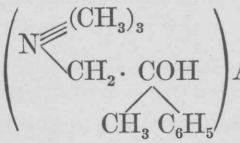
Compound III, β, β-methylphenylcholine chloride, was subsequently prepared by the same method applied to the preparation of the β-dimethyl compound—that is, the chlorhydrine of methylphenylglycol was mixed in a Carius tube with a calculated quantity (slightly in excess of theory) of a 33 per cent solution of trimethylamine in absolute alcohol. The tube was sealed, heated in a boiling water bath for about 4½ hours, and, incidentally, was allowed to stand in the cooling bath for about 40 hours. The crude product of reaction was a reddish yellow oil. It was transferred to a distilling flask, the tube rinsed with methyl alcohol, and the rinsings added to the main product in the flask. The alcohol and excess of trimethyl-

amine was evaporated off, *in vacuo*, with the aid of gentle heat over a water bath. The residue was washed several times with methyl alcohol (each wash portion being evaporated off *in vacuo*) and finally with absolute alcohol. The product thus obtained was a white crystalline solid somewhat contaminated by a reddish-brown oil. Although the white solid is readily soluble in absolute alcohol a small portion of it was obtained perfectly clean by carefully washing by decantation several times with small portions of absolute alcohol. A better method of purification, however, consisted in gradually adding dry ether, with stirring, to a concentrated solution of the product in absolute alcohol. At a certain point the choline compound began to crystallize out and, on rubbing with a glass rod, continued to separate quite abundantly without further addition of ether. This was filtered with the aid of suction, washed first with a mixture of absolute alcohol and dry ether, then with dry ether, yielding a perfectly white crystalline product, which, as indicated by analyses of platinum and gold salts, proved to be pure $\beta$, $\beta$-methylphenylcholine chloride. By concentrating the mother liquor and repeating the treatment the yield of the pure choline compound was materially increased. It is readily soluble in cold absolute alcohol and in cold water and can be recrystallized, in small measure, from a very concentrated hot solution in absolute alcohol (crystals under low power appear to be rhombohedral plates).

The platinum salt,  $PtCl_6$, was prepared by dissolving the chloride of the base in a little dilute alcohol (about 80 per cent) and adding an alcoholic solution of chlorplatinic acid. The salt was precipitated as a yellow, more or less waxy, solid. It is moderately soluble in cold, readily in hot, water and very slightly soluble in absolute alcohol. It is readily recrystallized from 50 per cent alcohol. On heating in a capillary tube the salt gradually sinters and darkens above 209° and decomposes with effervescence at 223.5° to 224° (corr.). Ignition for platinum in two different samples gave the following results:

I. 0.1005 gram salt gave 0.0245 gram Pt.
II. 0.1175 gram salt gave 0.0289 gram Pt.

| Calculated for | Found | |
|---|---|---|
| $C_{24}H_{40}O_2N_2Cl_6Pt$ | I. | II. |
| Pt....24.49 per cent | 24.38 per cent | 24.57 per cent. |

The gold salt of $\beta,\beta$-methylphenycholine,  $AuCl_4$,

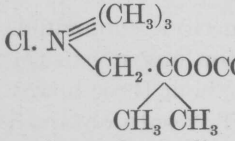
was prepared in a manner analogous to the preparation of the platinum salt, but less readily. The addition of alcoholic chlorauric acid to a fairly concentrated solution of the chloride of the base in about 80 per cent alcohol caused practically no precipitation. Further addition to the mixture of a very concentrated solution of the choline chloride in absolute alcohol appeared to have no effect until agitation of the liquid suddenly induced separation of crystals (plates) of the pale yellow salt in considerable abundance. Concentration of the mother liquor on the water bath resulted, on cooling, in the separation of more salt, but further concentration of the second mother liquor caused reduction with separation of metallic gold. The salt is quite soluble in absolute alcohol, in water, and even in dry ether. On heating the dry salt in a capillary tube the effect is perceptible at about 134° and it melts slowly at 137.5°–138.5° (corr.). Analysis for gold by ignition resulted as follows:

0.1114 gram salt gave 0.0414 gram Au.

| | Calculated for $C_{12}H_{20}ONCl_4Au$ | Found |
|---|---|---|
| Au | 37.00 per cent | 37.16 per cent. |

In his second paper on this work the writer called attention to the greater stability toward saponification of the phenylacetyl derivative of β-methylcholine as compared with the corresponding derivative of α-methylcholine, the former being an ester of a secondary alcohol and the latter an ester of a primary alcohol. The greater ease of esterification for the α-methylcholine was also indicated.[1] In accordance with these facts it was anticipated that the introduction of acyl groups in the tertiary hydroxyl of the three β-disubstituted cholines described above would prove more difficult than with either of the other two classes; and, in most of the few attempts made, this proved to be true.

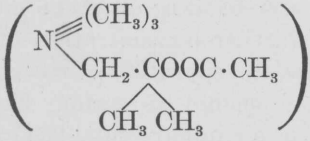
The acetyl derivative of β-dimethylcholine chloride,



$Cl.\ N(CH_3)_3CH_2\cdot COOCCH_3$, $CH_3\ CH_3$, in striking contrast to all other attempts to acylate these disubstituted cholines, was easily prepared by procedure similar to that applied for the preparation of the same derivative of the α-methylcholine—i. e., β-dimethylcholine chloride was mixed with a large excess of acetic anhydride and heated to gentle boiling for about three hours. The reaction product on cooling was poured into a relatively large volume of dry ether, whereupon the

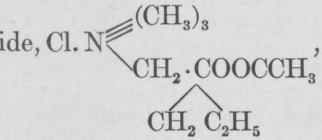
[1] These facts are in harmony with the published work of a large number of distinguished investigators in the field of esterification, including Dr. E. Emmet Reid, to whom the writer is very gratefully indebted for generous response to a recent inquiry on this subject.

acetyl derivative separated as an oil which, on rubbing with a glass rod, was gradually transformed to a white, more or less waxy, solid. The ether solution was decanted, the solid repeatedly washed by decantation with dry ether to remove excess of acetic anhydride and finally dried in a vacuum desiccator. The white solid thus obtained is moderately hygroscopic, readily soluble in absolute alcohol and was identified as the desired acetyl derivative by means of the platinum salt.

The platinum salt,  , was prepared by dissolving in absolute alcohol a portion of the product described above and adding a solution, in absolute alcohol, of chlorplatinic acid. By this procedure the salt is precipitated as a finely divided, pale yellow, solid. It is practically insoluble in boiling absolute alcohol, the solubility steadily increasing with additions of water. It is fairly soluble in cold, readily in hot, water. The dry salt heated in a capillary tube decomposes with vigorous effervescence at about 233°–234°, gradually shrinking over a considerable range below that point. Analysis for platinum by ignition resulted as follows:

0.1185 gram salt gave 0.0305 gram Pt.

| | Calculated for $C_{18}H_{40}O_4N_2Cl_6Pt$ | Found |
|---|---|---|
| Pt .... | 25.78 per cent | 25.74 per cent. |

Two attempts to prepare the acetyl derivative of $\beta$, $\beta$-methylethylcholine chloride,  , by the same procedure as just described for the corresponding $\beta$-dimethyl compound, resulted in failure. In the first attempt the reaction mixture in the acetylization flask was heated to gentle boiling for about three hours, and in the second attempt for about eight hours. The results obtained in the two cases (based upon analyses of the platinum salt and assuming high results to be due to platinum salt of the unaltered base), indicated respectively about 35 per cent and 50 per cent esterification. An attempt was made to purify the 50 per cent mixture of platinum salts by careful recrystallization. The small quantity of material to work with, however, made complete separation difficult. Analysis of the recrystallized salt still indicated about 20 per cent contamination by unacetylated base, as shown in the following data:

0.1327 gram salt gave 0.033 gram Pt.

| Calculated for $C_{20}H_{44}O_4N_2Cl_6Pt$ | Found |
|---|---|
| Pt ..... 24.85 per cent | 25.55 per cent. |

A third attempt to prepare this acetyl derivative consisted in heating the chloride of the base with an excess of acetyl chloride, in a sealed tube, to 100° for about five hours. The product, worked up as previously described, was converted into the platinum salt, analysis of which gave a result for platinum (27.99 per cent), practically theoretical for the platinum salt of the unaltered base (27.84 per cent), indicating that the acetylization had not proceeded at all. Evidence contradictory to such a conclusion, however, was obtained by determination of the melting point. The platinum salt under discussion melts with decomposition at about 208°, while that of the unaltered base behaves similarly at 242°–243°—too great a divergence to be explained by a trace of impurity or the erratic character of the decomposition point. The true explanation of such contradictory data, however, could only be determined by further investigation, opportunity for which has not offered.

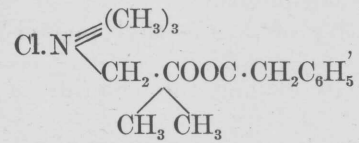
The preparation of the acetyl derivative of $\beta$, $\beta$-methyl phenylcholine chloride, $Cl.N\begin{matrix}(CH_3)_3\\ CH_2\cdot COOCCH_3\\ CH_3\quad C_6H_5\end{matrix}$, by boiling the chloride of the base with a large excess of acetic anhydride in an acetylization flask, was tried and yielded results which, as was to be expected, indicate a lower limit of acteylization, for the same period of time, than was obtained in the corresponding attempt to prepare the same derivative of the $\beta$, $\beta$-methylethylcholine, described above. The compound was successfully prepared, however, by heating a mixture of the $\beta$, $\beta$-methylphenylcholine chloride and acetylchloride in a sealed tube to 100° (water bath) for about 24 hours. The reaction product poured into dry ether yielded a nearly white oily precipitate which, washed with dry ether as previously described, was converted to a white solid with a pale yellow tinge. A portion of this solid dissolved in 95 per cent alcohol (readily soluble) was treated with an alcoholic solution of chlorplatinic acid, causing the separation of the platinum salt as a pale yellow flocculent precipitate. This salt, on filtering, washing, and drying, was analyzed directly for platinum and indicated complete acetylization, as shown in the following data:

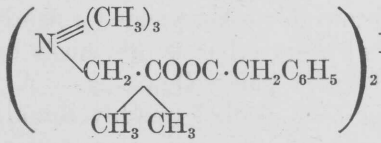
0.1055 gram salt gave 0.0234 gram Pt.

| Calculated for $C_{28}H_{44}O_4N_2Cl_6Pt$ | Found |
|---|---|
| Pt ..... 22.14 per cent | 22.18 per cent. |

These three β-disubstituted cholines (I, II, and III, p. 40) considered as alcohols are all of the same class, tertiary, but, as indicated by the behavior of each toward acetic anhydride in experiments described above, they show a distinct gradation in their resistance to esterification, increasing with increased molecular complexity. The results obtained with acetyl chloride, in two cases to which it was applied, are not considered either sufficiently extensive, or comparative, or (in the one case) dependable to either corroborate or contradict such a generalization.

Further attempts to prepare acyl derivatives of these three compounds were limited to the phenylacetyl and the benzoyl derivatives. The former was successful only in the case of the β-dimethylcholine and was less easily prepared than the acetyl derivative; the latter was unsuccessful in all cases. The method in all cases consisted in heating the acyl chlorides with the respective choline compounds in sealed tubes to 100° and working up the products as described in the following paragraph.

The phenylacetyl derivative of β-dimethylcholine chloride,  , was finally obtained in approximate purity by heating a mixture of the chloride of the base with a considerable excess of phenylacetylchloride in a sealed tube to 100° (water bath) for about seven hours. The cold reaction product, a greenish-yellow oil, poured into a large volume of dry ether caused a milky solution from which a semi-solid oil gradually settled. This product, by repeated thorough washing with dry ether, was gradually converted to a nearly white flaky solid. It is readily soluble in absolute alcohol and was identified as the desired phenylacetyl derivative by means of the platinum salt.

The platinum salt  , separates from a mixture of alcoholic solutions of the choline compound and chlorplatinic acid as a pale yellow, very finely divided, waxy precipitate; difficult to filter and wash free of the excess of chlorplatinic acid. The salt is very difficultly soluble in both alcohol and water. Heated in the capillary tube it sinters sharply at 220° and decomposes with effervescence at 221°. On ignition for platinum the following duplicate results were obtained:

I. 0.1123 gram salt gave 0.0246 gram Pt.
II. 0.1097 gram salt gave 0.0239 gram Pt.

| | Calculated for $C_{30}H_{48}O_4N_2Cl_6Pt$ | Found I. | Found II. |
|---|---|---|---|
| Pt .... | 21.47 per cent. | 21.82 per cent. | 21.79 per cent. |

The slightly high results might be due to incomplete esterification, leaving about 2 per cent unaltered chloride of the base, or to imperfect washing of the platinum salt, leaving a trace of chlorplatinic acid.

From the pharmacological point of view the $\beta$-disubstituted cholines, to the limited extent that they have yet been tested, appear not to be as active as the $\alpha$-methylcholine or the $\beta$-methylcholine ("$\beta$-homocholine") and their derivatives. The comparative pharmacological study of these three types of choline compounds has, therefore, made especially desirable and interesting the further development of $\alpha$-monosubstituted cholines and the development of $\alpha$-disubstituted cholines.

The key to success in such development seems to the writer to be found in the preliminary preparation of chlorhydrines of the types $\begin{matrix}\mathrm{Cl}\\ \\ \mathrm{R}\end{matrix}\!\!>\!\mathrm{CH}\cdot\mathrm{CH_2OH}$ and $\mathrm{Cl}\cdot\mathrm{C}(\mathrm{R}\,\mathrm{R}')\cdot\mathrm{CH_2OH}$. Two plans of procedure have been applied to that end. The first consisted in the well-known condensation of the olefin hydrocarbons with hypochlorous acid, which theoretically offers two possibilities of development. The second plan is an adaptation to chlorhydrines of the type $\mathrm{Cl}\cdot\mathrm{CH_2}\cdot\mathrm{C}(\mathrm{R}\,\mathrm{R})\cdot\mathrm{OH}$, of part of the procedure applied to the development of $\alpha$-methylcholine, previously described. The two plans are outlined in the following scheme:

I. (*a*) $\mathrm{R_2C{=}CH_2} \xrightarrow{\mathrm{HOCl}} \mathrm{R_2C(Cl)\cdot CH_2OH}$

(*b*) $\mathrm{R_2C{=}CH_2} \xrightarrow{\mathrm{HOCl}} \mathrm{R_2C(OH)\cdot CH_2Cl}$

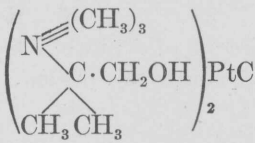
II. $\mathrm{Cl\cdot CH_2\cdot CR_2\cdot OH} \xrightarrow{\mathrm{KC_2H_3O_2}} \mathrm{CH_3COOCH_2\cdot CR_2\cdot OH} \xrightarrow{\mathrm{PCl_3}}$

$\mathrm{CH_3COOCH_2\cdot CR_2\cdot Cl} \xrightarrow[\mathrm{HCl}]{\mathrm{CH_3OH}} \mathrm{CH_2OH\cdot CR_2\cdot Cl}$

The two theoretical possibilities indicated in the first plan have been the subject of much controversy in the literature by different able investigators, leading to much confusion of conclusions, of which the truth probably is that the reaction produces a mixture of the two isomeric chlorhydrines. Upon this assumption the writer applied the reaction to isobutylene, according to the method of Butlerow,[1] with the purpose of converting the mixed chlorhydrines to the corresponding choline compounds and separating the two by fractional recrystallization of their platinum salts. The mixture of platinum salts actually obtained, from the procedure outlined, analyzed high for the platinum content of the isomeric cholines (31.15 per cent, against 28.97 per cent calculated), indicating contamination either by trimethylamine or a by-product. The separation of this mixture into pure fractions by recrystallization proved to be extremely difficult. Two small fractions were finally obtained pure, the one corresponding in platinum content to trimethylammoniumchlorplatinate and the other to the platinum salts of the choline compounds sought, as indicated in the following analytical data:

0.1267 gram salt gave 0.0366 gram Pt.

| | Calculated for $C_{14}H_{36}O_2N_2Cl_6Pt$ | Found |
|---|---|---|
| Pt ..... | 28.997 per cent | 28.89 per cent. |

The small amount of pure salt obtained did not permit of elaborate investigation to determine whether it represented only one of the two possible isomers or a mixture of both. A melting point determination, however, offered fairly definite evidence of a pure compound and to that extent identified it as the platinum salt of $\alpha$-dimethylcholine,  , as indicated in the following data:

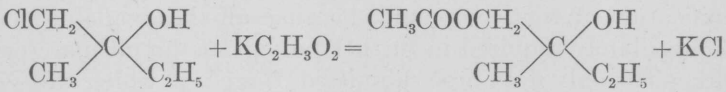
Heated in a capillary tube, with careful manipulation, the above salt sinters gradually above 205° and melts sharply with effervescence at 213.5°–214.5°; while the platinum salt of $\beta$-dimethylcholine, carefully subjected to similar treatment, gradually gets darker in color, begins to blacken at about 240°, and decomposes with vigorous effervescence at 245°. Granting, however, that the pure platinum salt described above is that of $\alpha$-dimethylcholine, nevertheless, based upon the one imperfect experiment, the procedure by which it was obtained could not be recommended as a practical method for the preparation of $\alpha$-disubstituted cholines.

The procedure outlined in scheme II (above) was applied in the attempted development of $\alpha$, $\alpha$-methylethylcholine, but, to the extent

[1] Ann. d. chem., 144, pp. 1–45.

that the work has proceeded, without success. The first step in the process, however, yielded a compound, the monoacetate of asymmetrical methylethylglycol, in such pure condition as to make possible a much more complete and accurate description of it than the literature now contains. Richter's Lexicon credits the only previous preparation of this compound to Kling,[1] who claims to have obtained it, together with methyldiethylcarbinol, by the action of ethyl-magnesium bromide on "acetate of acetal." He describes the compound rather meagerly as "a product of ethereal odor, distilling about 145°–147° at 10 mm. pressure, of percentage composition approximately that of the monoacetate of amylglycol, but which could not be purified by vacuum distillation because it decomposes with each fractionation." Further description states that the product on saponification was converted into amylglycol.

The writer has prepared the compound under discussion as follows: To 74 grams of freshly prepared pure methylethylglycolchlordrine, in a small round-bottom flask, was added freshly fused potassium acetate (in slight excess of monomolecular proportion). The flask was connected with a reflux condenser and the mixture heated in an air bath to gentle boiling for about three and one-half hours, during which the solid portion of the reaction mixture gradually became distinctly granular, suggesting the formation of potassium chloride in accordance with the following equation:



The reaction product on cooling was thoroughly extracted with dry ether. A small portion of the solid residue, dissolved in water to form a dilute solution, gave a striking test for chloride.

The ether extract, freed from ether by careful evaporation in vacuo, yielded a residual oil somewhat contaminated by "schmiere." From this product was obtained by vacuum distillation a perfectly clear, colorless, limpid oil, of ethereal odor, to the amount of 62 grams (representing about a 70.5 per cent yield of the desired acetate). This oil distilled at 94°–95° and about 16 mm. pressure (though much the larger part of it was collected at 94°–94.5°), while the unaltered chlorhydrine distills mostly at 50°–51° and 15 mm. pressure. About 20 c. c. of the dry oil was very carefully and slowly redistilled to obtain a fraction as pure as possible for tests and analysis. This procedure yielded a fraction of about 12 c. c., distilling at 92.8°–93° and about 15 mm. pressure, with which the following data were obtained:

Specific gravity at 25°, 0.9989.

[1] Compt. rend., 137, 758.

Several samples subjected to appropriate treatment gave definite indication of (1) absence of chlorine (compound decomposed with sodium, extracted with $H_2O$ and tested for chloride); (2) presence of the acetate group (compound saponified, resulting acetate isolated and tested); (3) presence of the hydroxyl group (compound treated with phosphorus trichloride, product isolated, and tested for chlorine).

By means of an improvised apparatus a molecular weight determination by the freezing point method was made:

| | |
|---|---|
| Solvent, benzol, $C_6H_6$ (K=4900) | 15.61 grams (L) |
| Sample, acetate, | 0.2320[1] gram (S) |
| Depression of freezing point | 0.460° ($\Delta$) |
| Theoretical for | Found |
| $C_7H_{14}O_3$ | $\left(\text{calculated by } M=\frac{KS}{\Delta L}\right)$ |
| Molecular weight (M) 164 | 158 |

Duplicate analyses for carbon and hydrogen (by combustion) gave the following results:

I. 0.5343 [1] gram sample gave 1.1173 g $CO_2$; 0.4674 g $H_2O$.
II. 0.5177 gram sample gave 1.0832 g $CO_2$; 0.4490 g $H_2O$.

| Calculated for | Found. | |
|---|---|---|
| $C_7H_{14}O_3$ | I. | II. |
| C—57.53 per cent. | 57.03 per cent. | 57.06 per cent. |
| H— 9.59 per cent. | 9.72 per cent. | 9.64 per cent. |

No attempt was made to isolate and determine the product of saponification of this compound, because all the available material was immediately required in further pursuit of the ultimate product sought. The only data here submitted, therefore, which offers direct comparison with that furnished by Kling is the boiling point, which, considered in connection with other data here submitted, seems to the writer to require no discussion.

The next step in the second plan of procedure, outlined as Scheme II above—that is, the introduction of chlorine in place of hydroxyl—it is believed was successfully accomplished, though no special effort was made to purify and identify the product other than to determine the presence of the chlorine, which was found in proportion (20.53 per cent) roughly approximating the calculated (21.55 per cent). Subsequent development, however, was negative and demands of other work prevented renewed attack.

The writer takes great pleasure in making grateful acknowledgment to the Bureau of Chemistry, Department of Agriculture, for the use, in connection with this work, of certain apparatus with which this laboratory was not at that time equipped.

[1] Weighings of oil were subject to more or less inaccuracy because of tendency to absorb moisture from air.

# THE DETECTION OF WHITE PHOSPHORUS IN MATCHES.[1]

By EARLE B. PHELPS, *Professor of Chemistry.*

In accordance with the provisions of an act of Congress approved April 9, 1912, entitled "An act to provide for a tax upon white phosphorus matches and for other purposes," a tax is imposed upon the manufacture of matches containing white or yellow phosphorus. Regulations No. 30 of the United States Internal-Revenue Service relative to the enforcement of this law provides that every manufacturer of matches shall file with the collector of revenue of the district a statement under oath setting forth the names of materials used in the matches produced by him and shall furnish samples of the materials named and of the finished product to the office of the Commissioner of Internal Revenue and to the Surgeon General, United States Public-Health Service, for examination. The Surgeon General is furthermore authorized to purchase matches from time to time upon the open market and to cause them to be examined in the Hygienic Laboratory to determine whether they contain white or yellow phosphorus.

The law specifically applies to "the common poisonous white or yellow phosphorus used in matches and not to include the nonpoisonous forms or the nonpoisonous compounds."

Three forms of phosphorus are commonly employed in the manufacture of matches, the crystalline white or yellow elementary phosphorus, the amorphous red elementary phosphorus, and the so-called sesquisulphide, $P_4S_3$.

It became necessary, therefore, to investigate methods for the detection of the white or yellow elementary phosphorus in the presence of various ingredients that enter into the composition of matches and especially to distinguish this particular form of phosphorus from the allotropic elementary red phosphorus and also from various phosphorus compounds, especially the sulphide.

An investigation of existing methods for the qualitative separation of yellow phosphorus under these conditions was made during the summer of 1912 by Dr. George A. Menge, of the Hygienic Laboratory. The most satisfactory method that had been proposed at that time was the so-called Thorp method.[2] A bulb about 1 inch in internal diameter is blown on the end of a piece of glass tubing, one-half inch in diameter and 12 inches in length. About 200 match heads are

[1] Manuscript submitted for publication May 15, 1914.

[2] F. E. Thorp, J. Chem. Soc. (Trans.) **95**, 1909, 440.

placed in the bulb, the tube evacuated and sealed off and the bulb heated in an oil bath at 40°–60° for two or three hours. If yellow phosphorus is present it sublimes upon the walls of the tube in well defined and characteristic crystals. These become luminescent if the seal be broken in a dark room.

This method gave satisfactory results upon matches and match materials tested. It did not appear to be sufficiently sensitive, however, for the detection of small amounts of phosphorus, and in view of the considerable number of samples that would have to be examined, it seemed desirable to develop a more simple and rapid method and if possible one that would possess greater sensitiveness.

A review of the properties of phosphorus and phosphorus compounds indicated that the combined properties of volatility in steam, and of luminescence distinguishes the yellow phosphorus from all other forms. A qualitative test based upon the luminescence of phosphorus had recently been described by Schröder,[1] and doubtless this property had previously been used for the purpose. Schröder's method involved extraction in benzol, and it was hoped that this could be avoided by taking advantage of the volatility in steam.

Small quantities of yellow phosphorus were placed in a test tube of water and the tube closed with a rubber stopper through which projected a glass tube of small bore. Upon heating, the issuing jet of steam was faintly luminous in the dark room. A more satisfactory result was obtained by the use of a stream of hydrogen gas, the stream being allowed to bubble through the watery suspension, heated to about 100°.

Further studies of the method upon every small known amounts of phosphorus (in a volumetric benzol solution) showed that the luminescence or glow was due to oxidation and did not occur if there were enough oxygen in the apparatus to complete the reaction before the stream of gas emerged. The hydrogen was, therefore, passed through a wash-bottle containing a strong solution of sodium pyrogallate. After this gas had passed through the hot aqueous suspension of phosphorus a sufficient time to displace the air within the test tube, the issuing jet was faintly luminous in the laboratory and beautifully luminous in the dark room with as little as 0.1 mg. of yellow phosphorus in suspension in 10 c. c. of water.

It was also discovered that the intensity of the glow could be increased by allowing the gas to issue through a fine capillary at a relatively high velocity, thus bringing about a maximum of reaction within a minimum space.

The apparatus which was finally developed for the purpose is shown in figure 1.

[1] Arb. Kais. Gesundh., **44**, 1913, 1–29.

The dark room has been reduced to a small chamber upon which is mounted a small microscope of about 12 cm. focal length. Within the chamber and focused in the field of the microscope is the jet, a glass tube drawn out to a capillary about 0.2 mm. in diameter. For about 2 inches back of the jet the glass tube is electrically heated to prevent condensation. Covered "Climax" resistance wire No. 33 B. & S. was wound about the tube and then covered with plaster of Paris and the whole inclosed in a brass tube half an inch in diameter. A rubber stopper for attaching the test tube containing the match heads, a Drexel wash bottle filled with sodium pyrogallate, and a Kipp generator for hydrogen complete the apparatus. The test tube is immersed in a dish of water kept boiling by a small electric heater. Direct electric heating of the test tube would be advantageous. The glass tube by which hydrogen enters the test tube is drawn out to a small tip in order to produce an even flow of gas and to improve the volatilization by breaking up the air into small bubbles. The bottom of the chamber is removable for focusing the jet before making the observation.

The procedure is as follows: Ten or twelve match heads or an equivalent amount of the phosphorus or other compound to be examined are placed in a 5-inch test tube, which is then half filled with water and plugged with cotton and placed in a bath of boiling water. From 6 to 12 samples are thus prepared before making the tests. One of the prepared tubes is then fitted to the rubber stopper, the glass tip extending an inch or so below the surface of the water. The dish of boiling water is placed as shown. Hydrogen is allowed to bubble through for a moment, and an observation is made through the microscope. A luminous flame at the tip of the capillary jet is evidence of the presence of yellow phosphorus. If a negative result is obtained the hydrogen is shut off for a minute, to allow an accumulation of phosphorus vapor. It is then turned on again, observations being made of the first gas to issue.

For ordinary phosphorus matches the refinements of washing the hydrogen and superheating the vapor after leaving the test tube are unnecessary, but with these refinements negative results assure the absence of yellow phosphorus down to about 0.1 mg. in the quantity of match heads taken.

It was thought probable at the beginning that it might be necessary to separate certain of the match constituents which might interfere with the test. In particular, nitrates and chlorates were feared by reason of their oxidizing properties. An exhaustive study of the test as applied to about 25 specimens of matches and to several times that number of materials entering into the manufacture of matches has shown thus far little, if any, interference. In every instance, after obtaining a negative result, a portion of an emulsion correspond-

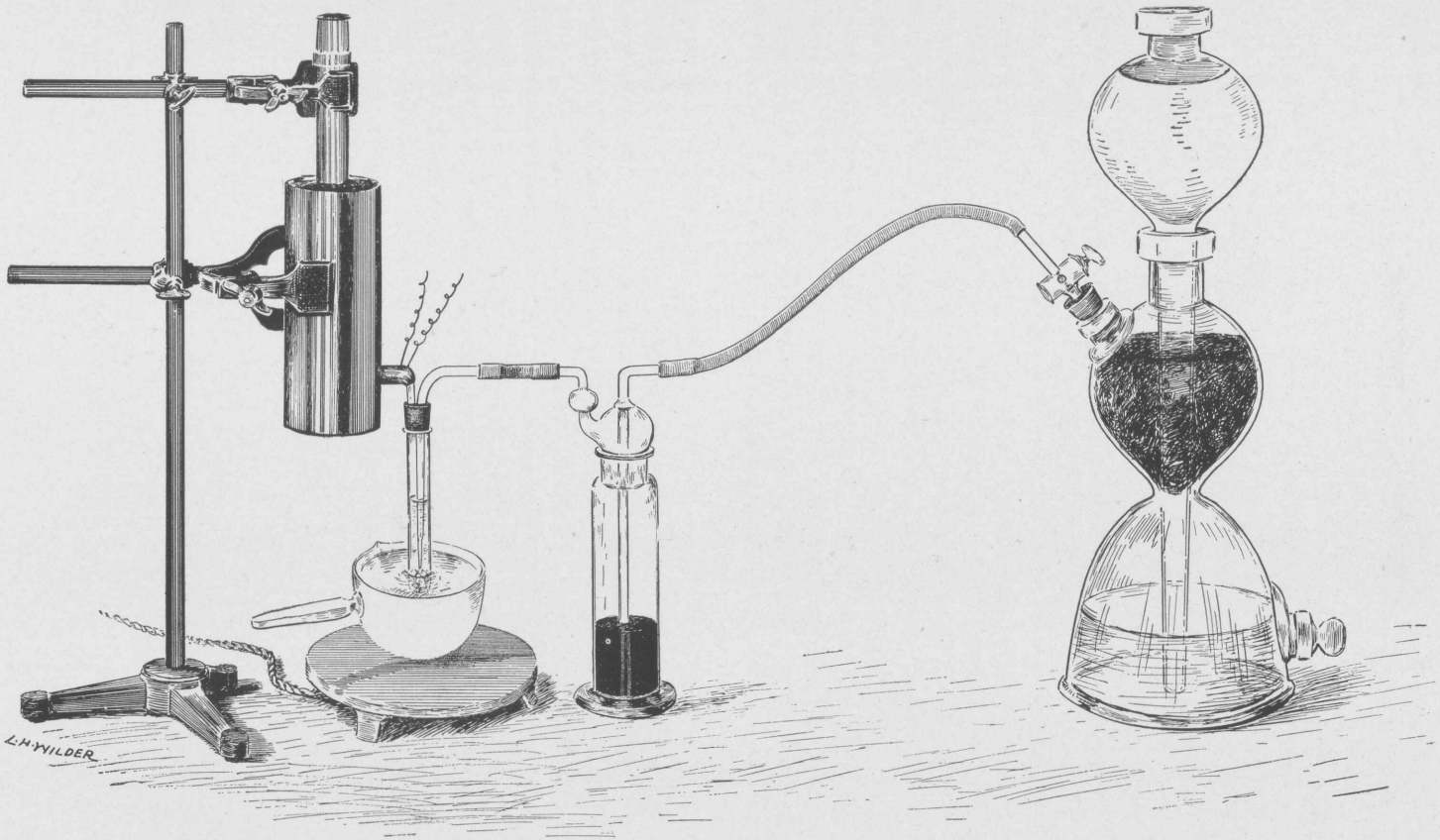
ing to one-tenth of one match head made of matches containing phosphorus was added, and in no instance did the test fail.

In the examinations of the sesquisulphide, which always contains hydrogen sulphide, a positive result was always obtained after the addition of the above emulsion in the quantity stated. This fact is of interest in view of the statement in Watt's Dictionary and other reference works that hydrogen sulphide prevents the glow. Similar statements are made concerning carbon disulphide, but these were found to be equally without foundation under this particular technique.

It is possible, therefore, to extract the phosphorus in carbon disulphide or in benzol, and in this way to increase the sensitiveness of the test to almost any desired degree, but in no case was this found necessary in routine examinations. Matches or materials giving a negative result with the quantities used failed to give positive results after most careful and diverse treatment of much greater quantities, while all such cases gave a pronounced test after the addition to the mixture of two drops of the control emulsion, equivalent to one-tenth of a match head known to contain yellow phosphorus.

For the routine examination of matches, therefore, this test is simple, rapid, and sufficiently accurate and delicate for practical purposes. It requires no extraction with solvents, and no elaborate apparatus or dark room.

No extensive search of the literature has been made to determine whether a similar test has been described. There is nothing novel in the principles involved, and they have doubtless been made use of in other connections. The details of the method probably constitute an improvement over other methods depending upon the luminescence or glow of yellow phosphorus. The examination of matches will probably become a matter of some importance in various laboratories engaged in public-health work, and the publication of the details of the method which will be used by the Hygienic Laboratory of the United States Public Health Service in the official examination of matches and match materials under the new Federal law seems therefore to be appropriate.




APPARATUS FOR THE DETECTION OF WHITE OR YELLOW PHOSPHORUS IN MATCHES OR MATCH MATERIAL.

# THE CHEMICAL COMPOSITION OF RUBBER USED IN NURSING NIPPLES AND IN SOME RUBBER TOYS.[1]

By EARLE B. PHELPS, *Professor of Chemistry*, and ALBERT F. STEVENSON, *Sanitary Chemist*.

Pure india rubber, or caoutchouc, is a gummy product derived from the juice of certain tropical trees. In its natural state it is entirely unsuited for commercial use. It is soft, plastic, and sticky, and readily undergoes spontaneous oxidation to a hard, brittle, resinous substance. Its commercial value depends largely upon its property of combining chemically with certain mineral substances. Such compounds are obtained commercially by heating mixtures of the gum with sulphur, or metallic sulphides or oxides, a process known as vulcanization.

Vulcanized rubber differs from the natural gum in that it has lost its plastic and sticky properties, is much more permanently elastic, and is less subject to spontaneous oxidation or other change. The simplest type of a vulcanized rubber is obtained from a mixture of pure gum and sulphur, properly heated. Such a compound is highly elastic and quite soft, having little body and offering no resistance to rough usage. It is approximated in a good grade of rubber band. Various other qualities are imparted to a rubber compound by the addition of other ingredients before vulcanization. Metallic oxides, such as the oxides of lead and of zinc, produce a rubber of tougher qualities and with lower elastic limits. Various pigments are also added for the purpose of giving the product a desirable color. Among those commonly used are venetian red (oxide of iron), ultramarine (in white rubber), lamp black, and various lakes prepared with organic dyes. These added compounds which take part in the vulcanization or give desirable qualities to the finished product can not be regarded in any sense as adulterants but are necessary constituents in a pure rubber compound of the required characteristics.

A large number of additional materials are used in rubber compounding simply to reduce the cost of the product. Among these may be mentioned whiting (calcium carbonate), barytes (sulphate of barium), clay, and various rubber substitutes, such as artificial rubber, oils, and tar products. Finally, so-called recovered rubber, or shoddy, is used to a large extent in rubber compounds, resulting in a varying and generally unknown composition of the mineral constituents of the finished product.

[1] Manuscript submitted for publication May 15, 1914.

It has been found that sulphide of antimony can replace the sulphur, wholly or in part, in a simple vulcanized rubber, the resulting product being a brilliantly colored terra-cotta rubber. As the metallic base also gives a certain toughness and durability to the product the use of antimony sulphide in place of sulphur was formerly considered highly desirable in the production of a rubber having wearing qualities. Despite the fact that even better wearing qualities can now be obtained by the use of other metallic compounds, especially the oxide of zinc, red rubber is still popularly regarded as a superior product. This in turn has led to the artificial coloring of ordinary rubber by the use of other compounds, such as iron oxide and organic coloring matter, so that one may find upon the market many varieties of red rubber that are free from antimony.

The actual chemical relation of the sulphur and metallic sulphides and oxides which take part in the vulcanization, to the molecule of the finished product has never been satisfactorily determined. There is evidence that the sulphur attaches itself directly to the rubber molecule probably by substitution. In commercial processes, however, an excess of sulphur is always used, so that the finished product contains free sulphur, a portion of which appears as the "bloom" on black rubber. In view of the various characteristics that are imparted to the vulcanized rubber by the addition of metallic compounds it is probable that they too constitute an integral part of the molecule. But it is also to be noted that such compounded rubbers yield to proper solvents, small quantities of these added metals so that they too appear to be in excess in the product. It is not improbable that the reaction between the pure gum and the vulcanizing agents is one which comes to equilibrium before its completion, thus leaving at all times a certain uncombined residue. The analytical difficulties of determining definitely the so-called free sulphur in a rubber compound and the apparent ease with which certain metallic ingredients are removed, together with the fact that the complete removal of either sulphur or the metals is impossible short of the total destruction of the rubber, lends weight to this point of view.

In view of this fact and of the common use of various heavy metals in rubber compounds in general, it seemed to be a matter of some importance to investigate the composition of the rubbers used in the manufacture of nursing nipples and in certain small rubber toys. It is obvious that these rubbers should contain no toxic substances in such form of combination that they could by any possibility be extracted under conditions of normal use either in the saliva or by the gastric juice of the stomach, following accidental swallowing of small pieces of the rubber. This matter was recently brought to the attention of the Surgeon General of the Public Health Service by a communication from a physician on the Pacific coast, who described

an attack of acute gastro-enteritis in a nine week's infant following the use of a certain well-known and widely advertised nipple. The physician had noted in these nipples a considerable excess of free sulphur. Other nipples of the same brand, but without the same physical evidences of excess sulphur, were used without harmful effect.

While it is improbable that the results observed were due directly to the sulphur obtained from these carefully washed nipples, it seemed quite possible that the excess sulphur was associated with some other unusual characteristic of the compound used which may have been directly responsible for the results.

About the same time there was reported in the French Bulletin of Pharmacology [1] certain results of examinations of nipples in which artificial rubbers had been used. The rubber used contained rubber substitute, a compound prepared from linseed oil and chloride of sulphur. A residue of the latter was imperfectly neutralized and left a residual acidity in the finished product, which in turn led to the ready solubility of mercuric and other metallic compounds. The process of manufacture of so-called white or English rubber substitute involves the use of chloride of sulphur and if due care is not taken may lead to a considerable residue of hydrochloric acid in the finished product. The use of such a substitute in combination with metallic oxides might lead to the formation of metallic salts of poisonous character. As the use of white substitute is not uncommon even in high grade rubber goods, it is quite possible that an excess amount of this material may have been used in the batch of nipples referred to previously. This would fully account for the appearancs of excess sulphur "bloom," and assuming the presence of other metallic compounds would satisfactorily account for the unfortunate effect recorded.

It was determined, therefore, to obtain some knowledge of the general characteristics of rubber used in nipples and toys with special reference to the use of heavy metals and to the presence of residual acidity in the compound.

Thirteen different varieties of nipples were purchased in the market of which seven were of black rubber and six of red rubber. In addition there were obtained two varieties of teething rings and two small rubber toys. These articles were carefully prepared for analysis by being cut into small shreds and were then digested with red fuming nitric acid and ammonium persulphate. The digestion was accelerated by heating and was usually complete in from four to five hours, leaving a clear solution with a light-colored sediment. The contents of the flask were then submitted to a complete qualitative analysis

[1] Bull. Sci. Pharmacolog., 20, 459–75.

for the metals and the alkaline earths. Slight traces of metals, other than those known to have toxic properties, were regarded as accidental impurities and were not recorded.

A summary of the results of the analysis is given in Table I:

TABLE I.—*Summary of qualitative analyses of rubber nipples and toys.*

| No. | Color. | Aluminum. | Antimony. | Barium. | Calcium. | Iron. | Magnesium. | Zinc. |
|---|---|---|---|---|---|---|---|---|
| NIPPLES. | | | | | | | | |
| 1 | Black | + | + | + | − | + | − | + |
| 2 | do | + | + | − | − | + | − | + |
| 3 | do | + | − | + | − | + | − | − |
| 4 | do | + | − | − | − | + | − | − |
| 5 | do | + | − | + | − | + | − | − |
| 6 | do | + | + | − | + | + | − | − |
| 7 | do | + | + | − | − | + | + | − |
| 8 | Red | + | + | − | − | + | + | − |
| 9 | do | + | − | − | − | + | − | + |
| 10 | do | + | + | − | − | + | + | − |
| 11 | do | + | − | − | + | + | + | + |
| 12 | do | + | + | − | + | + | − | + |
| 13 | do | + | + | − | − | + | + | + |
| RINGS. | | | | | | | | |
| 14 | Red | + | − | − | + | + | − | + |
| 15 | do | + | + | + | + | + | − | + |
| TOYS. | | | | | | | | |
| 16 | White | + | − | − | − | + | − | + |
| 17 | do | + | − | + | + | + | − | + |

The following points of interest are to be noted. All specimens examined contained both iron and aluminum in considerable quantities. This indicates a rather general use of clay as a filling material. In the red rubbers either zinc oxide or magnesia was used in addition, while in the black rubbers two samples contained zinc oxide and three samples contained barytes. Antimony was found in four cases out of seven among the black rubbers and in five cases out of eight among the red rubbers. The three remaining specimens of red rubber were colored with Venetian red. The white rubber toys contained clay and zinc oxide, and one of them contained barytes. No traces of arsenic, lead, or mercury were found in any of the samples examined.

The occurrence of antimony in black rubber can be explained only upon the hypothesis that rubber scrap or recovered rubber has been used in the compounding. This fact is not of special significance by itself and if vulcanized rubber scrap from the same mill and of known chemical composition be used, there can be no possible objection upon hygienic grounds. The use of miscellaneous scrap, and especially of recovered rubber or shoddy, is objectionable because of its un-

known chemical composition. The use of lead compounds in rubber preparations is common, and salts of mercury are used to some extent. Either of these metals would be highly objectionable in a compound to be used for nipples or children's toys.

The principal question suggested by these analyses is the desirability of the use of antimony in articles of this kind. The salts of antimony have a powerful emetic action, and while there is little information upon the toxic effects of minute doses taken regularly, it is reasonable to assume that such treatment is not desirable in the case of very young infants. Such a conclusion is further strengthened by the knowledge that nursing infants are, in a great many cases, laboring under extreme difficulty in adjusting their digestive system to the conditions of artificial feeding, so that even very slight disturbing factors may suffice to bring about serious digestive troubles. It becomes a matter of considerable importance, therefore, to inquire to what extent the antimony used in nipples can be extracted under ordinary conditions of use.

This matter was first investigated under rather extreme conditions by the digestion of about 6 grams of shredded rubber in lactic-acid and in hydrochloric-acid solutions each having a strength of 0.5 per cent acid. Lactic acid was employed to simulate the common acid condition of the mouths of infants and the hydrochloric digestions were made to determine the effect of gastric juices upon a portion of the nipple which might have been accidentally swallowed. The digestion was carried on for five days at a temperature of 37°. All the specimens which had been found to contain antimony were submitted to this digestive experiment. The two acid solutions were not dissimilar in their solvent effect. Except in two cases quantities of antimony between 0 and 0.02 mg. were dissolved. The two exceptions were sample No. 15, a red rubber teething, ring and sample No. 12, a red nipple. The teething ring yielded 0.1 mg. of antimony to the hydrochloric acid solution and 0.6 to the lactic acid solution. Red nipple No. 12 gave 0.1 mg. to the hydrochloric acid solution and 0.2 mg. to the lactic acid solution. Similar digestions were also made in distilled water to determine what, if any, acidity could be extracted from the rubber compounds. Specimens No. 2 and 8 showed 0.02 and 0.01 acidity, respectively. The other specimens gave neutral solutions.

The extraction of antimony by this process does not seem to bear any relation to the quantity of metal originally present, being as great in most of the black-rubber samples as in the red-rubber samples. The exceptional cases can only be explained by some difference in the process of vulcanization or in the other chemical constituents of the compound. It is noticeable, especially in the

case of lactic acid, that the presence of zinc oxide seems to be associated with the ready extraction of antimony and the presence of magnesium seems to retard the extraction. It is also probable that the relation between sulphur and antimony sulphide in the original compound influences the solubility of the metal after vulcanization. This factor, however, could not be determined in the finished product. The slight, but definite, solubility of antimony compounds, especially in the dilute lactic acid solutions used, indicated the desirability of still further studying this action under conditions more closely approximating those of actual service.

For this purpose one of the red rubber nipples, namely, specimen No. 12, which had shown a considerable solubility of antimony in lactic acid was fitted to the end of an agate pestle and gently worked at intervals during the course of one and one-half hours in some fresh saliva which had been made very slightly acid with lactic acid. The saliva became pinkish in color and at the end of the experiment a quantitative determination of antimony showed the presence of 0.2 mg. of that metal. A second experiment with another nipple of the same lot made in exactly the same manner resulted in the extraction of 0.8 mg. of antimony. Similar experiments in normal saliva without any added acidity gave an average extraction of 0.1 mg. of antimony.

The average emetic dose of tartar emetic, the double tartrate of antimony and potassium is given by the United States Pharmacopœia as 30 mg. for an adult. This would give roughly 3 mg. as an emetic dose for a 15-pound infant, or approximately 0.5 mg. of metallic antimony. Our experiments, therefore, under conditions closely simulating those actually used showed an extraction of quantities of antimony of 20 per cent of an emetic dose in normal saliva and of from 40 to 160 per cent of an emetic dose in saliva of an acidity commonly found in an infant's mouth. It is apparent, therefore, without further discussion of the possibilities of a cumulative action of slight dosings resulting from the absorption of small amounts of antimony over a considerable period of time, that the use of antimony rubber in nursing nipples, teething rings, and other rubber materials used by young infants is at least undesirable. Such a conclusion seems all the more justifiable because it imposes no hardship.

The properties of antimony rubber are in no way superior to those of a good black rubber and the former possesses no possible advantage over the latter which at all compensates for the possible injurious effects of the antimony. The occurrence of antimony in black rubber compounds is especially unfortunate, since its presence can only be detected by chemical analysis. A purchaser desiring to avoid the use of antimony should at least have the privilege of so

doing. While our analysis did not disclose the presence of other and even more injurious substances, such as lead or mercury, the indiscriminate use of rubber shoddy or even of scrap unvulcanized rubber from the various compounds used at the mill is likely at any time to lead to the presence of these metals and is accordingly equally undesirable.

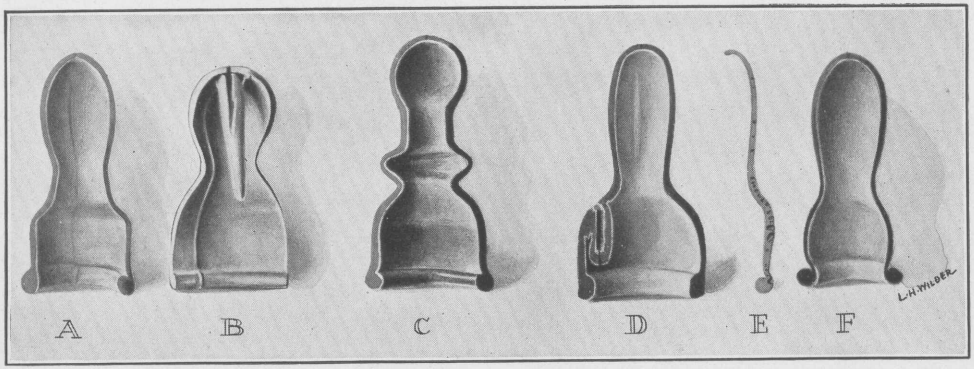
In addition to these chemical studies certain observations on the general hygienic properties of the various nipples were made. Considerable attention has been given by the various manufacturers to the matter of collapsing nipple under service. In some cases special devices such as a separate air inlet valve and a special reenforcement of the upper part of the nipple have been adopted. Some of these devices are shown upon the accompanying plate.

The plate is also designed to show certain typical shapes and to bring out other matters of hygienic importance. Chief among these is the possibility of easy and thorough cleansing. Special attention should be given to the general smoothness of the interior finish and to the absence of reentrant angles or sharp crevices. The nipple labeled "F" is of excellent shape and smooth interior finish. The reenforcement at the base is rolled outward, care having been taken to make the inside rather than the outside smooth. In all the other figures shown this reenforcement has been rolled inward, giving a smooth exterior but leaving a place on the inner surface which is exceedingly difficult to properly cleanse. The two noncollapsible-feature figures are also open to the same objection. Most of the nipples examined showed prominent mold lines such as are indicated in figures "A" and "B," and many of them had exceedingly rough interiors. In several cases the rubber was exceedingly porous, as shown in the small sliver "E." In the case of odd shapes like that of nipple "C" there seems to be no possible advantage which at all compensates for the additional difficulty of cleaning. The advertising material that accompanies many of these nipples lays special emphasis upon their noncollapsing features as being preventives of colic. While noncollapsing nipples are in most cases an aid to the parent or nurse, there is no possible relationship between the collapsing of a nipple and the production of colic except a possible negative one. The occasional collapse of the nipple certainly conduces to slower feeding, and even an occasional crying spell during the nursing period in protest against the collapse of the nipple can hardly be considered detrimental.

On the whole the ideal nursing nipple should be sufficiently stiff to resist collapse to a reasonable extent, should be simply curved in its general outlines, easily turned inside out for cleaning, and present a perfectly smooth interior surface. It should be made of a good

grade of black rubber, free from shoddy and from antimony, lead, arsenic, and mercury. Of the fillers commonly employed, magnesia, zinc oxide, and clay are less undesirable and barytes is probably without harmful influence. There can be no objection to a red rubber colored with oxide of iron, although the advantages of such a compound will probably not compensate for the disadvantages of its being confused with antimony rubber.

The same general conclusions regarding the chemical composition apply to teething rings and to other rubber articles which are given to young children.




SECTIONS OF VARIOUS TYPES OF RUBBER NIPPLES.

# THE ANALYSIS OF THYMOL CAPSULES.[1]

By ATHERTON SEIDELL, *Technical Assistant.*

Since the beginning of the campaigns directed by the various State health authorities against the hookworm, immense quantities of thymol capsules have been used. These are of the 5 and 10 grain sizes filled with a mixture of equal parts of thymol and milk sugar. From time to time accidents, sometimes fatal, following the thymol treatment have been reported. Although it is probable that the cause of these accidents is in practically all cases attributable to idiosyncrasy or the giving in spite of warnings of substances known to increase the absorption of thymol, such as oils or alcohol, it has appeared of interest to obtain some data upon the composition of the thymol capsules so largely used at present. Accordingly, at the request of Dr. C. W. Stiles, of the Hygienic Laboratory, health officers in North and South Carolina and in Georgia submitted samples of the capsules in use by them. In all, nine samples from six different localities were submitted.

The average weight of the contents of the 10-grain capsules (=0.648 gram) was found to be 0.64 gram with maximum variations from 0.58 to 0.69 gram; that of the 5-grain capsules (=0.324 gram) was 0.32 with maximum variations of 0.29 to 0.34 grams. As far as weight of contents is concerned, the samples were all close to the amount specified.

Since the thymol mixture contained in the capsules was understood to consist of equal parts of thymol and milk sugar, an experiment was first made to ascertain whether the thymol could be titrated by the bromine hydrobromic acid method (Seidell, Am. Chem. J., **47**, 520, 1912) in presence of milk sugar. The following results show that the presence of milk sugar exerts no appreciable effect upon the thymol titration:

| Sample of thymol. | Added milk sugar. | C. c. thiosulphate for— | | Difference. | Thymol found. | Per cent thymol. |
|---|---|---|---|---|---|---|
| | | Excess. | Free. | | | |
| *Grams.* | *Grams.* | *Br.* | *HBr.* | | *Grams.* | |
| 0.0 | 0.5 | 9.15 | 9.25 | 0.1 | ..... | ..... |
| .1544 | .1 | 6.1 | 45.9 | 39.8 | 0.153 | 98.9 |
| .1620 | .5 | 3.8 | 45.2 | 41.4 | .159 | 98.1 |
| .1330 | 1.0 | 10.3 | 45.1 | 34.8 | .134 | 100.3 |

Readings standard thiosulphate × 1.823 = 0.05 N. exactly.

[1] Manuscript submitted for publication May 15, 1914.

In view of the above results the percentage of thymol in each of the mixtures contained in the nine samples of capsules was determined by the bromine hydrobromic acid method. The results were as follows:

| Sample No. | Weight of sample. | C. c. thiosulphate for— | | Difference. | Thymol found. | Per cent of thymol in sample. |
|---|---|---|---|---|---|---|
| | | Excess. | Free. | | | |
| | *Grams.* | *Br.* | *HBr.* | | *Grams.* | |
| 539 | 0.3004 | 12.8 | 52.6 | 39.8 | 0.1527 | 50.8 |
| 540 | .6025 | 16.7 | 95.5 | 78.8 | .3024 | 50.2 |
| 541 | .3143 | 19.2 | 59.8 | 40.6 | .1558 | 49.6 |
| 542 | .2397 | 22.6 | 54.4 | 31.8 | .1220 | 50.9 |
| 543 | .3129 | 26.6 | 66.6 | 40.0 | .1535 | 49.1 |
| 544 | .1331 | 20.8 | 37.2 | 16.4 | .0629 | 47.3 |
| 545 | .2347 | 14.7 | 44.8 | 30.1 | .1155 | 49.2 |
| 546 | .3192 | 16.1 | 57.8 | 41.7 | .1600 | 50.1 |
| 547 | .3281 | 12.6 | 52.1 | 39.5 | .1516 | 46.2 |

Readings standard thiosulphate $\times$ 1.023 = 0.05 N. exactly.

On the basis of these results it is apparent that the thymol mixtures vary very little from the 50 per cent of thymol they are reputed to contain.

The only other points upon which information appears desirable are in regard to the amount of milk sugar present and the purity of the thymol used in the capsules. The method used for these determinations was as follows: A sample of the thymol-milk sugar mixture was placed in a weighed Gooch crucible and the thymol extracted with absolute ether, using about 5 c. c. for each extraction and repeating the process five or more times. Separate tests showed this procedure to completely remove all the thymol from a 1-gram sample. After drying the Gooch crucible in a vacuum desiccator over night no further loss in weight occurred on drying two hours in a water bath at about 99°. The percentage of milk sugar was calculated from the weight of the residue not dissolved by ether. The purity was tested by extracting the milk-sugar residue with water and weighing the cuprous oxide precipitated from Fehling's solution by an aliquot of the aqueous milk-sugar solution, according to the details prescribed in the method of the Association of Official Agricultural Chemists. The weights of cuprous oxide corresponded in most cases to somewhat more than the theory for pure lactose. The ethereal extracts containing the thymol were allowed to evaporate spontaneously in a cool place, and the resulting crystalline thymol was powdered and dried over concentrated sulphuric acid for several days and the melting point then determined.

| Sample No. | Weight of Gooch crucible— | | Weight of sample. | Weight of Gooch after ether extraction. | Weight of extracted residue. | Calculated per cent milk sugar. | Melting point of extracted thymol. |
|---|---|---|---|---|---|---|---|
| | Empty. | +Sample. | | | | | |
| 539 | 12.8352 | 13.4900 | 0.6548 | 13.1583 | 0.3231 | 49.3 | 49.0°–49.5° |
| 540 | 12.2878 | 12.8706 | .5828 | 12.5730 | .2852 | 49.9 | 49.5°–50.0° |
| 541 | 13.7315 | 14.3630 | .6315 | 14.0460 | .3145 | 49.8 | 49.5°–50.0° |
| 542 | 14.3277 | 15.0252 | .6975 | 14.6723 | .3446 | 49.4 | 49.0°–49.5° |
| 543 | 12.2657 | 12.9037 | .6380 | 12.5984 | .3327 | 52.1 | 49.5°–50.0° |
| 544 | 12.2575 | 13.3020 | 1.0445 | 12.7886 | .5311 | 50.9 | 49.5°–50.0° |
| 545 | 11.7384 | 12.4159 | .6775 | 12.0839 | .3455 | 51.0 | 49.5°–50.0° |
| 546 | 13.0691 | 13.7078 | .6387 | 13.3959 | .3268 | 51.2 | 49.4°–49.6° |
| 547 | 13.2517 | 13.8941 | .6424 | 13.5878 | .3357 | 52.3 | 49.4°–49.6° |

The melting point of a sample of pure thymol on hand in the laboratory was made simultaneously with the above determinations and gave the figures 49.7–50.3°, which is just appreciably higher than for the thymol obtained from the capsules. These slightly lower results may be due to the retarded and apparently imperfect crystallization from the ethereal solutions, and would therefore not necessarily indicate an impurity in the thymol as present in the capsules.

An ash determination was made in the case of one of the samples by igniting the contents of one capsule in a platinum crucible. No detectable residue was obtained, therefore confirming the absence of inorganic impurities in the sample.

A test for possible poisonous constituents was made by extracting the thymol from the mixed contents of 25 capsules with ether and administering the milk-sugar residue to a dog. No noticeable effects were produced.

## SUMMARY.

The determinations made upon the nine samples of thymol capsules used for hookworm treatment show that they contain approximately equal weights of thymol and milk sugar, each of satisfactory purity. No indication was obtained of harmful impurities which might account for accidental cases of poisoning sometimes noted in the thymol treatment of hookworm.

# SEASONAL VARIATION IN THE COMPOSITION OF THE THYROID GLAND.[1]

By Atherton Seidell and Frederic Fenger.

In a previous paper [2] on this subject we have shown that a very marked seasonal variation exists in the iodine content of the thyroid gland of the sheep, beef, and hog. This work has now been extended for another year and additional determinations made which show that other constituents than the iodine vary regularly with season.

The plan of collecting, preparing, and analyzing the samples was practically the same as mentioned in our former paper. In order to increase the number of individual animals represented by a given sample without increasing the bulk of the material to be handled, it was decided to use only one lobe from each animal instead of the whole gland. This could not be done, however, in the case of hog thyroids since the gland grows in a single mass from which one lobe can not easily be separated. The material was collected as before on Tuesdays and Thursdays of each week and the four lots representing a two-week period mixed. On account of the present methods of slaughtering it was not possible to secure perfectly whole glands in all cases. For weighing purposes, only entire glands were used and the numbers weighed stated in the tables. The maximum and minimum weights per lobe were ascertained by selecting the largest and smallest glands from each lot and weighing these individually. The highest and lowest figure in each case is reported in the tables.

The cleaned glands were finely minced, dried at 65° and degreased by extraction with petroleum ether in a Soxhlet apparatus. The slightly higher temperature above that previously used for drying resulted in somewhat more uniform and lower figures for the moisture content of the finished gland powder. On account of the difficulty experienced last year with the porcelain ball mills, due to the gradual wearing away of their inner surfaces and consequent contamination of the samples with silicious material, which rendered the ash determinations valueless, it was necessary to use mills provided with steel grinding surfaces. Even with these, considerable wear occurred and it was necessary to remove the iron from the ground powder by means of a magnet. The resulting powder yielded an ash in which

[1] Manuscript submitted for publication May 15, 1914.

[2] J. Biol. Chem., 13, 517 (1913).

there was a detectable trace of iron, but apparently not enough to appreciably affect the accuracy of the results.

The determinations of iodine, phosphorous, moisture and ash in the desiccated gland powder were made independently by each of us. The two sets of iodine determinations made by the Hunter method agreed in all cases to the second decimal place, showing again the reliability of this method in the hands of different analysts. Contrary to the experience of last year with the moisture determinations, constant results could not be obtained in all cases. A somewhat higher temperature was selected for the drying, namely, 105°–108° instead of approximately 98°, but differences of as much as 0.8 per cent between duplicate determinations were sometimes obtained. In such cases the higher result was always used for calculating the other values to the dry basis.

In the case of the ash determinations it was realized that only approximate results could be obtained even under the best conditions. The last traces of carbon are removed only by intense ignition and at the very high temperatures a fusion and partial volatilization of some inorganic constituents will undoubtedly occur. Duplicate determinations made by igniting the samples in a muffle furnace in groups of 10 gave results in the hands of one of us varying in some cases by as much as 0.6 per cent. The two sets of determinations made by each of us independently varied in a few cases by as much as 1 per cent. In selecting the figures quoted in the tables the lower results were given preference since it was considered that the principal error is that due to incomplete combustion of the carbon, and this would of course lead to high results.

The determinations of the phosphorous (expressed as pentoxide) were made by the well-known Neumann acid ashing method,[1] followed by a volumetric titration of the yellow ammonium molybdate precipitate according to the details prescribed in the methods of the Association of Official Agricultural Chemists. One gram of the sample was digested in a Kjeldahl flask with 5 c.c. of concentrated sulphuric acid plus 5 c.c of concentrated nitric acid until all red fumes disappeared, an additional 3 to 5 c.c. of nitric acid was then added and the heating continued until the red fumes again disappeared. This was repeated a second and third time if found necessary for the complete destruction of the organic matter, as indicated by disappearance of practically all dark color from the residual liquid. After cooling, distilled water was added and the solution transferred to a beaker, about 25 c.c of strong ammonia and a slight excess of nitric acid added. The precipitation with the ammonium molybdate reagent, filtration, washing and titration of the yellow precipitate

[1] Z. Physiol. Chem. 37, 115, 103; Arch. f. (Anat. u.) Physiol. *1905*, 208.

was made according to the prescribed details of the official method. It was found that very satisfactory duplicate determinations were obtained by this procedure. Attention has been called by Richardson [1] to the probable effect of sulphuric acid in causing high results by the volumetric method. Comparative experiments made by digesting a thyroid sample as above described and by alkaline ashing, indicated that a positive error of not exceeding 0.04 per cent $P_2O_5$ may be ascribed to the sulphuric acid used in the acid ashing method. This difference was considered to be within the limit of accuracy required for the present work.

TABLE I.—*Showing analytical results obtained upon composite samples of beef thyroid glands.*

[Collected at biweekly periods during one year.]

| No. of sample in series. | Mid-date of its biweekly collection period. | Single thyroid lobes (1 from each animal). | | Weight per single fresh lobe. | | | Per cent moisture in the prepared fat-free dried gland powder. | Constituents of gland powder calculated to dry basis. | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Number collected. | Number weighed. | Average. | Maximum. | Minimum. | | Per cent iodine. | Per cent $P_2O_5$. | Per cent ash. |
| | 1912. | | | | | | | | | |
| 1..... | Dec. 11 | 396 | 238 | 13.5 | 26.0 | 9.0 | 4.2 | 0.300 | 1.27 | 3.18 |
| 2..... | Dec. 29 | 402 | 57 | 18.6 | 25.0 | 9.0 | 4.4 | .316 | 1.20 | 3.02 |
| | 1913. | | | | | | | | | |
| 3..... | Jan. 12 | 370 | 58 | 13.4 | 23.0 | 7.0 | 4.6 | .264 | 1.17 | 3.15 |
| 4..... | Jan. 26 | 402 | 97 | 15.4 | 47.0 | 5.0 | 6.2 | .216 | 1.61 | 3.88 |
| 5..... | Feb. 9 | 397 | 137 | 22.2 | 72.0 | 8.0 | 11.5 | .127 | 1.93 | 4.67 |
| 6..... | Feb. 23 | 453 | 158 | 22.1 | 74.0 | 8.0 | 6.3 | .121 | 1.80 | 4.26 |
| 7..... | Mar. 9 | 469 | 151 | 24.6 | 46.0 | 8.0 | 4.3 | .119 | 1.70 | 4.36 |
| 8..... | Mar. 23 | 473 | 137 | 22.1 | 60.0 | 10.1 | 3.1 | .119 | 1.77 | 4.38 |
| 9..... | Apr. 6 | 422 | 192 | 21.0 | 73.2 | 8.2 | 3.0 | .095 | 1.80 | 4.79 |
| 10..... | Apr. 20 | 342 | 143 | 31.9 | 70.0 | 8.6 | 3.3 | .049 | 1.87 | 5.02 |
| 11..... | May 4 | 422 | 257 | 23.4 | 73.5 | 8.5 | 4.1 | .071 | 2.01 | 5.19 |
| 12..... | May 18 | 489 | 253 | 23.2 | 63.0 | 7.5 | 3.9 | .124 | 1.82 | 4.71 |
| 13..... | June 1 | 369 | 254 | 25.7 | 65.0 | 8.0 | 3.3 | .144 | 1.76 | 4.55 |
| 14..... | June 15 | 414 | 259 | 22.7 | 70.0 | 7.5 | 4.4 | .195 | 1.65 | 3.91 |
| 15..... | June 29 | 494 | 314 | 17.3 | 40.0 | 7.5 | 5.2 | .273 | 1.30 | 3.93 |
| 16..... | July 13 | 397 | 234 | 18.6 | 65.0 | 7.5 | 3.2 | .317 | 1.34 | 3.85 |
| 17..... | July 27 | 435 | 316 | 14.5 | 45.0 | 8.5 | 3.7 | .366 | 1.25 | 3.42 |
| 18..... | Aug. 10 | 366 | 200 | 14.9 | 65.0 | 6.5 | 2.8 | .353 | 1.13 | 3.83 |
| 19..... | Aug. 24 | 416 | 244 | 14.3 | 56.5 | 7.5 | 1.7 | .376 | 1.08 | 2.56 |
| 20..... | Sept. 7 | 491 | 280 | 13.1 | 28.0 | 7.5 | 2.2 | .426 | 1.07 | 2.35 |
| 21..... | Sept. 21 | 425 | 242 | 15.9 | 39.0 | 7.0 | 3.1 | .419 | 1.07 | 2.62 |
| 22..... | Oct. 5 | 396 | 231 | 14.7 | 50.0 | 7.5 | 4.0 | .380 | 1.12 | 2.62 |
| 23..... | Oct. 19 | 471 | 284 | 13.3 | 50.5 | 7.5 | 3.4 | .368 | 1.13 | 2.78 |
| 24..... | Nov. 2 | 365 | 202 | 15.9 | 49.5 | 8.5 | 4.6 | .366 | 1.18 | 3.20 |
| 25..... | Nov. 16 | 438 | 310 | 12.5 | 29.0 | 7.5 | 2.1 | .321 | 1.19 | 3.25 |
| 26..... | Nov. 30 | 317 | 208 | 17.1 | 59.5 | 7.0 | 2.0 | .276 | 1.25 | 2.93 |

[1] J. Am. Chem. Soc., **29**, 1314 (1907).

TABLE II.—*Showing analytical results obtained upon composite samples of hog thyroid glands.*

[Collected at biweekly periods during one year.]

| No. of sample in series. | Mid-date of its biweekly collection period. | Whole thyroid glands (both lobes). | | Weight per whole fresh gland. | | | Per cent moisture in the prepared fat-free dried gland powder. | Constituents of gland powder calculated to dry basis. | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Number collected. | Number weighed. | Average. | Maximum. | Minimum. | | Per cent iodine. | Per cent $P_2O_5$. | Per cent ash. |
| | 1912. | | | | | | | | | |
| 1 | Dec. 11 | 393 | 393 | 8.5 | 30.0 | 5.0 | 4.0 | 0.324 | 1.38 | 3.13 |
| 2 | Dec. 29 | 444 | 444 | 8.5 | 30.0 | 5.0 | 4.3 | .275 | 1.52 | 3.36 |
| | 1913. | | | | | | | | | |
| 3 | Jan. 12 | 288 | 155 | 8.5 | 20.0 | 5.0 | 4.7 | .310 | 1.43 | 3.16 |
| 4 | Jan. 26 | 446 | 268 | 8.7 | 26.0 | 5.0 | 5.7 | .298 | 1.47 | 3.30 |
| 5 | Feb. 9 | 463 | 236 | 9.8 | 29.0 | 5.0 | 6.2 | .211 | 1.61 | 3.84 |
| 6 | Feb. 23 | 482 | 241 | 10.2 | 30.0 | 5.0 | 5.4 | .239 | 1.62 | 3.85 |
| 7 | Mar. 9 | 419 | 239 | 10.6 | 26.0 | 5.0 | 2.9 | .213 | 1.56 | 3.79 |
| 8 | Mar. 23 | 439 | 272 | 10.7 | 20.0 | 3.8 | 2.0 | .222 | 1.60 | 3.85 |
| 9 | Apr. 6 | 409 | 286 | 10.7 | 23.4 | 4.5 | 2.3 | .259 | 1.59 | 3.82 |
| 10 | Apr. 20 | 426 | 279 | 9.1 | 20.0 | 3.7 | 2.2 | .297 | 1.42 | 3.40 |
| 11 | May 4 | 427 | 273 | 9.9 | 20.0 | 3.0 | 2.6 | .318 | 1.41 | 3.56 |
| 12 | May 18 | 397 | 273 | 9.5 | 20.0 | 3.0 | 3.1 | .301 | 1.38 | 3.78 |
| 13 | June 1 | 459 | 374 | 8.6 | 20.0 | 3.0 | 2.8 | .304 | 1.41 | 3.44 |
| 14 | June 15 | 438 | 359 | 8.7 | 20.0 | 3.0 | 3.4 | .337 | 1.28 | 3.41 |
| 15 | June 29 | 351 | 257 | 8.6 | 20.0 | 3.0 | 3.1 | .362 | 1.38 | 3.37 |
| 16 | July 13 | 399 | 289 | 8.9 | 25.0 | 2.5 | 3.7 | .440 | 1.15 | 2.94 |
| 17 | July 27 | 400 | 270 | 8.9 | 20.0 | 2.5 | 4.3 | .427 | 1.30 | 3.61 |
| 18 | Aug. 10 | 438 | 308 | 9.8 | 20.0 | 2.5 | 3.1 | .444 | 1.10 | 2.97 |
| 19 | Aug. 24 | 436 | 313 | 9.3 | 20.0 | 2.0 | 2.1 | .429 | .95 | 2.86 |
| 20 | Sept. 7 | 427 | 306 | 9.9 | 20.0 | 2.0 | 2.3 | .424 | .97 | 2.87 |
| 21 | Sept. 21 | 427 | 317 | 10.5 | 20.0 | 2.0 | 3.7 | .410 | .95 | 3.00 |
| 22 | Oct. 5 | 451 | 322 | 11.2 | 20.0 | 2.5 | 3.6 | .437 | 1.05 | 2.93 |
| 23 | Oct. 19 | 464 | 357 | 10.2 | 21.0 | 2.5 | 3.7 | .382 | 1.04 | 3.15 |
| 24 | Nov. 2 | 457 | 337 | 8.5 | 20.0 | 2.0 | 4.3 | .385 | 1.01 | 3.00 |
| 25 | Nov. 16 | 478 | 332 | 9.0 | 20.0 | 2.5 | 1.9 | .350 | 1.04 | 3.16 |
| 26 | Nov. 30 | 349 | 298 | 8.3 | 20.0 | 2.0 | 2.3 | .342 | .97 | 3.11 |

TABLE III.—*Showing analytical results obtained upon composite samples of sheep thyroid glands.*

[Collected at biweekly periods during one year.]

| No. of sample in series. | Mid-date of its biweekly collection period. | Single thyroid lobes collected and weighed (1 from each animal). | Weight per single fresh lobe. | | | Per cent moisture in the prepared fat-free dried gland powder. | Constituents of gland powder calculated to dry basis. | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | Average. | Maximum. | Minimum. | | Per cent iodine. | Per cent $P_2O_5$. | Per cent ash. |
| | 1912. | | | | | | | | |
| 1 | Dec. 11 | 606 | 2.6 | 12.0 | 1.0 | 4.3 | 0.205 | 1.50 | 3.59 |
| 2 | Dec. 29 | 446 | 3.3 | 14.0 | 2.0 | 5.6 | .194 | 1.65 | 3.78 |
| | 1913. | | | | | | | | |
| 3 | Jan. 12 | 742 | 3.3 | 13.0 | 2.0 | 5.0 | .188 | 1.67 | 3.92 |
| 4 | Jan. 26 | 780 | 3.4 | 12.0 | 2.0 | 5.8 | .162 | 1.70 | 4.38 |
| 5 | Feb. 9 | 773 | 2.8 | 13.0 | 2.0 | 7.3 | .119 | 2.02 | 4.52 |
| 6 | Feb. 23 | 661 | 2.8 | 20.0 | 2.0 | 5.3 | .108 | 2.00 | 5.40 |
| 7 | Mar. 9 | 712 | 2.9 | 20.0 | .9 | 3.0 | .082 | 2.20 | 4.67 |
| 8 | Mar. 23 | 628 | 3.6 | 18.2 | 1.0 | 4.7 | .099 | 2.00 | 4.83 |
| 9 | Apr. 6 | 532 | 5.2 | 20.0 | 1.4 | 4.6 | .049 | 2.50 | 5.36 |
| 10 | Apr. 20 | 644 | 4.8 | 22.4 | 1.0 | 3.7 | .085 | 2.02 | 4.49 |
| 11 | May 4 | 598 | 5.7 | 24.9 | 1.9 | 4.1 | .068 | 2.07 | 5.25 |
| 12 | May 18 | 517 | 6.3 | 25.0 | 1.3 | 3.9 | .073 | 2.17 | 5.09 |
| 13 | June 1 | 478 | 7.7 | 25.0 | 2.5 | 5.5 | .085 | 2.31 | 5.30 |
| 14 | June 15 | 790 | 4.7 | 20.5 | 1.2 | 5.3 | .143 | 1.91 | 4.88 |
| 15 | June 29 | 763 | 4.0 | 23.0 | 1.5 | 3.1 | .243 | 1.52 | 4.10 |
| 16 | July 13 | 774 | 3.6 | 20.0 | 2.0 | 2.5 | .260 | 1.54 | 4.07 |
| 17 | July 27 | 648 | 3.6 | 25.0 | 2.5 | 4.4 | .257 | 1.64 | 4.33 |
| 18 | Aug. 10 | 547 | 4.8 | 20.0 | 2.0 | 3.1 | .185 | 1.46 | 3.70 |
| 19 | Aug. 24 | 831 | 3.5 | 17.0 | 1.6 | 2.3 | .230 | 1.42 | 3.25 |
| 20 | Sept. 7 | 615 | 3.6 | 21.0 | 2.0 | 2.4 | .222 | 1.33 | 3.60 |
| 21 | Sept. 21 | 734 | 3.8 | 17.0 | 2.0 | 3.9 | .206 | 1.36 | 3.45 |
| 22 | Oct. 5 | 753 | 3.4 | 12.0 | 2.0 | 3.1 | .192 | 1.29 | 3.49 |
| 23 | Oct. 19 | 626 | 3.9 | 19.0 | 2.5 | 3.7 | .212 | 1.34 | 3.60 |
| 24 | Nov. 2 | 604 | 3.8 | 22.5 | 2.0 | 3.5 | .202 | 1.34 | 3.07 |
| 25 | Nov. 16 | 644 | 3.6 | 12.0 | 2.5 | 2.0 | .214 | 1.32 | 3.67 |
| 26 | Nov. 30 | 536 | 3.8 | 15.0 | 3.0 | 1.8 | .229 | 1.23 | 3.03 |

The results obtained upon the three series of samples are shown in the accompanying tables (Nos. I, II, and III). For the sake of continuity with the data reported in our preceding paper, the iodine results for 1912 and 1913 have been plotted on one diagram and average curves drawn through or near the greatest possible number of points. As will be seen by reference to figure No. 1 the descent or ascent of iodine content with time appears to follow very closely straight lines, and at the changes in direction only a slight amount of rounding is required. By drawing sinuous curves which were thought to more nearly represent the actual conditions, a larger number of discrepant points were obtained than by the above mentioned plan. The data, therefore, indicate that the activity of the thyroid diminishes regularly from about September to the following March or April, and then abruptly changes to a regular increase in activity from March or April to the following September. The maximum and minimum points vary somewhat with the three animals and with the year. The variation between the high and the low points in the case of beef is from 0.04–0.43 per cent iodine, of the hog

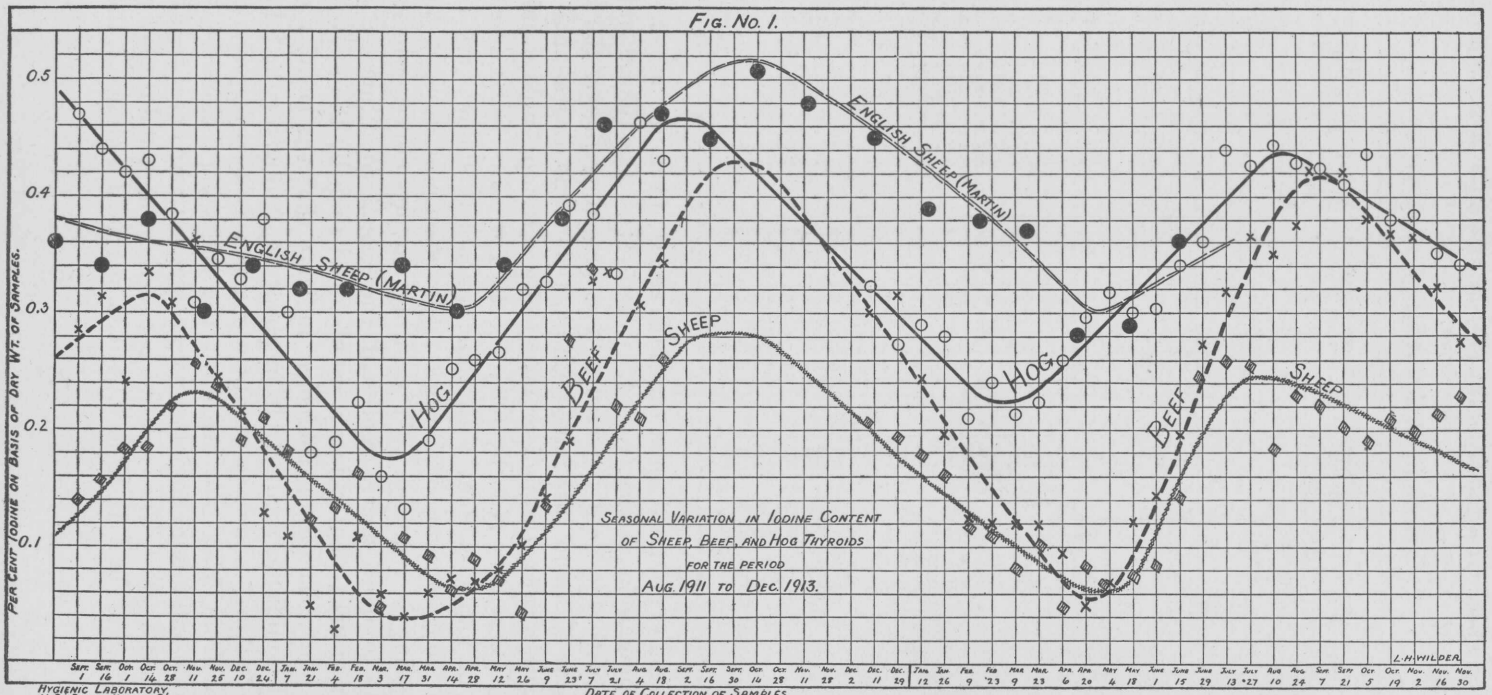
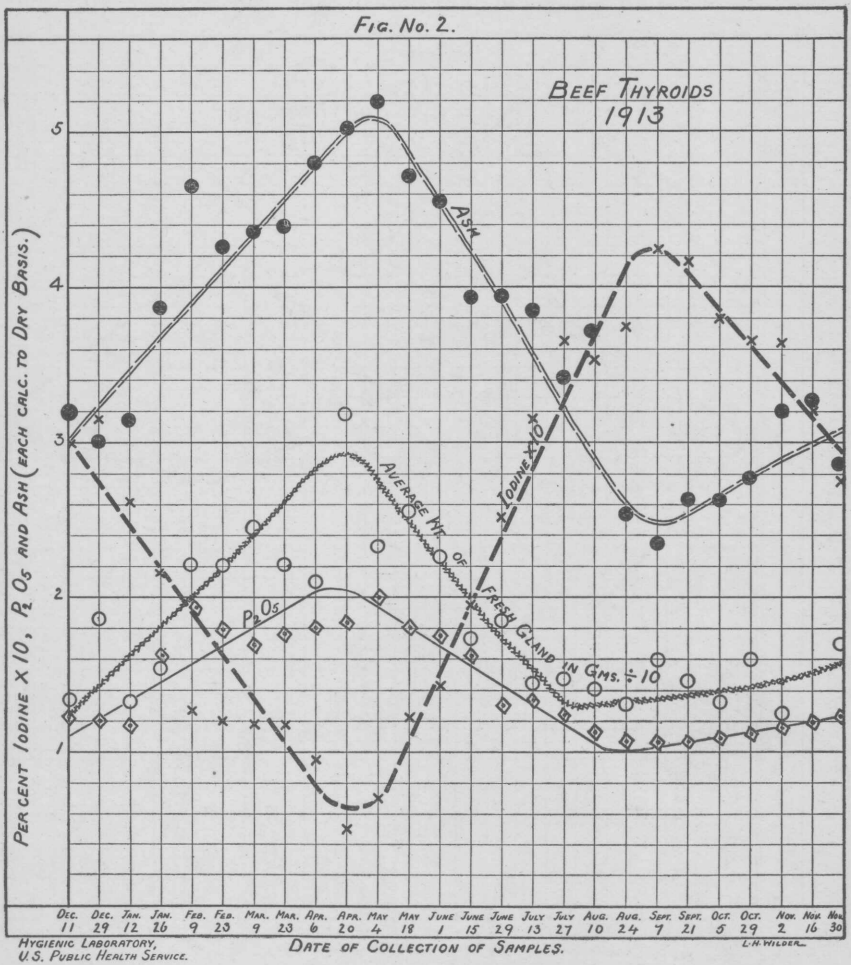


FIG. No. 1.
PER CENT IODINE ON BASIS OF DRY WT. OF SAMPLES.
0.5
0.4
0.3
0.2
0.1
ENGLISH SHEEP (MARTIN)
HOG
BEEF
SHEEP
ENGLISH SHEEP (MARTIN)
HOG
BEEF
SHEEP
SEASONAL VARIATION IN IODINE CONTENT
OF SHEEP, BEEF, AND HOG THYROIDS
FOR THE PERIOD
AUG. 1911 TO DEC. 1913.
L. H. WILDER.
HYGIENIC LABORATORY,
U. S. PUBLIC HEALTH SERVICE.
DATE OF COLLECTION OF SAMPLES.

0.17–0.47 per cent iodine, and of the sheep 0.06–0.28 per cent iodine. For the sake of comparison, the results of Martin [1] are also plotted. It is noteworthy that although the results of this investigator confirm our observations, he has apparently failed to observe that his results give evidence of the seasonal change in iodine content of the glands. Although the position of the curve for English sheep

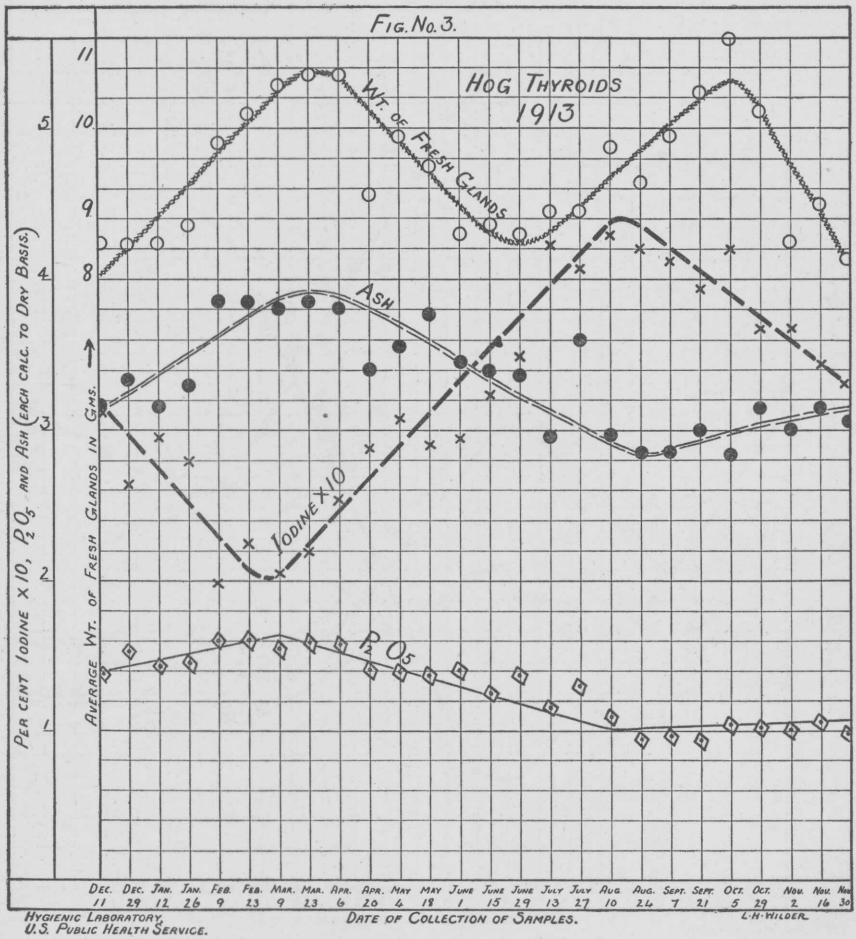



is above that for American sheep, due no doubt to particular climatic conditions, it is very interesting to note that the dates of the maximum and minimum points coincide fairly well with those found for American sheep.

The results of the phosphorus and ash determinations as well as the average weights of the fresh glands have been plotted in figures No. 2,

[1] Pharm. Jour. (Lond.), **89**, 144 (1912); **91**, 126 (1913).

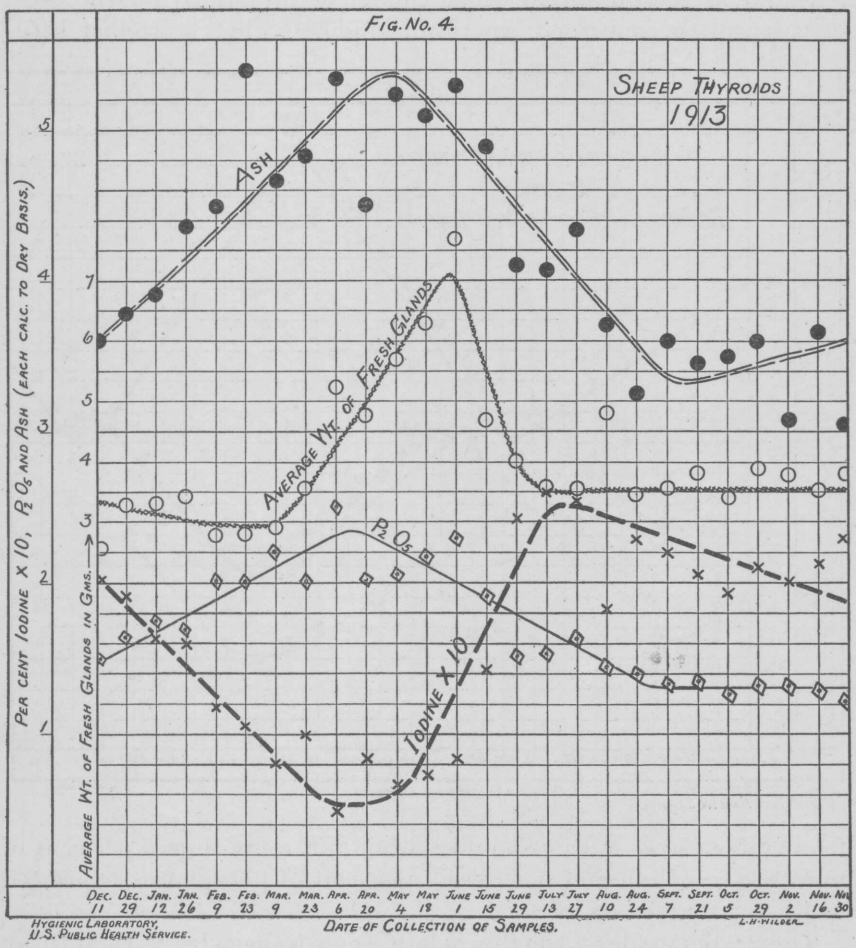
No. 3, and No. 4. For the sake of comparison, the iodine results multiplied by 10 are also given. It is seen that in each case the phosphorus results and the ash run more or less parallel and both vary inversely with the iodine. This inverse variation shows that phosphorus can not be a constituent of the physiologically active iodine compound of the thyroid gland. It is therefore probable that the observed seasonal change in phosphorus and ash is more apparent




than real and is due simply to the change, at various periods, in the relative amounts of the active iodine compound present in a glandular structure of fairly constant composition. According to this view neither the composition of the active iodine complex nor that of the supporting tissues of the gland changes with season, but only the relative amounts of the two. Consequently, for a given gland, an increase in the amount of its contained active iodine compound would

naturally be accompanied by an apparent decrease in the phosphorus content and vice versa.

A comparison of the curves for the three animals shows that the beef samples exhibit the greatest regularity. This appears to be especially true in connection with the results for the average weight of the fresh glands. Here, as would be expected, there is a fair parallelism between the weight of the glands and their ash and phos-

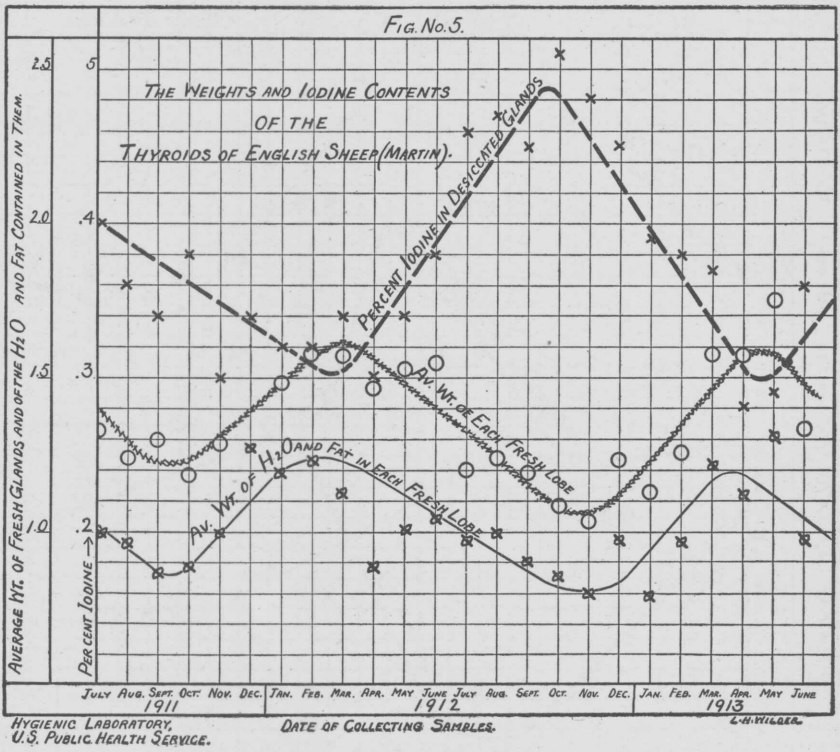



phorus content. In the case of the sheep this parallelism holds good only in a general sense, while with the hog there is a distinct variation for the latter half of the period. A second maximum occurs where a minimum would be expected.

Considering the results from the standpoint of the commercial production of desiccated thyroid of a prescribed composition, it is seen that the majority of the points for percentage of iodine lie above the

0.2 per cent line, and therefore, for only a short period of the year would it be impossibe to secure raw material which would yield a desiccated product containing at least 0.2 per cent iodine. If the material obtained during this period is reserved for mixing with the gland powder obtained during the remainder of the year, little difficulty should be experienced in supplying a product of quite uniform physiological activity.

As far as the limitation which should be prescribed for the other constituents is concerned, the results indicate that a product satis-




factory for use as a drug should contain not more than 5 per cent of moisture or of ash and not over 2 per cent of phosphorus expressed as the pentoxide.

The studies made on the thyroids of English sheep by N. H. Martin [1] and also by R. Glode Guyer [2], although undertaken especially to obtain data upon which to base a standard for a uniform thyroid preparation, appear of sufficient interest from the standpoint of seasonal variation to be briefly described in connection with the present experiments. The results of Martin, obtained during two one-

[1] Loc cit.

[2] Pharm. Jour. (Lond.), **91**, 123–126 (1913).

year periods, are combined in Table No. IV and plotted in figures Nos. 1 and 5. They refer to sheep bought in the open market at Newcastle-on-Tyne. It will be noted that the inverse proportionality between the iodine content and the weight of the fresh gland is even more consistent than that found for sheep from the United States. The average weight of water and fat in each fresh lobe runs in general parallel with the average weight of the fresh lobes. The percentage of dry material in the fresh glands apparently shows no regular change with season.

TABLE IV.—*Showing the percentage of iodine and the fresh and dry weights of thyroid glands obtained from English sheep (Martin).*[1]

| Date. | Number of lobes used. | Average weight of each fresh lobe. | Average weight $H_2O$ and fat in each fresh lobe. | Per cent dry material in fresh glands. | Per cent of iodine in dry material. |
|---|---|---|---|---|---|
| 1911. | | | | | |
| July | 590 | 1.33 | 1.00 | 24.8 | 0.40 |
| August | 885 | 1.24 | .97 | 21.8 | .36 |
| September | 490 | 1.29 | .88 | 31.8 | .34 |
| October | 490 | 1.19 | .89 | 25.2 | .38 |
| November | 190 | 1.28 | 1.00 | 21.9 | .30 |
| December | 253 | 1.61 | 1.33 | 17.3 | .34 |
| 1912. | | | | | |
| January | 216 | 1.48 | 1.19 | 19.6 | .32 |
| February | 184 | 1.57 | 1.24 | 21.0 | .32 |
| March | 393 | 1.57 | 1.13 | 28.0 | .34 |
| April | 1,081 | 1.46 | .89 | 39.0 | .30 |
| May | 958 | 1.52 | 1.01 | 33.5 | .34 |
| June | 830 | 1.55 | 1.04 | 32.9 | .38 |
| July | 1,223 | 1.21 | .98 | 23.1 | .46 |
| August | 1,944 | 1.23 | 1.00 | 18.7 | .47 |
| September | 1,220 | 1.19 | .91 | 23.5 | .45 |
| October | 1,318 | 1.08 | .86 | 20.4 | .51 |
| November | 942 | 1.04 | .80 | 23.1 | .48 |
| December | 564 | 1.23 | .97 | 21.1 | .45 |
| 1913. | | | | | |
| January | 470 | 1.13 | .79 | 30.1 | .39 |
| February | 1,172 | 1.26 | .97 | 23.0 | .38 |
| March | 1,032 | 1.58 | 1.22 | 22.8 | .37 |
| April | 1,584 | 1.58 | 1.12 | 29.1 | .28 |
| May | 1,826 | 1.75 | 1.31 | 25.2 | .29 |
| June | 632 | 1.32 | .97 | 26.5 | .36 |

[1] Pharm. Jour. (Lond.), **89**, 144, 1912; **91**, 126 (1913).

The experiments of Guyer were made upon the thyroids of sheep from the vicinity of Edinburgh. The daily supply of glands, amounting to several hundred single lobes on the average, were counted and the total weight determined, then dried at a temperature of 40° and the loss of moisture calculated. The weight after degreasing was also ascertained. The daily results are tabulated and the totals for each month given. Iodine determinations were made upon the bulked material collected during each one month period. The daily and monthly averages of weight per fresh lobe have been plotted in

figure No. 6, but only the monthly averages included in the following Table No. V:

TABLE V.—*Showing the average weight and iodine content of the thyroids of sheep from the vicinity of Edinburgh (Guyer).*

| Date. | Number of single lobes. | Average weight of each fresh lobe. | Per cent dried fat-free substance in fresh glands. | Per cent iodine in fat-free dried substance. |
|---|---|---|---|---|
| 1912. | | | | |
| December | 5,023 | 1.31 | 28.1 | 0.258 |
| 1913. | | | | |
| January | 7,220 | 1.40 | 29.9 | ..... |
| February | 3,966 | 1.50 | 28.1 | .222 |
| March | 4,142 | 1.71 | 27.0 | .266 |
| April | 5,374 | 2.22 | 26.4 | .229 |
| May | 5,981 | 2.27 | 27.2 | .251 |
| June | 4,262 | 1.96 | 22.1 | .279 |

As will be seen, there is a very marked variation from day to day in the average weight per lobe, due in part no doubt to failure to reject abnormal glands or those from immature animals. In spite of this daily variation there is an unmistakable seasonal change in the average weight per lobe, and the maximum point corresponds approximately with that found for the same year by Martin, but is about one month in advance of the maximum point found by us for American sheep. The iodine determinations are somewhat lower than those of Martin in the cases of Newcastle-on-Tyne sheep. If one point is omitted, the others agree in showing a minimum at approximately the date established on the basis of the results of Martin and the figures reported by us in the present paper.

Among the several factors which may be considered of importance in accounting for the seasonal change in composition and activity of the thyroid gland may be mentioned temperature, diet, and periods of activity of the reproductive function. Of these the temperature effect in connection with its influence upon changes in metabolism appears to be of principal importance. During the colder months of the year the increased physical activity of animals together with the necessity for maintenance of the normal body temperature in a colder surrounding medium requires an increased metabolism which in turn would no doubt call for an increased output of the active thyroid material. Conversely, in the summer months, the demands upon the thyroid being less than the normal rate of production of active principle, would lead to a storing up of the iodine compound. The observed regular decrease in percentage of iodine beginning with the cooler weather in September and continuing through the winter to the warmer weather of early spring and the

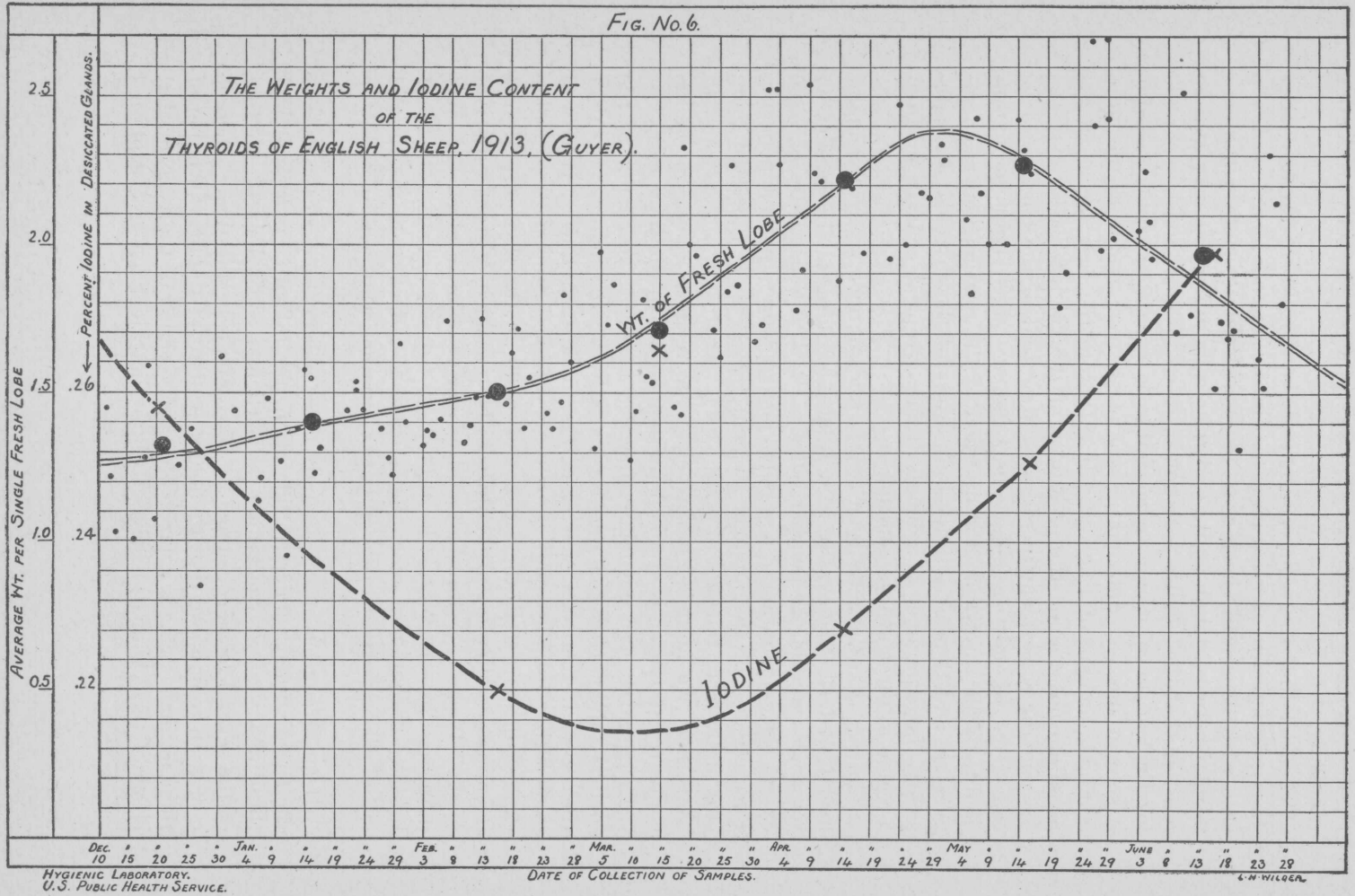


FIG. No. 6.
THE WEIGHTS AND IODINE CONTENT
OF THE
THYROIDS OF ENGLISH SHEEP, 1913, (GUYER).
AVERAGE WT. PER SINGLE FRESH LOBE
PERCENT IODINE IN DESICCATED GLANDS.
2.5
2.0
1.5
1.0
0.5
.26
.24
.22
WT. OF FRESH LOBE
IODINE
DEC. JAN. FEB. MAR. APR. MAY JUNE
10 15 20 25 30 4 9 14 19 24 29 3 8 13 18 23 28 5 10 15 20 25 30 4 9 14 19 24 29 4 9 14 19 24 29 3 8 13 18 23 28
DATE OF COLLECTION OF SAMPLES.
HYGIENIC LABORATORY.
U.S. PUBLIC HEALTH SERVICE.
L.N. WILDER.

regular increase thereafter, appears to accord exactly with this reasoning.

The fact that the iodine content of the thyroids of English sheep do not diminish to as great an extent in the summer months as is the case with American sheep may be considered to confirm the connection between temperature and thyroid activity. It may also be possible that the smaller variation between the high and low periods for English sheep, in contradistinction to the greater differences for American sheep at different seasons of the year is due to the more equitable climate of England. If this were the case the usual explanation based upon the proximity of the English pastures to the sea and the consequent increased salt content of the food could be considered of only secondary importance. Much light would undoubtedly be thrown on the question of the relation between temperature and thyroid activity by a study of the thyroids of a sufficiently large number of animals from a tropical country or from a region in the southern hemisphere.

The fact that the activity of the thyroids of mice, which live under nearly uniform temperature conditions the year round, also show a seasonal variation as pointed out by Dr. Hunt discredits to some extent the explanation of a seasonal change based upon temperature. Furthermore, of the animals studied, the sheep is protected more effectually from the cold of winter by its wool than is the case with the other two animals, nevertheless the relative change in iodine content with season is of about the same order in the three cases.

In regard to the possible connection between the change of the food at different seasons and the variation in the iodine content of the thyroid, there are several points to be mentioned. It is well known that feeding small amounts of various compounds of iodine causes an increase in the amount of iodine in the thyroid. Minute amounts of iodine are also known to be present in a great variety of foods. It can therefore be readily conceived that a given diet may influence the activity of the thyroid to a great extent. Dr. Hunt has also shown[1] that many foods cause an effect upon the thyroid out of all proportion to the quantity of iodine that could possibly be present.

If an attempt is made to apply these observations to the case of the three animals under consideration, the difficulty is encountered that little is known concerning the particular effect upon the thyroid of the several foods consumed by the animals in question. There is no reason to suppose that the iodine content of the food of either of these animals changes appreciably at any period of the year, or that either of these animals receives a winter diet which has an exceptional

[1] Hygenic Laboratory Bulletin No. 69, June, 1910.

effect in the direction of increasing the iodine content of the thyroid. In the case of the hog which might reasonably be expected to receive a more uniform diet throughout the year than either of the other animals, the magnitude of the variation in iodine content lies between that of the beef and the sheep. On the basis of our present knowledge it therefore seems improper to lay much emphasis upon the factor of diet as an explanation of the seasonal variations in thyroid activity.

There is also little to be said in regard to the connection between thyroid activity and the reproductive function. As shown by the curves, there is an unmistakable dimunition in the iodine content of the thyroids in the spring months and this of course is the time at which, in the case of each of the animals, birth is given to the larger number of offspring. The data of Fenger,[1] however, upon the influence of pregnancy upon the iodine content of the thyroid, fails to show that an increase in the activity of the gland can be ascribed to this factor.

When the very great individual variations in the iodine content of the thyroids of the same species of animals are considered in connection with the seasonal changes which have been demonstrated, it appears probable that the activity of the thyroid of individual animals is constantly fluctuating. The limits of the fluctuations change at different periods of the year, probably in much the same manner as indicated by the diagram, figure No. 6, representing the data of Guyer upon the average weights of fresh sheep glands collected at Edinburgh. From this it would follow that various circumstances may cause temporary fluctuations in the iodine content of the thyroid, especially those which affect metabolism.

## SUMMARY.

The evidence for a seasonal variation in the iodine content, and consequently the activity of the thyroid gland, has been confirmed by analyses of samples collected during another one-year period.

It has furthermore been shown that a regular change occurs in the phosporus and ash content of thyroids and the amounts of these constituents vary inversely with the iodine. This is explained on the assumption that phosphorus does not form a part of the active iodine complex of the gland, but only of the supporting glandular tissues. An increase in the percentage of iodine would therefore naturally be accompanied by a decrease of phosphorus. Consequently it appears that neither the composition of the active iodine complex nor that of the supporting tissues of the gland changes with season but only the relative amounts of the two.

[1] J. Biol. Chem., 17, 23 (1914).

In regard to the fresh weights of the glands the results show a more or less regular seasonal change coincident with the iodine in the case of the beef and sheep but not with the hog.

The results of Martin and Guyer upon the thyroids of English sheep confirm our observations in regard to the seasonal change in the activity of the gland.

A consideration of the several causes of the seasonal change in activity of the thyroid leads to the conclusion that the temperature factor is the most important of all.

# NOTE ON A NEW APPARATUS FOR USE WITH THE WINKLER METHOD FOR DISSOLVED OXYGEN IN WATER.[1]

By HYMAN L. SHOUB, *Sanitary Bacteriologist.*

The determination of dissolved oxgen in water by the Winkler method necessitates the use of a full bottle of water up to the point of final titration. The present Standard Methods procedure calls for the titration of the entire contents of the bottle. If $n$ cubic centimeters of the standard N/40 thiosulphate solution are used then the dissolved oxygen in parts per million is 200n/v in which $v$ is the volume of the bottle.

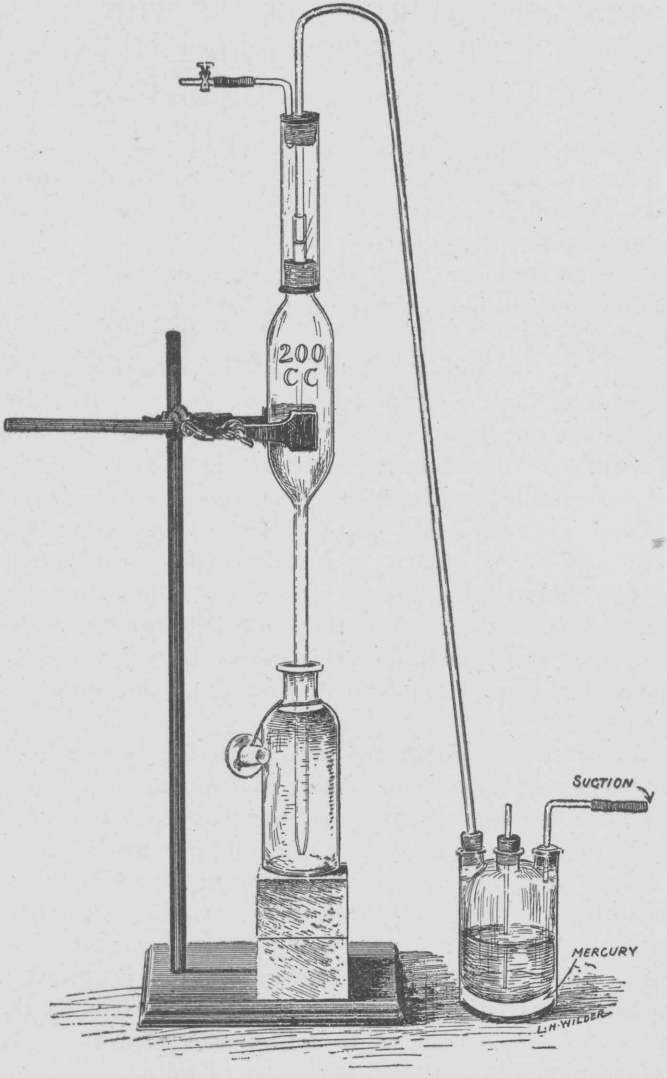
There are seldom any two bottles of the same lot having exactly the same capacities so that it becomes necessary to calibrate and mark each new bottle, to record the capacity with the titration and finally to divide the titration value by the capacity. Where large numbers of samples are being handled, this procedure involves considerable unnecessary labor and several avoidable opportunities for accidental error. At many laboratories this procedure has been modified by the withdrawal and titration of a definite volume of the solution. At the Hygienic Laboratory 200 cubic centimeters are taken, and, as it is desirable to withdraw the liquid with the least possible agitation and exposure to the air, a 200 cubic centimeter pipette is used.

The apparatus shown in the accompanying sketch has been designed for the purpose of economizing time and labor in this operation.

The pipette extends through a rubber stopper into a suction chamber made of 1-inch glass tubing. From the top of this chamber there project two tubes, one terminating in a glass cock open to the air, the other in the pressure regulator bottle shown below. The latter tube extends into the suction chamber and to a point within the end of the pipette which is exactly opposite its gradation mark. The pressure regulator bottle is connected directly to the suction system or may be connected to a Richard's aspirator. It holds a layer of mercury above which is a layer of water. A capillary tube enters this bottle through the center hole and projects slightly beneath the mercury surface. The submergence of this tube is so arranged that when the suction is applied at a constant and slow rate, water will rise in the pipette and part way into the suction

[1] Manuscript submitted for publication May 15, 1914.

tube. When it has reached a point about opposite the upper end of the suction chamber air enters the regulator bottle through the submerged capillary and further increase in the height of the water column is thereby prevented. An approximate adjustment of this




Apparatus for use with the Winkler method of determining dissolved oxygen in water.

level is made by submerging the capillary in the mercury and it is finally adjusted by adding water above the mercury until the desired result is obtained.

Having regulated the apparatus in this way, its operation is as follows: The bottle of water which has been prepared for titration is

placed in position beneath the pipette and suction applied at such a rate that the pipette will just about fill in the time required for a titration. The stopcock leading to the air is closed. Titration of the previous sample is then proceeded with without further attention to the apparatus. The liquid fills the pipette to its mark and then enters the upper tube up to a point about on a level with the upper rubber stopper, where it comes into equilibrium with the submerged capillary in the regulator bottle, air being admitted through the latter and the water level remaining constant in the suction tube. The whole system is now in equilibrium, and will remain so indefinitely. The bottle is next removed and a flask placed in a similar position. The stopcock is then opened to the air. The contents of the pipette are discharged rapidly into the flask, while the contents of the suction tube above its lower point are drawn over into the regulator bottle. The next sample bottle is then put in place, the stopcock closed, and the titration of the present sample proceeded with.

# THE PHARMACOLOGICAL ACTION OF SOME SERUM PRESERVATIVES.[1]

By CARL VOEGTLIN, *Professor of Pharmacology.*

## 1. PREVIOUS EXPERIMENTAL WORK AND CLINICAL OBSERVATIONS.

It is a well-established fact that since the introduction of the serum treatment in cerebrospinal meningitis the mortality from this disease has been greatly reduced. Although no untoward effects are observed in the great majority of cases treated with antimeningitis serum, a few cases react to the serum with sudden collapse characterized by stoppage of the respiration and low-blood pressure. Frost (1), Parmelee (2), Kramer (3), Litterer (4), and others have described such accidents in the course of treatment. These symptoms were all attributed by Kramer to the toxic action of the trikresol used for the preservation of the serum. This author described experiments on dogs in which 0.5 per cent trikresol serum, if injected into the subarachnoid space of these animals, caused stoppage of the respiration and a rapid fall of blood pressure. He explains the toxic action of the trikresol as being due to a patent central canal of the cord which permits the trikresol to reach the medullary centers; a view which seems hardly justified. Flexner (5), in a more recent article, attributes the symptoms to increased intracranial pressure, a view also held by Sophian (6). Other explanations such as the lytic action of the serum on the meningococcus present in the cerebrospinal fluid with a subsequent liberation of toxin, have been advanced. Anaphylaxis was thought to be responsible for the occurrence of collapse. Of all these explanations Kramer's hypothesis of the preservative being the cause of the symptoms, seems to be the most important; Hale (7), in a recent bulletin from this laboratory, was able to demonstrate beyond doubt the toxic action of trikresol serum, if introduced into the subarachnoid space of dogs and cats. The animals were anæsthetized by means of morphine-ethyl carbamate-chloral hydrate or ethyl carbamate-

[1] Manuscript submitted for publication May 15, 1914.

chloral hydrate. Normal serum, if injected by means of the syringe into the subarachnoid space, produced in these animals symptoms due to increased intracranial pressure, such as described by Cushing (**8**) and Eyster (**9**), namely, stoppage of respiration and considerable rise in blood pressure. On the contrary, serum containing 0.3 and 0.5 per cent trikresol caused always a fall in blood pressure (only in a few instances a slight preliminary rise was observed) and cessation of the respiration. Hale arrives at the conclusion that "the danger from trikresol seems much greater and more certain than that which might possibly arise from increased intracranial pressure."

The purpose of the present investigation was (*a*) to confirm the previous results obtained with trikresol serum (Kramer and Hale), (*b*) to study the quantitative effect of various concentrations of trikresol in serum on blood pressure and respiration by means of the syringe and gravity methods, (*c*) to extend the work to other species of animals, and (*d*) to test other preservatives besides trikresol in order to find a more desirable substitute for the phenol preservative.

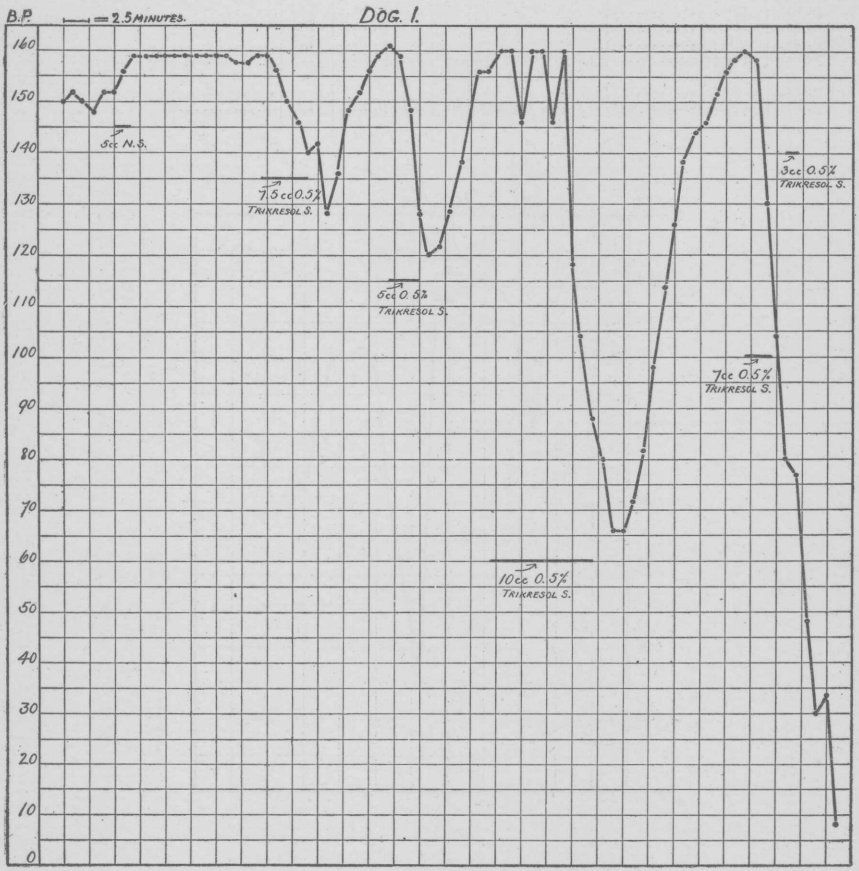
We selected dogs and monkeys (*Rhesus macacus*) for the experiments. Throughout the work a light ether anæsthesia was used. Tracheotomy was performed and warm ether vapors from a bottle were introduced. It seemed very desirable to make use of a light ether anæsthesia instead of the nonvolatile anæsthetics used by Hale in order to rule out, as far as possible, the depressing effect of these latter anæsthetics on the medullary centers. The degree of anæsthesia obtained with ether was fairly constant and the changes of blood pressure and respiration due to the anæsthetic were very slight and did not interfere with the correct interpretation of the action of the serum studied in each case. Respiratory movements were recorded by means of a canula inserted in the pleural cavity and connected by means of a rubber tube with a tambour. Blood pressure was measured by means of a mercury manometer connected with a glass canula which was inserted into the carotid artery.

For the injection of the serum into monkeys, a syringe needle connected with a short piece of rubber tube was introduced through the skin and muscles into the spinal canal in the upper lumbar region. With some experience one is able to get the needle into the proper situation, making use of the same directions as in lumbar puncture in the human. In dogs it was necessary to expose about four of the lumbar vertebræ; the needle could then be introduced without great difficulty between two vertebræ by the lateral route. Cerebrospinal fluid escaped through the needle. The needle was then connected with a syringe or a graduated burette according to the method to be employed. It was left in place for a number of injections,

care being taken not to change its position. For the gravity method an average injection pressure of 40 to 50 cm. was found to be very satisfactory. The results of some of the experiments are given in the following tables. For the sake of clearness charts were prepared illustrating the relation between the length of time of injection to the concentration of the preservative and the effect on the blood pressure. As will be seen from an examination of the charts, there exists a definite relation between the degree of change in blood pressure and the time used for the injection of the serum and the concentration of the preservative in the serum. As a rule, it can be said that, having a given volume of serum, the fall or rise in blood pressure increased with the rate of injection. The fall of blood pressure after serum containing a phenol preservative increased with the concentration of the preservative in the serum under otherwise constant conditions of injection.

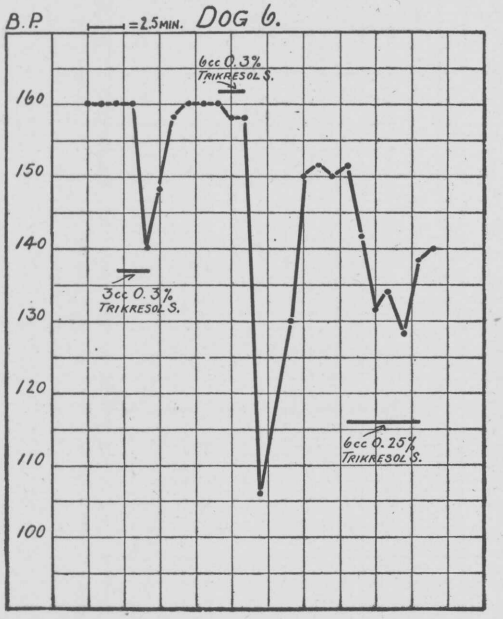
*Dog 1, 17 kilos; ether anesthesia; gravity method; trikresol serum.*

| Time. | Blood pressure. | Duration of injection. | Remarks. |
|---|---|---|---|
| 11.39 a. m. | 150 | ..... | |
| 11.40 a. m. | 152 | ..... | |
| 11.41 a. m. | 150 | ..... | |
| 11.42 a. m. | 148 | ..... | |
| 11.43 a. m. | 152 | ..... | |
| 11.44 a. m. | 152 | ..... | Normal serum. |
| 11.45 a. m. | 156 | ..... | |
| 11.46 a. m. | 160 | 1.5 minutes. | 5 c. c. |
| 11.47 a. m. | 160 | ..... | |
| 11.48 a. m. | 160 | ..... | |
| 11.49 a. m. | 160 | ..... | |
| 11.50 a. m. | 160 | ..... | |
| 11.51 a. m. | 160 | ..... | |
| 11.52 a. m. | 160 | ..... | |
| 11.53 a. m. | 160 | ..... | |
| 11.54 a. m. | 160 | ..... | |
| 11.55 a. m. | 160 | ..... | |
| 11.56 a. m. | 158 | ..... | |
| 11.57 a. m. | 158 | ..... | |
| 11.58 a. m. | 160 | ..... | 0.5 per cent trikresol. |
| 11.59 a. m. | 160 | ..... | |
| 12.00 m.... | 156 | ..... | |
| 12.01 p. m. | 150 | ..... | |
| 12.02 p. m. | 146 | ..... | |
| 12.03 p. m. | 140 | 4 minutes and 32 seconds. | 7.5 c. c. |
| 12.04 p. m. | 142 | ..... | |
| 12.05 p. m. | 128 | ..... | |
| 12.06 p. m. | 136 | ..... | |
| 12.07 p. m. | 148 | ..... | |
| 12.08 p. m. | 152 | ..... | |
| 12.09 p. m. | 156 | ..... | |
| 12.10 p. m. | 158 | ..... | |
| 12.11 p. m. | 160 | ..... | 0.5 per cent trikresol serum. |
| 12.12 p. m. | 158 | ..... | |
| 12.13 p. m. | 148 | ..... | |
| 12.14 p. m. | 128 | 3 minutes and 17 seconds. | 5 c. c. |
| 12.15 p. m. | 120 | ..... | |
| 12.16 p. m. | 122 | ..... | |
| 12.17 p. m. | 128 | ..... | |
| 12.18 p. m. | 138 | ..... | |
| 12.19 p. m. | 150 | ..... | |
| 12.20 p. m. | 156 | ..... | |
| 12.21 p. m. | 156 | ..... | |
| 12.22 p. m. | 160 | ..... | |
| 12.23 p. m. | 160 | ..... | 0.5 per cent trikresol serum. |
| 12.24 p. m. | 146 | ..... | |
| 12.25 p. m. | 162 | ..... | |
| 12.26 p. m. | 118 | ..... | |
| 12.27 p. m. | 104 | ..... | |
| 12.28 p. m. | 88 | ..... | |
| 12.29 p. m. | 80 | 5 minutes and 15 seconds. | 10 c. c. |
| 12.30 p. m. | 66 | ..... | |
| 12.31 p. m. | 66 | ..... | |
| 12.32 p. m. | 72 | ..... | |
| 12.33 p. m. | 82 | ..... | |
| 12.34 p. m. | 98 | ..... | |
| 12.35 p. m. | 114 | ..... | |
| 12.36 p. m. | 126 | ..... | |
| 12.37 p. m. | 138 | ..... | |
| 12.38 p. m. | 144 | ..... | |
| 12.39 p. m. | 146 | ..... | |
| 12.40 p. m. | 152 | ..... | |
| 12.41 p. m. | 156 | ..... | |
| 12.42 p. m. | 158 | ..... | |
| 12.43 p. m. | 160 | ..... | 0.5 per cent trikresol serum, serum forced in. |
| 12.44 p. m. | 158 | ..... | |
| 12.45 p. m. | 130 | 2 minutes and 17 seconds. | 12:45: 11 respiration stops; 12:45: 22,7 c. c. |
| 12.46 p. m. | 104 | ..... | Respiration resumed. |
| 12.47 p. m. | 80 | ..... | 0.5 per cent trikresol forced in 3 c.c.; respiration stops. |
| 12.48 p. m. | 77 | 50 seconds.. | |
| 12.49 p. m. | 48 | ..... | |
| 12.50 p. m. | 30 | ..... | |
| 12.51 p. m. | 34 | ..... | |
| 12.52 p. m. | 8 | ..... | Animal dies. |


B.P.
= 2.5 MINUTES.
DOG. I.
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
5cc N.S.
7.5 cc 0.5% TRIKRESOL S.
5cc 0.5% TRIKRESOL S.
10cc 0.5% TRIKRESOL S.
7cc 0.5% TRIKRESOL S.
3cc 0.5% TRIKRESOL S.

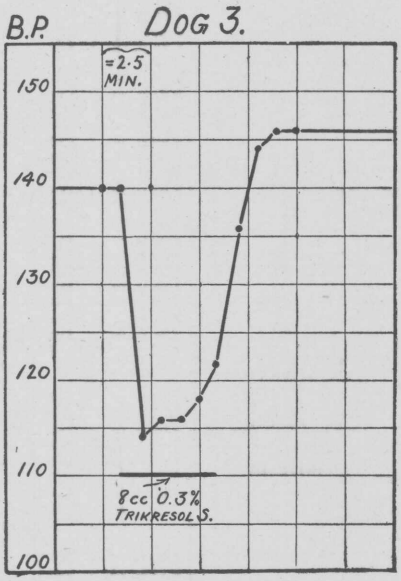
*Dog 6, 10.95 kilos; ether anesthesia; gravity method; trikresol serum (0.3 and 0.25 per cent).*

| Time. | Blood pressure. | Duration of injection. | Remarks. | Time. | Blood pressure. | Duration of injection. | Remarks. |
|---|---|---|---|---|---|---|---|
| 3.22 p. m.. | 160 | ..... | | 3.34 p. m.. | 106 | ..... | |
| 3.23 p. m.. | 160 | ..... | | 3.35 p. m.. | 106 | ..... | |
| 3.24 p. m.. | 160 | ..... | 0.3 per cent trikresol serum. | 3.36 p. m.. | 130 | ..... | |
| | | | | 3.37 p. m.. | 150 | ..... | |
| 3.25 p, m.. | 160 | ..... | | 3.38 p. m.. | 152 | ..... | |
| 3.26 p. m.. | 140 | 1 minute and 50 seconds. | 3 c. c. | 3.39 p. m.. | 150 | ..... | |
| | | | | 3.40 p. m.. | 152 | ..... | 0.25 per cent trikresol serum. |
| 3.27 p. m.. | 148 | ..... | | 3.41 p. m.. | 142 | ..... | |
| 3.28 p. m.. | 158 | ..... | | 3.42 p. m.. | 132 | ..... | |
| 3.29 p. m.. | 160 | ..... | | 3.43 p. m.. | 134 | ..... | |
| 3.30 p. m.. | 160 | ..... | | 3.44 p. m.. | 128 | ..... | |
| 3.31 p. m.. | 160 | ..... | 0.3 per cent trikresol serum. | 3.45 p. m.. | 138 | 4 minutes and 58 seconds. | 6 c. c. |
| 3.32 p. m.. | 158 | ..... | | | | | |
| 3.33 p. m.. | 158 | 2 minutes.. | 6 c. c.; respiration depressed. | 3.46 p. m.. | 140 | ..... | |



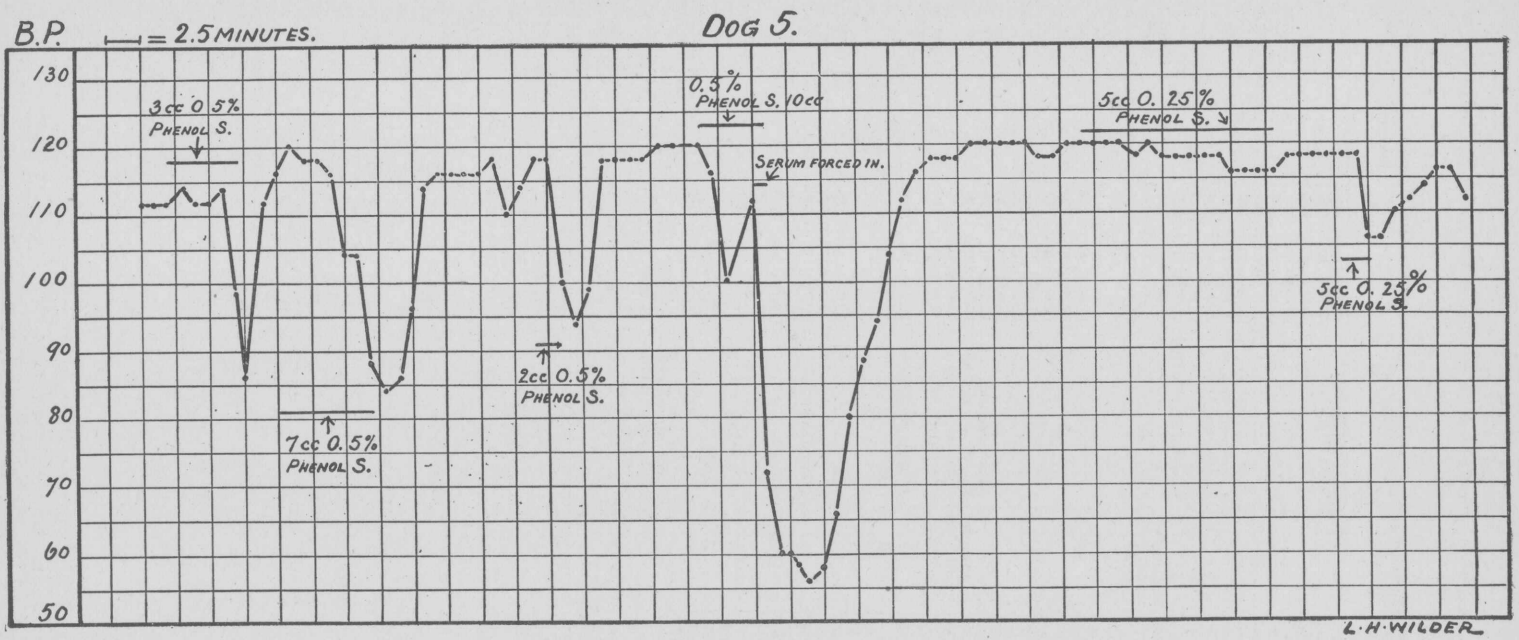
*Dog 3, 10.3 kilos; ether anesthesia; gravity method; trikresol serum (0.3 per cent).*

| Time. | Blood pressure. | Duration of injection. | Remarks. | Time. | Blood pressure. | Duration of injection. | Remarks. |
|---|---|---|---|---|---|---|---|
| 10.03 a. m. | 140 | ..... | Trikresol serum (0.3 per cent). | 10.09 a. m. | 122 | 4 minutes and 40 seconds. | 8 c. c. |
| 10.04 a. m. | 140 | ..... | | 10.10 a. m. | 136 | ..... | |
| 10.05 a. m. | 114 | ..... | | 10.11 a. m. | 144 | ..... | |
| 10.06 a. m. | 116 | ..... | | 10.12 a. m. | 146 | ..... | |
| 10.07 a. m. | 116 | ..... | | 10.13 a. m. | 146 | ..... | |
| 10.08 a. m. | 118 | ..... | | | | | |



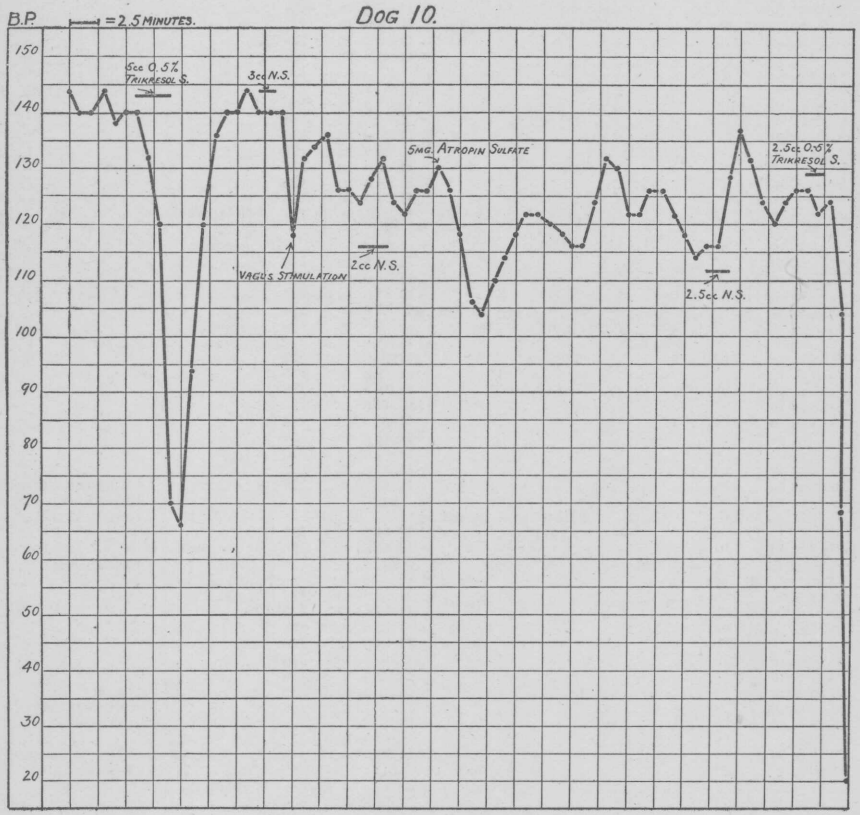
*Dog 5, 6.7 kilos; ether anesthesia; gravity method; phenol serum.*

| Time. | Blood pressure. | Duration of injection. | Remarks. |
|---|---|---|---|
| 2.38 p. m. | 112 | | |
| 2.39 p. m. | 112 | | |
| 2.40 p. m. | 112 | | 0.5 per cent phenol serum. |
| 2.41 p. m. | 114 | | |
| 2.42 p. m. | 112 | | |
| 2.43 p. m. | 112 | | |
| 2.44 p. m. | 114 | | |
| 2.45 p. m. | 98 | 5 minutes and 3 seconds. | 3 c. c. |
| 2.45½ p. m. | 86 | | |
| 2.46 p. m. | | | |
| 2.47 p. m. | 112 | | |
| 2.48 p. m. | 116 | | 0.5 per cent phenol serum. |
| 2.49 p. m. | 120 | | |
| 2.50 p. m. | 118 | | |
| 2.51 p. m. | 118 | | |
| 2.52 p. m. | 116 | | |
| 2.52 p. m. | 104 | | |
| 2.54 p. m. | 104 | | |
| 2.55 p. m. | 88 | 7 minutes and 2 seconds. | 7 c. c. |
| 2.56 p. m. | 84 | | |
| 2.57 p. m. | 86 | | Respiration depressed. |
| 2.58 p. m. | 96 | | |
| 2.59 p. m. | 114 | | |
| 3 p. m. | 116 | | |
| 3.01 p. m. | 116 | | |
| 3.02 p. m. | 116 | | |
| 3.03 p. m. | 116 | | |
| 3.04 p. m. | 118 | | |
| 3.05 p. m. | 110 | | |
| 3.06 p. m. | 114 | | |
| 3.07 p. m. | 118 | | 0.5 per cent phenol serum. |
| 3.08 p. m. | 118 | | |
| 3.09 p. m. | 100 | 1 minute and 45 seconds. | 2 c. c. |
| 3.10 p. m. | 94 | | |
| 3.11 p. m. | 104 | | |
| 3.13 p. m. | 118 | | |
| 3.14 p. m. | 118 | | |
| 3.15 p. m. | 118 | | |
| 3.16 p. m. | 118 | | |
| 3.17 p. m. | 120 | | |
| 3.18 p. m. | 120 | | |
| 3.19 p. m. | 120 | | |
| 3.20 p. m. | 120 | | 0.5 per cent phenol serum. |
| 3.21 p. m. | 116 | | |
| 3.22 p. m. | 100 | | Serum running in very slowly. |
| 3.23 p. m. | 100 | | |
| 3.42 p. m. | 112 | | Serum forced in. |
| 3.25 p. m. | 72 | 5 minutes and 20 seconds. | 10 c. c.; respiration much depressed. |
| 3.26 p. m. | 60 | | |
| 3.27 p. m. | 58 | | |
| 3.28 p. m. | 56 | | |
| 3.29 p. m. | 58 | | |
| 3.30 p. m. | 66 | | |
| 3.31 p. m. | 80 | | |
| 3.32 p. m. | 88 | | |
| 3.33 p. m. | 94 | | |
| 3.34 p. m. | 104 | | |
| 3.35 p. m. | 112 | | |
| 3.36 p. m. | 116 | | |
| 3.37 p. m. | 118 | | |
| 3.38 p. m. | 118 | | |
| 3.39 p. m. | 118 | | |
| 3.40 p. m. | 120 | | |
| 3.41 p. m. | 120 | | |
| 3.42 p. m. | 120 | | |
| 3.43 p. m. | 120 | | |
| 3.44 p. m. | 120 | | |
| 3.45 p. m. | 118 | | |
| 3.46 p. m. | 118 | | |
| 3.47 p. m. | 120 | | |
| 3.48 p. m. | 120 | | 0.25 per cent phenol serum. |
| 3.49 p. m. | 120 | | |
| 3.50 p. m. | 120 | | |
| 3.51 p. m. | 120 | | |
| 3.52 p. m. | 118 | | |
| 3.53 p. m. | 120 | | |
| 3.54 p. m. | 118 | | |
| 3.55 p. m. | 118 | | |
| 3.56 p. m. | 118 | | |
| 3.57 p. m. | 118 | | |
| 3.58 p. m. | 118 | | |
| 3.59 p. m. | 116 | | |
| 4 p. m. | 116 | | |
| 4.01 p. m. | 116 | | |
| 4.02 p. m. | 116 | 13 minutes and 53 seconds. | 5 c. c. |
| 4.03 p. m. | 118 | | |
| 4.04 p. m. | 118 | | |
| 4.05 p. m. | 118 | | |
| 4.06 p. m. | 118 | | |
| 4.07 p. m. | 118 | | 0.25 per cent phenol serum. |
| 4.08 p. m. | 118 | | |
| 4.09 p. m. | 106 | 1 minute and 50 seconds | 5 c. c. |
| 4.10 p. m. | 106 | | |
| 4.11 p. m. | 110 | | |
| 4.12 p. m. | 112 | | |
| 4.13 p. m. | 114 | | |
| 4.14 p. m. | 116 | | |
| 4.15 p. m. | 116 | | |
| 4.16 p. m. | 112 | | |
| 4.17 p. m. | 114 | | 0.25 per cent phenol serum. |
| 4.18 p. m. | 102 | | |
| 4.19 p. m. | 96 | | |
| 4.20 p. m. | 96 | 3 minutes and 5 seconds. | 10 c. c. |
| 4.21 p. m. | 88 | | |
| 4.22 p. m. | 94 | | |
| 4.23 p. m. | 100 | | |
| 4.24 p. m. | 106 | | |
| 4.25 p. m. | 112 | | |
| 4.26 p. m. | 112 | | |
| 4.27 p. m. | 116 | | |


B.P.
= 2.5 MINUTES.
DOG 5.
130
120
110
100
90
80
70
60
50
3cc 0.5% PHENOL S.
7cc 0.5% PHENOL S.
2cc 0.5% PHENOL S.
0.5% PHENOL S. 10cc
SERUM FORCED IN.
5cc 0.25% PHENOL S.
5cc 0.25% PHENOL S.
L·H·WILDER

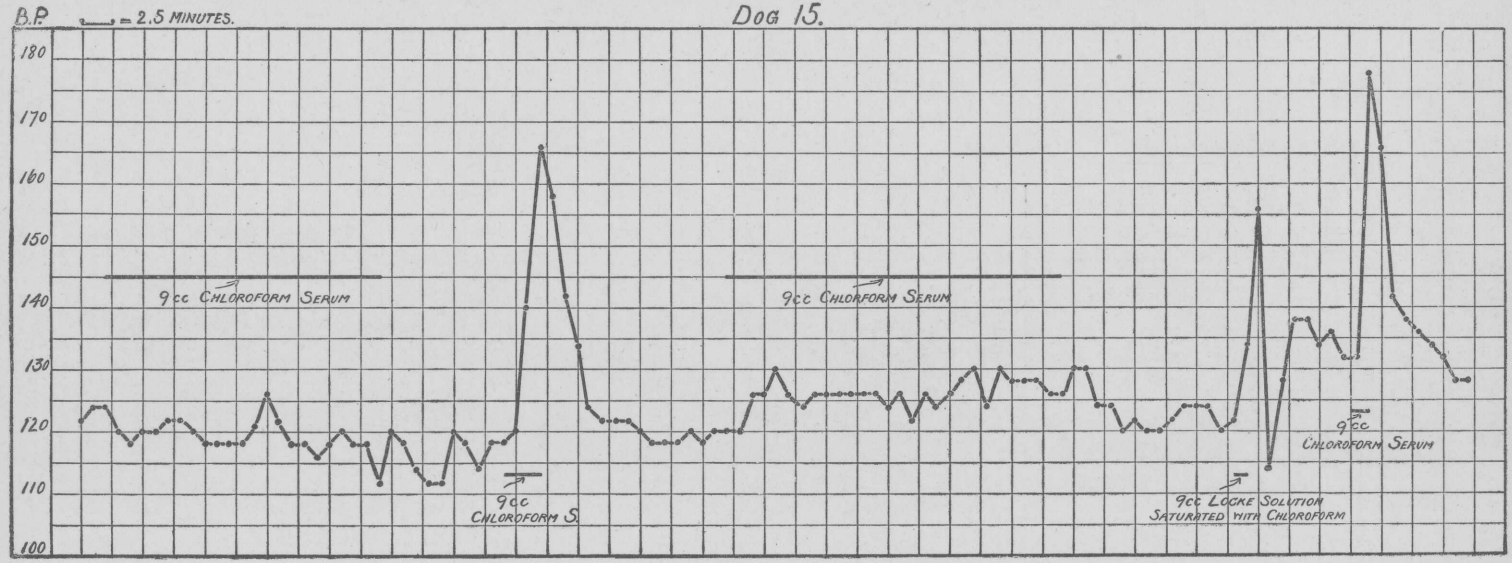
*Dog 10; ether anesthesia; gravity method; normal serum and 0.5 per cent trikresol serum.*

| Time. | Blood pressure. | Duration of injection. | Remarks. |
|---|---|---|---|
| 11.15 a. m. | 144 | ..... | |
| 11.16 a. m. | 140 | ..... | |
| 11.17 a. m. | 140 | ..... | |
| 11.18 a. m. | 144 | ..... | |
| 11.19 a. m. | 138 | ..... | |
| 11.20 a. m. | 140 | ..... | |
| 11.21 a. m. | 140 | ..... | 0.5 per cent trikresol serum. |
| 11.22 a. m. | 132 | ..... | |
| 11.23 a. m. | 120 | ..... | Respiration slowed. |
| 11.24 a. m. | 70 | 3 minutes and 16 seconds. | 5 c. c. |
| 11.25 a. m. | 66 | ..... | |
| 11.26 a. m. | 94 | ..... | |
| 11.27 a. m. | 120 | ..... | |
| 11.28 a. m. | 136 | ..... | |
| 11.29 a. m. | 140 | ..... | |
| 11.30 a. m. | 140 | ..... | |
| 11.31 a. m. | 144 | ..... | |
| 11.50 a. m. | 140 | ..... | |
| 11.51 a. m. | 140 | ..... | Normal serum. |
| 11.52 a. m. | 140 | 1 minute and 26 seconds. | 3 c. c.; respiration slowed; vagus stimulation. |
| 11.53 a. m. | 118 | ..... | |
| 11.54 a. m. | 132 | ..... | Respiration recovered. |
| 11.55 a. m. | 134 | ..... | |
| 11.56 a. m. | 136 | ..... | |
| 12.01 p. m. | 126 | ..... | |
| 12.02 p. m. | 126 | ..... | |
| 12.03 p. m. | 124 | ..... | Normal serum. |
| 12.04 p. m. | 128 | ..... | |
| 12.05 p. m. | 132 | 2 minutes and 30 seconds. | 2 c. c. |
| 12.06 p. m. | 124 | ..... | |
| 12.07 p. m. | 122 | ..... | |
| 12.08 p. m. | 126 | ..... | |
| 12.09 p. m. | 126 | ..... | |
| 12.10 p. m. | 130 | ..... | 5 mgr. atropine sulphate intramuscularly. |
| 12.11 p. m. | 126 | ..... | |
| 12.12 p. m. | 118 | ..... | |
| 12.13 p. m. | 106 | ..... | |
| 12.14 p. m. | 104 | ..... | |
| 12.15 p. m. | 110 | ..... | |
| 12.16 p. m. | 114 | ..... | |
| 12.17 p. m. | 118 | ..... | |
| 12.18 p. m. | 122 | ..... | |
| 12.19 p. m. | 122 | ..... | |
| 12.20 p. m. | 120 | ..... | |
| 12.21 p. m. | 118 | ..... | |
| 12.22 p. m. | 116 | ..... | |
| 12.23 p. m. | 116 | ..... | Normal horse serum. |
| 12.24 p. m. | 124 | 1 minute and 20 seconds. | Respiration slowed; 2.5 c. c. |
| 12.25 p. m. | 132 | ..... | |
| 12.26 p. m. | 130 | ..... | |
| 12.27 p. m. | 122 | ..... | |
| 12.28 p. m. | 122 | ..... | |
| 12.29 p. m. | 126 | ..... | |
| 12.30 p. m. | 126 | ..... | |
| 12.31 p. m. | 122 | ..... | |
| 12.32 p. m. | 118 | ..... | |
| 12.33 p. m. | 114 | ..... | |
| 12.34 p. m. | 116 | ..... | Normal horse serum. |
| 12.35 p. m. | 116 | ..... | |
| 12.36 p. m. | 128 | 1 minute and 38 seconds. | 2.5 c. c. |
| 12.37 p. m. | 138 | ..... | Respiration. |
| 12.38 p. m. | 132 | ..... | |
| 12.39 p. m. | 124 | ..... | |
| 12.40 p. m. | 120 | ..... | |
| 12.41 p. m. | 124 | ..... | |
| 12.42 p. m. | 126 | ..... | |
| 12.43 p. m. | 126 | ..... | 0.5 per cent trikresol serum. |
| 12.44 p. m. | 122 | ..... | |
| 12.45 p. m. | 124 | 1 minute and 25 seconds. | 2.5 c. c.; respiration stopped. |
| 12.46 p. m. | 104 | ..... | |
| 12.47 p. m. | 68 | ..... | |
| 12.48 p. m. | 20 | ..... | Animal dies. |


B.P.
= 2.5 MINUTES.
DOG 10.
150
140
130
120
110
100
90
80
70
60
50
40
30
20
5cc 0.5% TRIKRESOL S.
3cc N.S.
5MG. ATROPIN SULFATE
2.5cc 0.5% TRIKRESOL S.
VAGUS STIMULATION
2cc N.S.
2.5cc N.S.

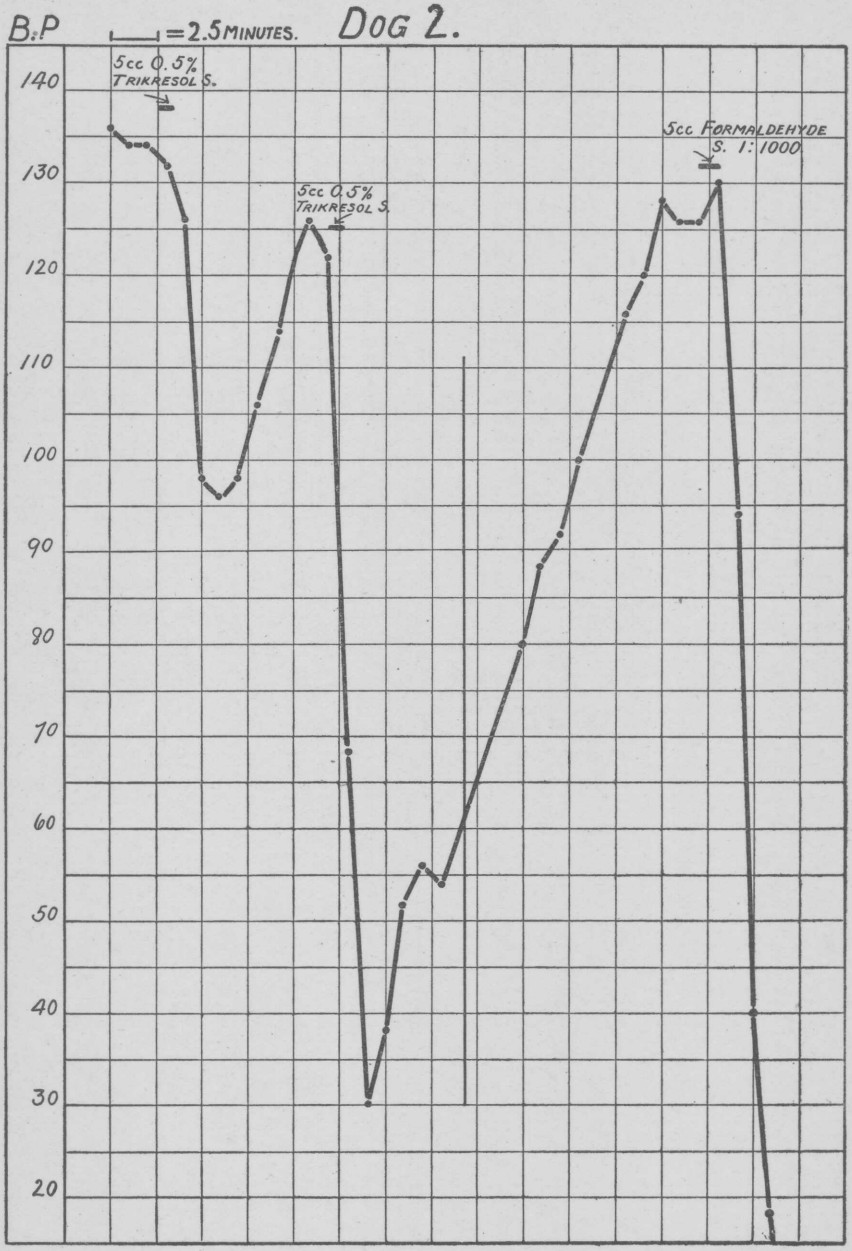
*Dog 15; 7.75 kilos; light ether anesthesia; gravity and syringe method; chloroform serum (saturated); Locke solution saturated with chloroform.*

| Time. | Blood pressure. | Duration of injection. | Remarks. |
|---|---|---|---|
| 10.13 a. m. | 122 | ..... | |
| 10.14 a. m. | 124 | ..... | |
| 10.14.22 a. m. | 124 | ..... | Chloroform serum (gravity). |
| 10.15 a. m. | 122 | ..... | |
| 10.16 a. m. | 120 | ..... | |
| 10.17 a. m. | 118 | ..... | |
| 10.18 a. m. | 120 | ..... | |
| 10.19 a. m. | 120 | ..... | |
| 10.20 a. m. | 122 | ..... | |
| 10.21 a. m. | 122 | ..... | |
| 10.22 a. m. | 120 | ..... | |
| 10.23 a. m. | 118 | ..... | |
| 10.24 a. m. | 118 | ..... | |
| 10.25 a. m. | 118 | ..... | |
| 10.26 a. m. | 118 | ..... | |
| 10.27 a. m. | 122 | ..... | |
| 10.28 a. m. | 126 | ..... | |
| 10.29 a. m. | 122 | ..... | |
| 10.30 a. m. | 118 | ..... | |
| 10.31 a. m. | 118 | ..... | |
| 10.32 a. m. | 116 | ..... | |
| 10.33 a. m. | 118 | ..... | |
| 10.34 a. m. | 120 | ..... | |
| 10.35 a. m. | 118 | ..... | |
| 10.36 a. m. | 118 | ..... | |
| 10.37 a. m. | 112 | ..... | |
| 10.37.42 a. m. | ..... | 22 minutes and 20 seconds. | 9 c. c.; respiration slightly depressed. |
| 10.38 a. m. | 120 | ..... | |
| 10.39 a. m. | 118 | ..... | |
| 10.40 a. m. | 114 | ..... | |
| 10.41 a. m. | 112 | ..... | |
| 10.42 a. m. | 112 | ..... | |
| 10.43 a. m. | 120 | ..... | |
| 10.44 a. m. | 118 | ..... | |
| 10.45 a. m. | 114 | ..... | |
| 10.46 a. m. | 118 | ..... | |
| 10.47 a. m. | 118 | ..... | |
| 10.47.38 a. m. | 120 | ..... | Chloroform serum (syringe). |
| 10.48 a. m. | 140 | ..... | |
| 10.48.48 a. m. | 166 | 1 minute... | 9 c. c. |
| 10.49 a. m. | 158 | ..... | |
| 10.50 a. m. | 142 | ..... | |
| 10.51 a. m. | 134 | ..... | |
| 10.52 a. m. | 124 | ..... | |
| 10.53 a. m. | 122 | ..... | |
| 10.54 a. m. | 122 | ..... | |
| 10.55 a. m. | 122 | ..... | |
| 10.56 a. m. | 120 | ..... | |
| 10.57 a. m. | 118 | ..... | |
| 10.58 a. m. | 118 | ..... | |
| 10.59 a. m. | 118 | ..... | |
| 11.00 a. m. | 120 | ..... | |
| 11.01 a. m. | 118 | ..... | |
| 11.02 a. m. | 120 | ..... | |
| 11.03 a. m. | 120 | ..... | Chloroform serum (gravity). |
| 11.04 a. m. | 120 | ..... | |
| 11.05 a. m. | 126 | ..... | |
| 11.06 a. m. | 126 | ..... | |
| 11.07 a. m. | 130 | ..... | |
| 11.08 a. m. | 126 | ..... | |
| 11.09 a. m. | 124 | ..... | |
| 11.10 a. m. | 126 | ..... | |
| 11.11 a. m. | 126 | ..... | |
| 11.12 a. m. | 126 | ..... | |
| 11.13 a. m. | 126 | ..... | |
| 11.14 a. m. | 126 | ..... | |
| 11.15 a. m. | 126 | ..... | |
| 11.16 a. m. | 124 | ..... | |
| 11.17 a. m. | 126 | ..... | |
| 11.18 a. m. | 122 | ..... | |
| 11.19 a. m. | 126 | ..... | |
| 11.20 a. m. | 124 | ..... | |
| 11.21 a. m. | 126 | ..... | |
| 11.22 a. m. | 128 | ..... | |
| 11.23 a. m. | 130 | ..... | |
| 11.24 a. m. | 124 | ..... | |
| 11.25 a. m. | 130 | ..... | |
| 11.26 a. m. | 128 | ..... | |
| 11.27 a. m. | 128 | ..... | |
| 11.28 a. m. | 128 | ..... | |
| 11.29 a. m. | 126 | ..... | |
| 11.29.17 a. m. | 126 | 26 minutes and 17 seconds. | 9 c. c. |
| 11.30 a. m. | 130 | ..... | |
| 11.31 a. m. | 130 | ..... | |
| 11.32 a. m. | 124 | ..... | |
| 11.33 a. m. | 124 | ..... | |
| 11.34 a. m. | 120 | ..... | |
| 11.35 a. m. | 122 | ..... | |
| 11.36 a. m. | 120 | ..... | |
| 11.37 a. m. | 120 | ..... | |
| 11.38 a. m. | 122 | ..... | |
| 11.39 a. m. | 124 | ..... | |
| 11.40 a. m. | 124 | ..... | |
| 11.41 a. m. | 124 | ..... | |
| 11.42 a. m. | 120 | ..... | |
| 11.43 a. m. | 122 | ..... | |
| 11.43.25 a. m. | 118 | ..... | Locke solution saturated with chloroform (syringe). |
| 11.44 a. m. | 134 | ..... | |
| 11.44.31 a. m. | 156 | 1 minute and 6 seconds. | 9 c. c. Increased respiration. |
| 11.45 a. m. | 114 | ..... | |
| 11.46 a. m. | 128 | ..... | |
| 11.47 a. m. | 138 | ..... | |
| 11.48 a. m. | 138 | ..... | |
| 11.49 a. m. | 134 | ..... | |
| 11.50 a. m. | 136 | ..... | |
| 11.51 a. m. | 132 | ..... | |
| 11.51.44 a. m. | 130 | ..... | Chloroform serum (syringe). |
| 11.52 a. m. | 132 | ..... | |
| 11.52.55 a. m. | 178 | 1 minute and 11 seconds. | 9 c. c. Increased respiration. |
| 11.53 a. m. | 166 | ..... | |
| 11.54 a. m. | 142 | ..... | |
| 11.55 a. m. | 138 | ..... | |
| 11.56 a. m. | 136 | ..... | |
| 11.57 a. m. | 134 | ..... | |
| 11.58 a. m. | 132 | ..... | |
| 11.59 a. m. | 128 | ..... | |
| 12.00 a. m. | 128 | ..... | |


B.P. ⌒ = 2.5 MINUTES.
Dog 15.
180
170
160
150
140
130
120
110
100
9cc Chloroform Serum
9cc Chloroform S.
9cc Chlorform Serum
9cc Locke Solution Saturated with Chloroform
9cc Chloroform Serum

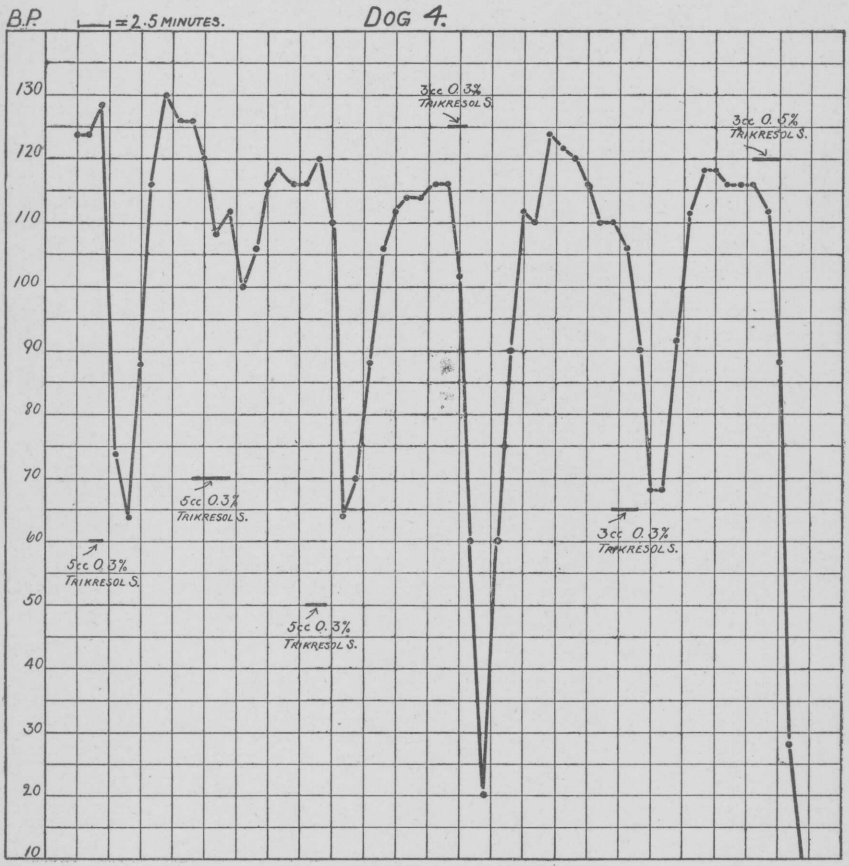
*Dog 2, 7 kilos; ether anesthesia; syringe method; trikresol serum and formaldehyde serum.*

| Time. | Blood pressure. | Duration of injection. | Remarks. | Time. | Blood pressure. | Duration of injection. | Remarks. |
|---|---|---|---|---|---|---|---|
| 2.42 p. m.. | 136 | ..... | | 2.58 p. m.. | 52 | ..... | |
| 2.43 p. m.. | 134 | ..... | | 2.59 p. m.. | 56 | ..... | |
| 2.44 p. m.. | 134 | ..... | | 3.00 p. m.. | 54 | ..... | |
| 2.45 p. m.. | 132 | ..... | 0.5 per cent trikresol serum. | 3.12 p .m.. | 80 | ..... | |
| | | | | 3.13 p. m.. | 88 | ..... | |
| 2.46 p. m.. | 126 | 35 seconds.. | 5 c. c. (some leakage). | 3.14 p. m.. | 92 | ..... | |
| | | | | 3.15 p. m.. | 100 | ..... | |
| 2.47 p. m.. | 98 | ..... | | 3.16 p. m.. | 116 | ..... | |
| 2.48 p. m.. | 96 | ..... | | 3.17 p. m.. | 120 | ..... | |
| 2.49 p. m.. | 98 | ..... | | 3.18 p. m.. | 128 | ..... | |
| 2.50 p. m.. | 106 | ..... | | 3.19 p. m.. | 126 | ..... | |
| 2.51 p. m.. | 114 | ..... | | 3.20 p. m.. | 126 | ..... | Formaldehyde serum 1:1000, 5 c. c. |
| 2.52 p. m.. | 122 | ..... | | | | | |
| 2.53 p. m.. | 126 | ..... | | | | | |
| 2.54 p. m.. | 122 | 24 seconds.. | 5 c. c.; trikresol serum (2.54.30 p. m.) (2.54.53 p. m.); respiration stops (2.55.10 p. m.); artificial respiration (2.55. 40 p. m.). | 3.21 p. m.. | 130 | 45 seconds.. | 5 c. c.; respiration stops (3.21.15). |
| | | | | 3.22 p. m.. | 94 | ..... | Vagus stimulation. |
| | | | | 3.23 p. m.. | 40 | ..... | Respiration resumed for 1 minute (3.23.30 p. m.). |
| | | | | 3.24 p. m.. | 18 | ..... | Animal dies after artificial respiration does not revive it. |
| 2.55 p. m.. | 68 | ..... | | | | | |
| 2.56 p. m.. | 30 | ..... | | | | | |
| 2.57 p. m.. | 38 | ..... | | | | | |


B.P
=2.5 MINUTES.
DOG 2.
5cc 0.5% TRIKRESOL S.
5cc 0.5% TRIKRESOL S.
5cc FORMALDEHYDE S. 1:1000
140
130
120
110
100
90
80
70
60
50
40
30
20

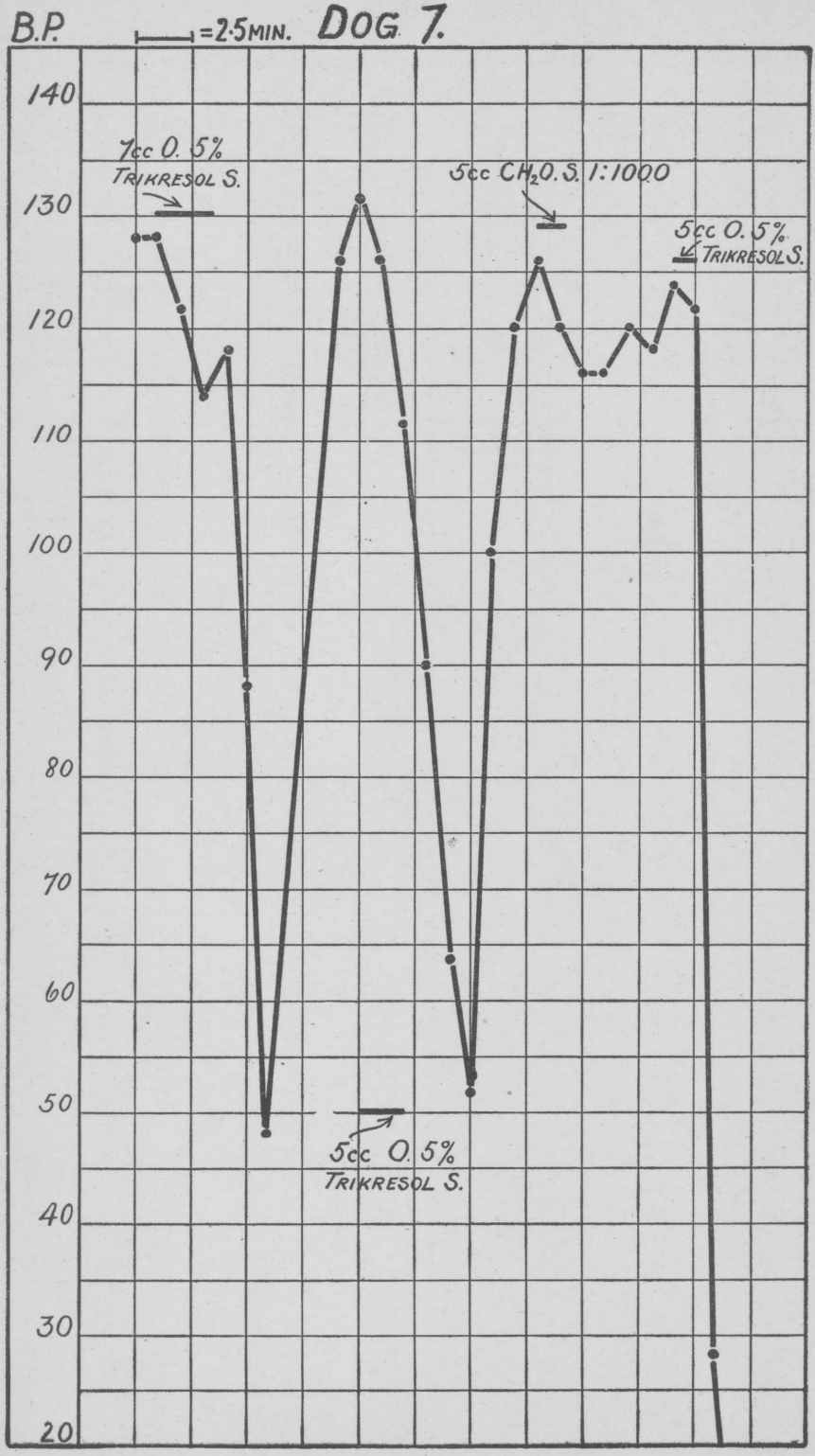
*Dog 4, 12.56 kilos; ether anesthesia; syringe method; trikresol serum (0.5 and 0.3 per cent).*

| Time. | Blood pressure. | Duration of injection. | Remarks. |
|---|---|---|---|
| 10.35 a. m. | 124 | ..... | |
| 10.36 a. m. | 124 | ..... | 0.3 per cent trikresol serum. |
| 10.37 a. m. | 128 | 55 seconds. | 5 c. c. |
| 10.38 a. m. | 74 | ..... | |
| 10.39 a. m. | 64 | ..... | |
| 10.40 a. m. | 88 | ..... | |
| 10.41 a. m. | 116 | ..... | |
| 10.42 a. m. | 130 | ..... | |
| 10.43 a. m. | 126 | ..... | |
| 10.44 a. m. | 126 | ..... | 0.3 per cent trikresol serum. |
| 10.45 a. m. | 120 | ..... | |
| 10.46 a. m. | 108 | ..... | |
| 10.47 a. m. | 112 | 3 minutes and 10 seconds. | 5 c. c. |
| 10.48 a. m. | 100 | ..... | |
| 10.49 a. m. | 106 | ..... | |
| 10.50 a. m. | 116 | ..... | |
| 10.51 a. m. | 118 | ..... | |
| 10.52 a. m. | 116 | ..... | |
| 10.53 a. m. | 116 | ..... | 0.3 per cent trikresol serum. |
| 10.54 a. m. | 120 | ..... | |
| 10.55 a. m. | 110 | 1 minute and 30 seconds. | 3 c. c. |
| 10.56 a. m. | 64 | ..... | |
| 10.57 a. m. | 70 | ..... | |
| 10.58 a. m. | 88 | ..... | |
| 10.59 a. m. | 106 | ..... | |
| 11.00 a. m. | 112 | ..... | |
| 11.01 a. m. | 114 | ..... | |
| 11.02 a. m. | 114 | ..... | |
| 11.03 a. m. | 116 | ..... | |
| 11.04 a. m. | 116 | ..... | 0.5 per cent trikresol serum. |
| 11.05 a. m. | 102 | 1 minute and 25 seconds. | 3 c. c.; respiration much slowed; respiration stops (11.05.40). |
| 11.06 a. m. | 60 | ..... | |
| 11.07 a. m. | 20 | ..... | Artificial respiration. |
| 11.08 a. m. | 60 | ..... | |
| 11.09 a. m. | 90 | ..... | Respiration resumed (11.09.50.) |
| 11.10 a. m. | 112 | ..... | |
| 11.11 a. m. | 110 | ..... | |
| 11.12 a. m. | 124 | ..... | |
| 11.13 a. m. | 122 | ..... | |
| 11.14 a. m. | 120 | ..... | |
| 11.15 a. m. | 116 | ..... | |
| 11.16 a. m. | 110 | ..... | |
| 11.17 a. m. | 110 | ..... | 0.3 per cent trikresol serum. |
| 11.18 a. m. | 106 | ..... | |
| 11.19 a. m. | 90 | 1 minute and 50 seconds. | 3 c. c. |
| 11.20 a. m. | 68 | ..... | |
| 11.21 a. m. | 68 | ..... | |
| 11.22 a. m. | 92 | ..... | |
| 11.23 a. m. | 112 | ..... | |
| 11.24 a. m. | 118 | ..... | |
| 11.25 a. m. | 118 | ..... | |
| 11.26 a. m. | 116 | ..... | |
| 11.27 a. m. | 116 | ..... | Respiration recovered. |
| 11.28 a. m. | 116 | ..... | 0.5 per cent trikresol serum. |
| 11.29 a. m. | 112 | ..... | Respiration stops (11.29.50). |
| 11.30 a. m. | 88 | 1 minute and 50 seconds. | 3 c. c. |
| 11.31 a. m. | 28 | ..... | |
| 11.32 a. m. | ..... | ..... | Animal dies. |


B.P.
= 2.5 MINUTES.
DOG 4.
130
120
110
100
90
80
70
60
50
40
30
20
10
5cc 0.3% TRIKRESOL S.
5cc 0.3% TRIKRESOL S.
5cc 0.3% TRIKRESOL S.
3cc 0.3% TRIKRESOL S.
3cc 0.3% TRIKRESOL S.
3cc 0.5% TRIKRESOL S.

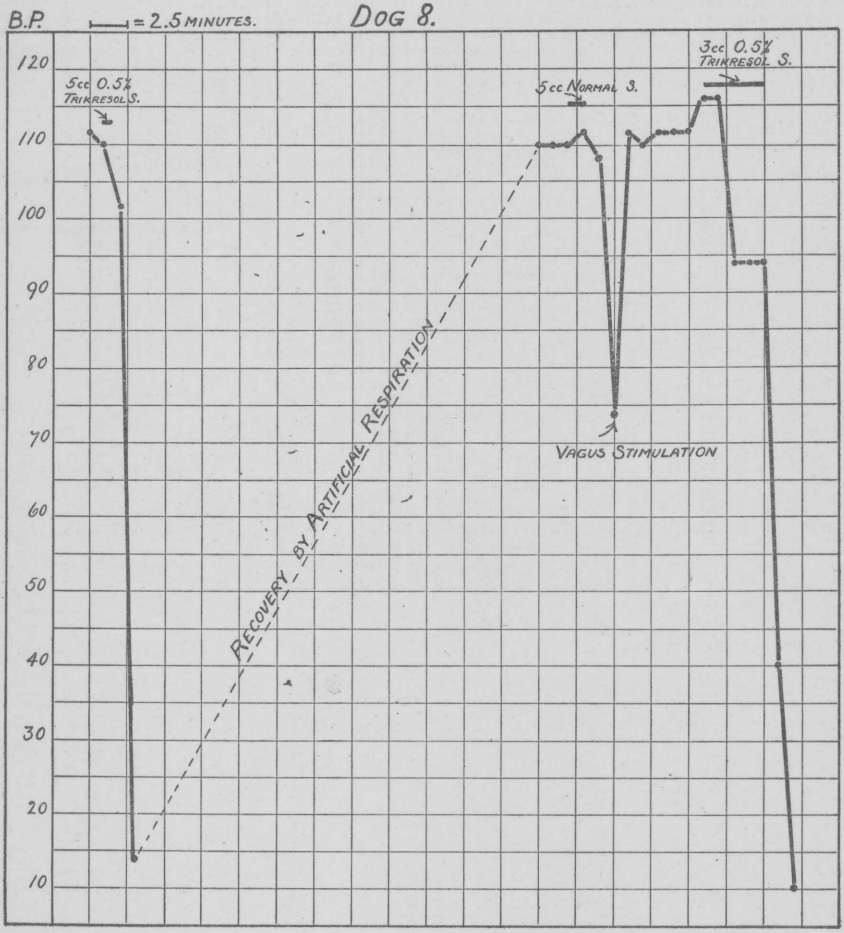
*Dog 7, 10.4 kilos; ether anesthesia; syringe method; trikresol serum (0.5 per cent) and formaldehyde serum (1:1000).*

| Time. | Blood pressure. | Duration of injection. | Remarks. | Time. | Blood pressure. | Duration of injection. | Remarks. |
|---|---|---|---|---|---|---|---|
| 2.10 p. m.. | 128 | ..... | | 2.25 p. m.. | 52 | ..... | |
| 2.11 p. m.. | 128 | ..... | 0.5 per cent trikresol serum. | 2.26 p. m.. | 100 | ..... | |
| | | | | 2.27 p. m.. | 120 | ..... | Respiration recovered. |
| 2.12 p. m.. | 122 | ..... | | | | | |
| 2.13 p. m.. | 114 | ..... | | 2.28 p. m.. | 126 | ..... | Formaldehyde serum (1:1000). |
| 2.14 p. m.. | 118 | 2 minutes and 48 seconds. | 7 c. c. | 2.29 p. m.. | 120 | 1 minute and 12 seconds. | 5 c. c. |
| 2.15 p. m.. | 88 | ..... | Respiration depressed. | | | | |
| | | | | 2.30 p. m.. | 116 | ..... | |
| 2.16 p. m.. | 48 | ..... | Respiration stops (2.16.12); artificial respiration (2.16.40). | 2.31 p. m.. | 116 | ..... | |
| | | | | 2.32 p. m.. | 120 | ..... | |
| | | | | 2.38 p. m.. | 118 | ..... | |
| 2.19 p. m.. | 126 | ..... | Respiration resumed. | 2.39 p. m.. | 124 | 58 seconds.. | 5 c. c. 0.5 per cent trikresol. |
| 2.20 p. m.. | 132 | ..... | 0.5 per cent trikresol serum. | 2.40 p. m.. | 122 | ..... | Respiration is depressed and slowed; stops (2.41.20 p. m.). |
| 2.21 p. m.. | 126 | ..... | | | | | |
| 2.22 p. m.. | 112 | 1 minute and 40 seconds. | 5 c. c. | 2.41 p. m.. | 28 | ..... | Dog dies in spite of artificial respiration. |
| 2.23 p. m.. | 90 | ..... | | | | | |
| 2.24 p. m.. | 64 | ..... | Respiration depressed. | | | | |


B.P.
=2·5 MIN.
DOG 7.
140
130
120
110
100
90
80
70
60
50
40
30
20
7cc 0.5% TRIKRESOL S.
5cc $CH_2O.S.$ 1:1000
5cc 0.5% TRIKRESOL S.
5cc 0.5% TRIKRESOL S.

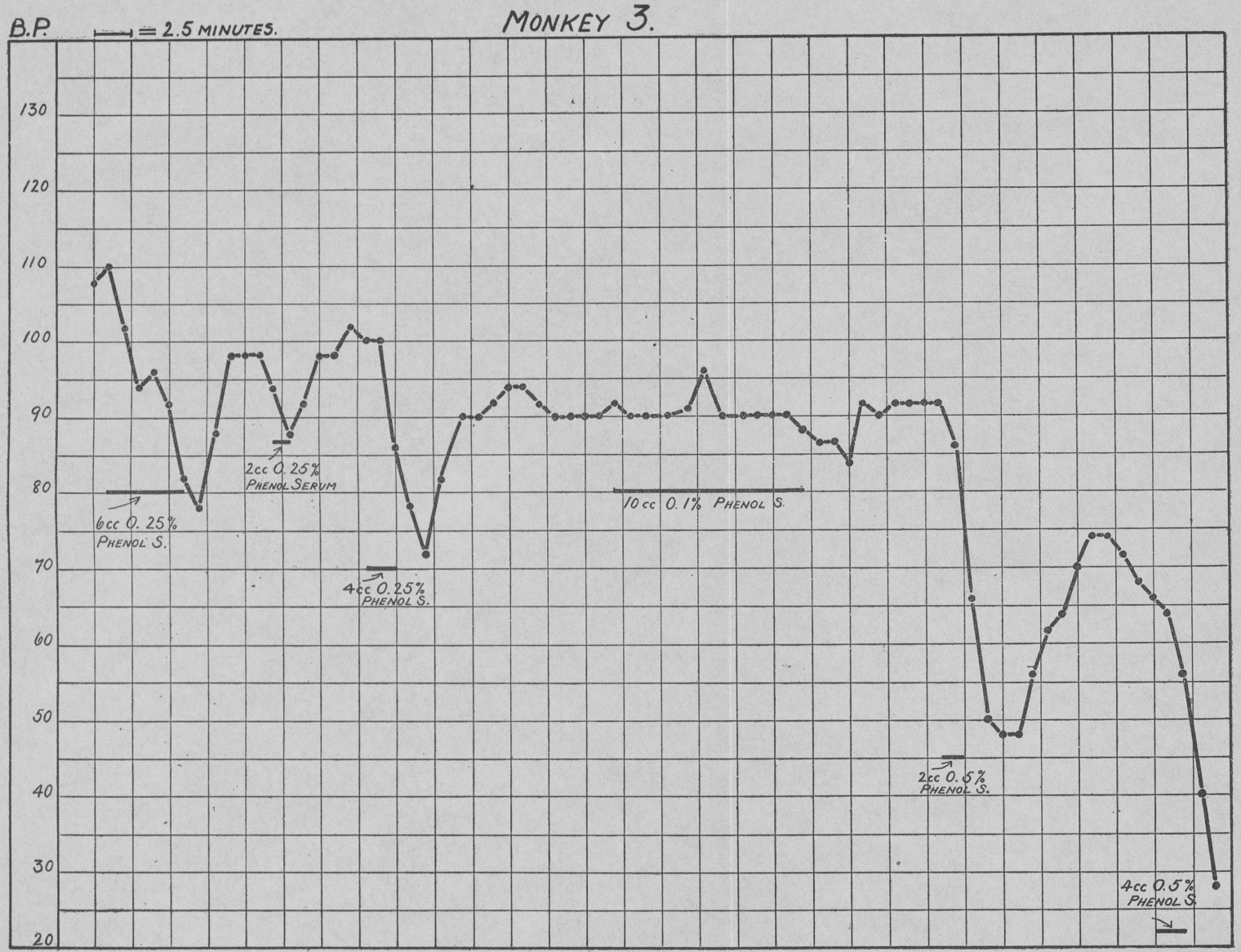
*Dog 8, 8 kilos; ether anesthesia; syringe method; normal and 0.5 per cent trikresol serum.*

| Time. | Blood pressure. | Duration of injection. | Remarks. | Time. | Blood pressure. | Duration of injection. | Remarks. |
|---|---|---|---|---|---|---|---|
| 2.28 p. m.. | 112 | ..... | | 2.54.25p.m. | 100 | ..... | |
| 2.30 p. m.. | 110 | ..... | 0.5 per cent trikresol serum. | 2.55 p. m.. | 112 | ..... | |
| | | | | 2.56 p. m.. | 110 | ..... | |
| 2.31 p. m.. | 102 | 31 seconds.. | 5 c. c.; respiration stops. | 2.57 p. m.. | 112 | ..... | |
| | | | | 2.58 p. m.. | 112 | ..... | |
| 2.32.35p.m | 14 | ..... | | 2.59 p. m.. | 112 | ..... | |
| 2.32 p. m.. | ..... | ..... | Artificial respiration; dog recovers slowly. | 3.00 p. m.. | 116 | ..... | 0.5 per cent trikresol serum. |
| | | | | 3.01 p. m.. | 116 | ..... | |
| 2.50 p. m.. | 110 | ..... | | 3.02 p. m.. | 94 | ..... | |
| 2.51 p. m.. | 110 | ..... | Respiration slow. | 3.03 p. m.. | 94 | ..... | |
| 2.52 p. m.. | 110 | ..... | Normal horse serum. | 3.04 p. m.. | 94 | 3 minutes and 42 seconds. | Respiration stops; 3 c. c. |
| 2.53 p. m.. | 112 | ..... | 5 c. c; respiration stops. | | | | |
| | | | | 3.05 p. m.. | 40 | ..... | |
| 2.53.20p.m. | 108 | ..... | Vagus stimulation. | 3.06 p. m.. | 10 | ..... | Animal dies in spite of artificial respiration. |
| 2.54 p. m.. | 74 | ..... | Vagus effect disappears; respiration resumed. | | | | |


B.P.
= 2.5 MINUTES.
DOG 8.
120
110
100
90
80
70
60
50
40
30
20
10
5cc 0.5% TRIKRESOL S.
5cc NORMAL S.
3cc 0.5% TRIKRESOL S.
VAGUS STIMULATION
RECOVERY BY ARTIFICIAL RESPIRATION

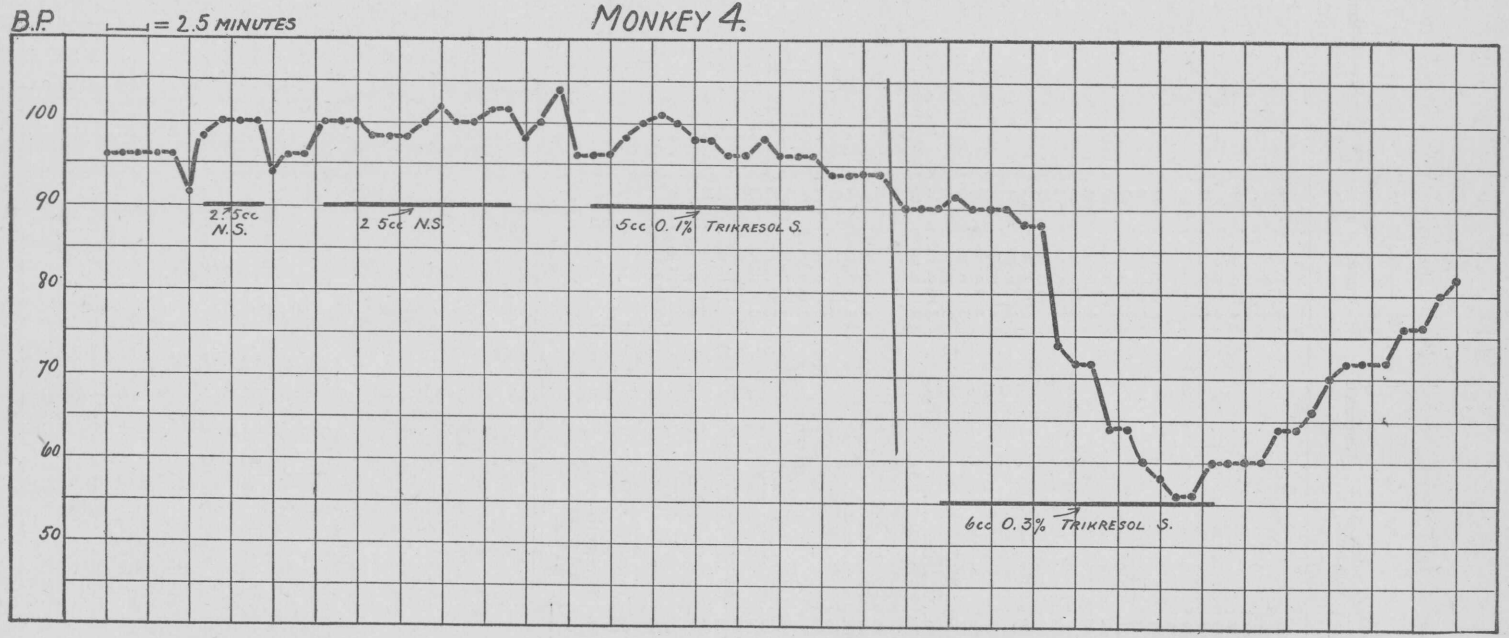
*Monkey 3, 1,650 grams; ether anesthesia; gravity method; phenol serum (0.5, 0.25, and 0.1 per cent).*

| Time. | Blood pressure. | Duration of injection. | Remarks. |
|---|---|---|---|
| 2.41 p. m. | 108 | | |
| 2.42 p. m. | 110 | | 0.25 per cent phenol serum. |
| 2.43 p. m. | 102 | | |
| 2.44 p. m. | 94 | | |
| 2.45 p. m. | 96 | | |
| 2.46 p. m. | 92 | | |
| 2.47 p. m. | 82 | 5 minutes | 6 c. c.; some of the serum leaked out. |
| 2.48 p. m. | 78 | | |
| 2.49 p. m. | 88 | | |
| 2.50 p. m. | 98 | | |
| 2.51 p. m. | 98 | | |
| 2.52 p. m. | 98 | | |
| 2.53 p. m. | 94 | | 0.25 per cent phenol serum. |
| 2.54 p. m. | 88 | 1 minute | 2 c. c. |
| 2.55 p. m. | 92 | | |
| 2.56 p. m. | 98 | | |
| 2.57 p. m. | 98 | | |
| 2.58 p. m. | 102 | | |
| 2.59 p. m. | 100 | | 0.25 per cent phenol serum. |
| 3.00 p. m. | 100 | | |
| 3.01 p. m. | 86 | 2.5 minutes | 4 c. c. |
| 3.02 p. m. | 78 | | |
| 3.03 p. m. | 72 | | |
| 3.04 p. m. | 82 | | |
| 3.05 p. m. | 90 | | |
| 3.06 p. m. | 90 | | |
| 3.07 p. m. | 92 | | |
| 3.08 p. m. | 94 | | |
| 3.09 p. m. | 94 | | |
| 3.10 p. m. | 92 | | |
| 3.11 p. m. | 90 | | |
| 3.12 p. m. | 90 | | |
| 3.13 p. m. | 90 | | |
| 3.14 p. m. | 90 | | |
| 3.15 p. m. | 92 | | 0.1 per cent phenol serum. |
| 3.16 p. m. | 90 | | |
| 3.17 p. m. | 90 | | |
| 3.18 p. m. | 90 | | |
| 3.19 p. m. | 92 | | |
| 3.20 p. m. | 96 | | |
| 3.21 p. m. | 90 | | |
| 3.22 p. m. | 90 | | |
| 3.23 p. m. | 90 | | |
| 3.24 p. m. | 90 | | Vagus stimulation. |
| 3.25 p. m. | 90 | | |
| 3.26 p. m. | 88 | 11 minutes | 10 c. c. |
| 3.27 p. m. | 86 | | |
| 3.28 p. m. | 86 | | |
| 3.29 p. m. | 84 | | |
| 3.30 p. m. | 92 | | |
| 3.31 p. m. | 90 | | |
| 3.32 p. m. | 92 | | |
| 3.33 p. m. | 92 | | |
| 3.34 p. m. | 92 | | |
| 3.35 p. m. | 92 | | 0.5 per cent phenol serum. |
| 3.36 p. m. | 86 | 1.5 minutes | 2 c. c. |
| 3.37 p. m. | 66 | | |
| 3.38 p. m. | 50 | | |
| 3.39 p. m. | 48 | | |
| 3.40 p. m. | 48 | | |
| 3.41 p. m. | 56 | | |
| 3.42 p. m. | 62 | | |
| 3.43 p. m. | 64 | | |
| 3.44 p. m. | 70 | | |
| 3.45 p. m. | 74 | | |
| 3.46 p. m. | 74 | | |
| 3.47 p. m. | 72 | | |
| 3.48 p. m. | 68 | | |
| 3.49 p. m. | 66 | | 0.5 per cent phenol serum. |
| 3.50 p. m. | 64 | | |
| 3.51 p. m. | 56 | 2 minutes | 4 c. c. |
| 3.52 p. m. | 40 | | Respiration stops. |
| 3.53 p. m. | 28 | | |
| 3.53.5 p. m | | | Animal dead. |


B.P.
= 2.5 MINUTES.
MONKEY 3.
130
120
110
100
90
80
70
60
50
40
30
20
6cc 0.25% PHENOL S.
2cc 0.25% PHENOL SERUM
4cc 0.25% PHENOL S.
10 cc 0.1% PHENOL S.
2cc 0.5% PHENOL S.
4cc 0.5% PHENOL S.

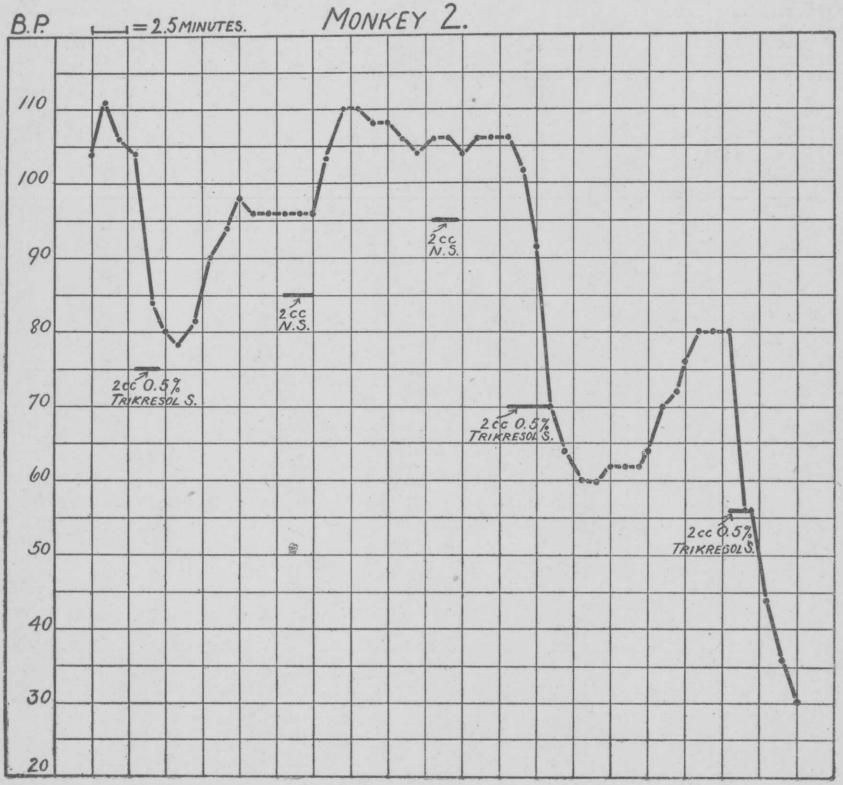
*Monkey 4, 2,400 grams; ether anesthesia; gravity method; trikresol serum.*

| Time. | Blood pressure. | Duraton of injection. | Remarks. | Time. | Blood pressure. | Duration of injection. | Remarks. |
|---|---|---|---|---|---|---|---|
| 2.01 p. m.. | 96 | ..... | | 2.44 p. m.. | 94 | ..... | |
| 2.02 p. m.. | 96 | ..... | | 2.45 p. m.. | 94 | ..... | |
| 2.03 p. m.. | 96 | ..... | | 2.46 p. m.. | 94 | ..... | |
| 2.04 p. m.. | 96 | ..... | | 2.47 p. m.. | 94 | ..... | |
| 2.05 p. m.. | 96 | ..... | | 2.55 p. m.. | 90 | ..... | |
| 2.06 p. m.. | 92 | ..... | | 2.56 p. m.. | 90 | ..... | |
| 2.07 p. m.. | 98 | ..... | Normal serum. | 2.57 p. m.. | 90 | ..... | 0.3 per cent trikresol serum. |
| 2.08 p. m.. | 100 | ..... | | | | | |
| 2.09 p. m.. | 100 | ..... | | 2.58 p. m.. | 92 | ..... | |
| 2.10 p. m.. | 100 | ..... | | 2.59 p. m.. | 90 | ..... | |
| 2.11 p. m.. | 94 | 3.5 minutes. | 2.5 c. c. | 3.00 p. m.. | 90 | ..... | |
| 2.12 p. m.. | 96 | ..... | | 3.01 p. m.. | 90 | ..... | |
| 2.13 p. m.. | 96 | ..... | | 3.02 p. m.. | 88 | ..... | |
| 2.14 p. m.. | 100 | ..... | Normal serum. | 3.03 p. m.. | 88 | ..... | |
| 2.15 p. m.. | 100 | ..... | | 3.04 p. m.. | 74 | ..... | |
| 2.16 p. m.. | 100 | ..... | | 3.05 p. m.. | 72 | ..... | |
| 2.17 p. m.. | 98 | ..... | | 3.06 p. m.. | 72 | ..... | |
| 2.18 p. m.. | 98 | ..... | | 3.07 p. m.. | 64 | ..... | |
| 2.19 p. m.. | 98 | ..... | | 3.08 p. m.. | 64 | ..... | |
| 2.20 p. m.. | 100 | ..... | | 3.09 p. m.. | 60 | ..... | |
| 2.21 p. m.. | 102 | ..... | | 310. p. m.. | 58 | ..... | |
| 2.22 p. m.. | 100 | ..... | | 3.11 p. m.. | 56 | ..... | |
| 2.23 p. m.. | 100 | ..... | | 3.12 p. m.. | 56 | ..... | |
| 2.24 p. m.. | 102 | 9.5 minutes. | 2.5 c. c. | 3.13 p. m.. | 60 | 15 minutes and 50 seconds. | 6 c. c. |
| 2.25 p. m.. | 102 | ..... | | | | | |
| 2.26 p. m.. | 98 | ..... | | | | | |
| 2.27 p. m.. | 100 | ..... | | 3.14 p. m.. | 60 | ..... | |
| 2.28 p. m.. | 104 | ..... | | 3.15 p. m.. | 60 | ..... | |
| 2.29 p. m.. | 96 | ..... | | 3.16 p. m.. | 60 | ..... | |
| 2.30 p. m.. | 96 | ..... | 0.1 per cent trikresol serum. | 3.17 p. m.. | 64 | ..... | |
| | | | | 3.18 p. m.. | 64 | ..... | |
| 2.31 p. m.. | 96 | ..... | | 3.19 p. m.. | 66 | ..... | |
| 2.32 p. m.. | 98 | ..... | | 3.20 p. m.. | 70 | ..... | |
| 2.33 p. m.. | 100 | ..... | | 3.21 p. m.. | 72 | ..... | |
| 2.34 p. m.. | 102 | ..... | | 3.22 p. m.. | 72 | ..... | |
| 2.35 p. m.. | 100 | ..... | | 3.23 p. m.. | 72 | ..... | |
| 2.36 p. m.. | 98 | ..... | | 3.24 p. m.. | 76 | ..... | |
| 2.37 p. m.. | 98 | ..... | | 3.25 p. m.. | 76 | ..... | |
| 2.38 p. m.. | 96 | ..... | | 3.32 p. m.. | 80 | ..... | |
| 2.39 p. m.. | 96 | ..... | | 3.45 p. m.. | 82 | ..... | Respiration never stopped during the entire experiment. |
| 2.40 p. m.. | 98 | ..... | | | | | |
| 2.41 p. m.. | 96 | ..... | | | | | |
| 2.42 p. m.. | 96 | ..... | | | | | |
| 2.43 p. m.. | 96 | 12 minutes and 25 seconds. | 5 c. c. | | | | |


B.P.
= 2.5 MINUTES
MONKEY 4.
100
90
80
70
60
50
2.5cc N.S.
2.5cc N.S.
5cc 0.1% TRIKRESOL S.
6cc 0.3% TRIKRESOL S.

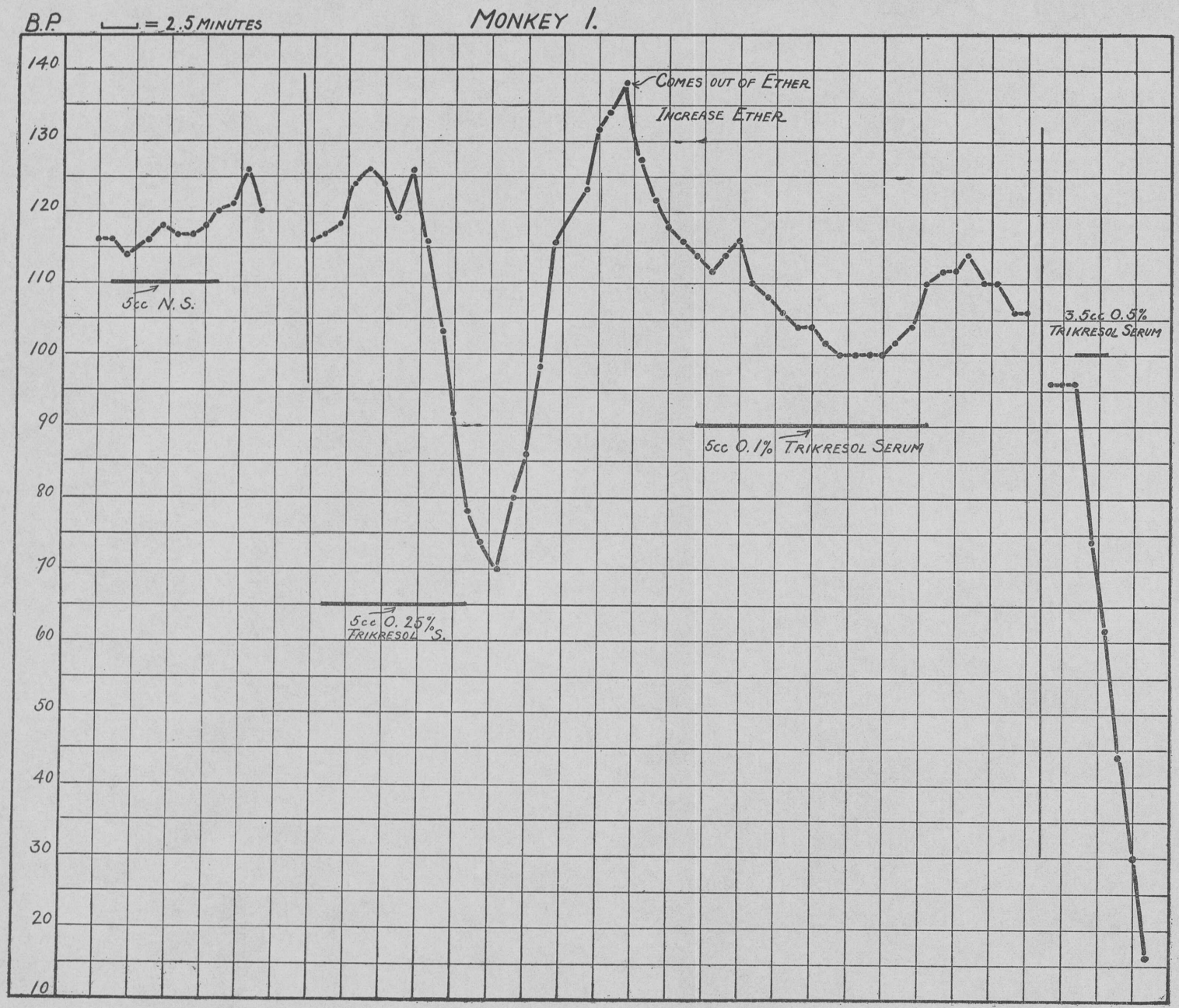
*Monkey 2, 1,800 grams; ether anesthesia; gravity method; trikresol serum and normal serum.*

| Time. | Blood pressure. | Duration of injection. | Remarks. | Time. | Blood pressure. | Duration of injection. | Remarks. |
|---|---|---|---|---|---|---|---|
| 2.09 p. m.. | 104 | ..... | | 2.36 p. m.. | 106 | ..... | |
| 2.10 p. m.. | 111 | ..... | 0.5 per cent trikresol serum. | 2.37 p. m.. | 106 | ..... | 0.5 per cent trikresol serum. |
| 2.11 p. m.. | 106 | ..... | | 2.38 p. m.. | 102 | ..... | |
| 2.12 p. m.. | 104 | 2.5 minutes. | 2 c. c. | 2.39 p. m.. | 92 | ..... | |
| 2.13 p. m.. | 84 | ..... | | 2.40 p. m.. | 70 | 3 minutes.. | 2 c. c.; respiration slightly depressed. |
| 2.14 p. m.. | 80 | ..... | | | | | |
| 2.15 p. m.. | 78 | ..... | | | | | |
| 2.16 p. m.. | 82 | ..... | | 2.41 p. m.. | 64 | ..... | |
| 2.17 p. m.. | 90 | ..... | | 2.42 p. m.. | 60 | ..... | |
| 2.18 p. m.. | 94 | ..... | | 2.43 p. m.. | 60 | ..... | |
| 2.19 p. m.. | 98 | ..... | | 2.44 p. m.. | 62 | ..... | |
| 2.20 p. m.. | 96 | ..... | | 2.45 p. m.. | 62 | ..... | |
| 2.21 p. m.. | 96 | ..... | | 2.46 p. m.. | 62 | ..... | |
| 2.22 p. m.. | 96 | ..... | Normal serum. | 2.47 p. m.. | 64 | ..... | |
| 2.23 p. m.. | 96 | ..... | | 2.48 p. m.. | 70 | ..... | |
| 2.24 p. m.. | 96 | 2 minutes.. | 2 c. c. | 2.49 p. m.. | 72 | ..... | |
| 2.25 p. m.. | 104 | ..... | | 2.50 p. m.. | 76 | ..... | |
| 2.26 p. m.. | 110 | ..... | | 2.51 p. m.. | 80 | ..... | |
| 2.27 p. m.. | 110 | ..... | | 3.18 p. m.. | 80 | ..... | |
| 2.28 p. m.. | 108 | ..... | | 3.19 p. m.. | 80 | ..... | 0.5 per cent trikresol serum. |
| 2.29 p. m.. | 108 | ..... | | | | | |
| 2.30 p. m.. | 106 | ..... | | 3.20 p. m.. | 56 | 1 minute... | 2 c. c. |
| 2.31 p. m.. | 104 | ..... | | 3.21 p. m.. | 44 | ..... | Respiration very slow. |
| 2.32 p. m.. | 106 | ..... | Normal serum. | | | | |
| 2.33 p. m.. | 106 | ..... | | 3.22 p. m.. | 36 | ..... | Respiration stops. |
| 2.34 p. m.. | 104 | 2 minutes.. | 2 c. c. | 3.23 p. m.. | 30 | ..... | |
| 2.35 p. m.. | 106 | ..... | | 3.24 p. m.. | ..... | ..... | Death. |



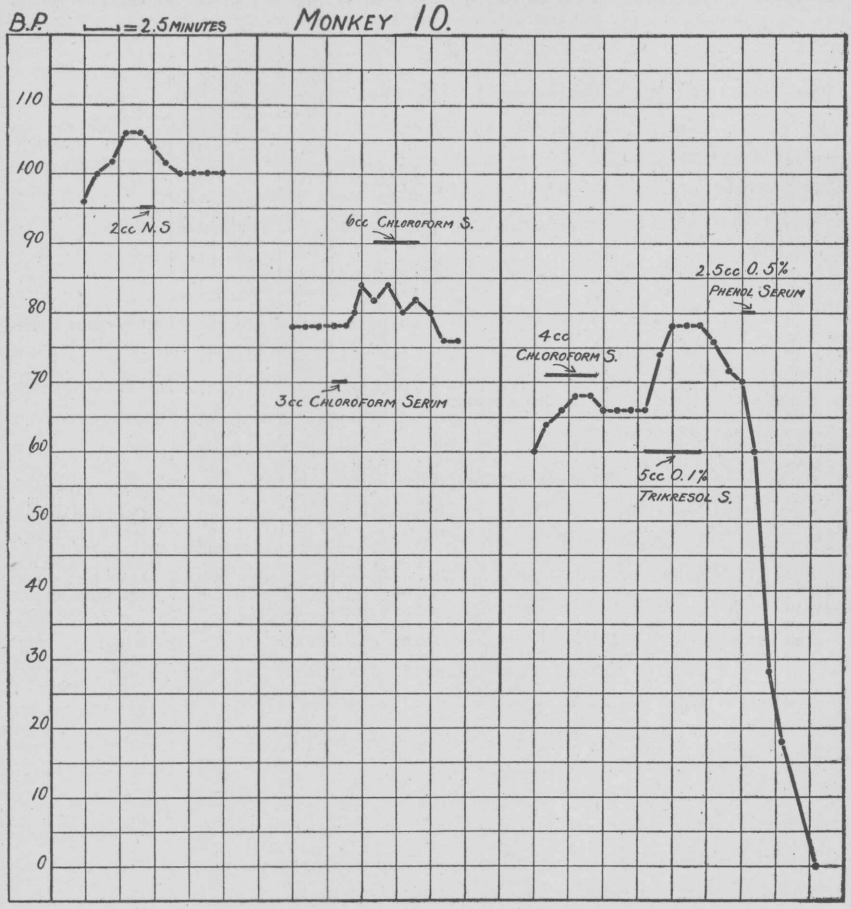
*Monkey 1, 2,010 grams; ether anesthesia; gravity method; trikresol serum and normal serum.*

| Time. | Blood pressure. | Duration of injection. | Remarks. | Time. | Blood pressure. | Duration of injection. | Remarks. |
|---|---|---|---|---|---|---|---|
| 2.02 p. m.. | 116 | ..... | ..... | 2.49 p. m.. | 116 | ..... | |
| | | | | 2.50 p. m.. | 114 | ..... | 0.1 per cent trikresol serum. |
| 2.04 p. m.. | 116 | ..... | Normal horse serum. | | | | |
| 2.05 p. m.. | 114 | ..... | | 2.51 p. m.. | 112 | ..... | |
| 2.07 p. m.. | 116 | ..... | | 2.52 p. m.. | 114 | ..... | |
| 2.08 p. m.. | 118 | ..... | | 2.53 p. m.. | 116 | ..... | |
| 2.09 p. m.. | 116 | ..... | | 2.54 p. m.. | 110 | ..... | |
| 2.10 p. m.. | 116 | ..... | | 2.55 p. m.. | 108 | ..... | |
| 2 11 p. m.. | 118 | ..... | | 2.56 p. m.. | 106 | ..... | |
| 2.12 p. m.. | 122 | 8 minutes.. | 5 c. c. | 2.57 p. m.. | 106 | ..... | |
| 2.13 p. m.. | 122 | ..... | | 2.58 p. m.. | 106 | ..... | |
| 2.14 p. m.. | 126 | ..... | | 2.59 p. m.. | 102 | ..... | |
| 2.15 p. m.. | 120 | ..... | | 3.00 p. m.. | 100 | ..... | |
| 2.23 p. m.. | 116 | ..... | | 3.01 p. m.. | 100 | ..... | |
| 2.24 p. m.. | 118 | ..... | 0.25 per cent trikresol serum. | 3.02 p. m.. | 100 | ..... | |
| | | | | 3.03 p. m.. | 100 | ..... | |
| 2.25 p. m.. | 118 | ..... | Animal struggles. | 3.04 p. m.. | 102 | ..... | |
| 2.26 p. m.. | 124 | ..... | | 3.05 p. m.. | 104 | ..... | |
| 2.27 p. m.. | 126 | ..... | | 3.06 p. m.. | 110 | 16 minutes. | 5 c. c. |
| 2.28 p. m.. | 124 | ..... | | 3.07 p. m.. | 112 | ..... | |
| 2.29 p. m.. | 118 | ..... | | 3.08 p. m.. | 112 | ..... | |
| 2.30 p. m.. | 126 | ..... | | 3.09 p. m.. | 114 | ..... | |
| 2.31 p. m.. | 116 | ..... | | 3.10 p. m.. | 110 | ..... | |
| 2.32 p. m.. | 102 | ..... | | 3.11 p. m.. | 110 | ..... | |
| 2.33 p. m.. | 92 | ..... | | 3.12 p. m.. | 106 | ..... | |
| 2.34 p. m.. | 78 | 11 minutes. | 5 c. c. | 3.13 p. m.. | 106 | ..... | |
| 2.35 p. m.. | 74 | ..... | | 3.26 p. m.. | 96 | ..... | |
| 2.36 p. m.. | 70 | ..... | | 3.27 p. m.. | 96 | ..... | |
| 2.37 p. m.. | 80 | ..... | | 3.28 p. m.. | 96 | ..... | 0.5 per cent trikresol serum. |
| 2.38 p. m.. | 86 | ..... | | | | | |
| 2.39 p. m.. | 98 | ..... | | 3.29 p. m.. | 74 | ..... | Respiration depressed. |
| 2.40 p. m.. | 116 | ..... | | | | | |
| 2.41 p. m.. | 120 | ..... | | 3.30 p. m.. | 62 | 2.5 minutes. | 3.5 c. c.; respiration further depressed. |
| 2.42 p. m.. | 123 | ..... | | | | | |
| 2.43 p. m.. | 132 | ..... | Animal comes out of ether. | 3.31 p. m.. | 44 | ..... | Respiration very slow and shallow. |
| 2.44 p. m.. | 134 | ..... | | 3.32 p. m.. | 30 | ..... | Respiration practically stopped. |
| 2.45 p. m.. | 138 | ..... | | | | | |
| 2.46 p. m.. | 126 | ..... | | 3.33 p. m.. | 16 | ..... | Respiration stopped. |
| 2.47 p. m.. | 122 | ..... | | | | | |
| 2.48 p. m.. | 118 | ..... | | 3.34 p. m.. | ..... | ..... | Animal dead. |


B.P.
= 2.5 MINUTES
MONKEY 1.
140
130
120
110
100
90
80
70
60
50
40
30
20
10
COMES OUT OF ETHER
INCREASE ETHER
5cc N.S.
5cc 0.25% TRIKRESOL S.
5cc 0.1% TRIKRESOL SERUM
3.5cc 0.5% TRIKRESOL SERUM

*Monkey 10, 2.05 kilos; ether anesthesia; gravity method; normal serum; chloroform serum (saturated); 0.1 per cent trikresol serum; 0.5 per cent phenol serum.*

| Time. | Blood pressure. | Duration of injection. | Remarks. | Time. | Blood pressure. | Duration of injection. | Remarks. |
|---|---|---|---|---|---|---|---|
| 3.38 p. m.. | 96 | ..... | | 4.51.4 p.m. | ..... | ..... | Chloroform serum. |
| 3.39 p. m.. | 100 | ..... | | 4.52 p. m.. | 66 | ..... | |
| 3.40 p. m.. | 102 | ..... | | 4.53 p. m.. | 68 | ..... | |
| 3.41 p. m.. | 106 | ..... | | 4.54 p. m.. | 68 | ..... | |
| 3.42 p. m.. | 106 | ..... | Normal serum. | 4.54.25 p.m | ..... | 3 minutes and 21 seconds. | 4 c. c. |
| 3.42.59 p.m | 104 | 59 seconds.. | 2 c. c. | | | | |
| 3.43 p. m.. | 104 | ..... | | | | | |
| 3.44 p. m.. | 102 | ..... | | 4.55 p. m.. | 66 | ..... | |
| 3.45 p. m.. | 100 | ..... | | 4.56 p. m.. | 66 | ..... | |
| 3.46 p. m.. | 100 | ..... | | 4.57 p. m.. | 66 | ..... | |
| 3.47 p. m.. | 100 | ..... | | 4.58 p. m.. | 66 | ..... | 0.1 per cent trikresol serum. |
| 3.48 p. m.. | 100 | ..... | | | | | |
| 4.34 p. m.. | 78 | ..... | | 4.59 p. m.. | 74 | ..... | |
| 4.35 p. m.. | 78 | ..... | | 5.00 p. m.. | 78 | ..... | |
| 4.36 p. m.. | 78 | ..... | | 5.01 p. m.. | 78 | ..... | |
| 4.36.55 p.m | 78 | ..... | Chloroform serum. | 5.02 p. m.. | 78 | ..... | |
| 4.37 p. m.. | 78 | ..... | | 5.02.10 p.m | ..... | 5 minutes and 10 seconds. | 5 c. c. |
| 4.37.45 p.m | 80 | 50 seconds.. | 3 c. c. | | | | |
| 4.38 p. m.. | 84 | ..... | | | | | |
| 4.39 p. m.. | 82 | ..... | | 5.03 p. m.. | 76 | ..... | |
| 4.39 10 p.m | ..... | ..... | Chloroform serum. | 5.04 p. m.. | 72 | ..... | |
| 4.40 p. m.. | 84 | ..... | | 5.05 p. m.. | 70 | ..... | |
| 4.41 p. m.. | 80 | ..... | | 5.05.10 p.m | ..... | ..... | 0.5 per cent phenol serum (syringe). |
| 4.42 p. m.. | 82 | ..... | | | | | |
| 4.42.23 p.m | ..... | 3 minutes and 13 seconds. | 6 c. c. | 5.05.52 p.m | ..... | 42 seconds.. | 2.5 c. c. |
| | | | | 5.06 p. m.. | 60 | ..... | Respiration slowed. |
| 4.43 p. m.. | 80 | ..... | | 5.06.10 p.m | ..... | ..... | Respiration stops. |
| 4.44 p. m.. | 76 | ..... | | 5.07 p. m.. | 28 | ..... | |
| 4.45 p. m.. | 76 | ..... | | 5.08 p. m.. | 18 | ..... | |
| 4.50 p. m.. | 60 | ..... | | 5.09..... | ..... | ..... | Death. |
| 4.51 p. m.. | 64 | ..... | | | | | |




**2. EFFECT OF NORMAL SERUM (FREE FROM PRESERVATIVE).**

All of the symptoms arising in our experiments with normal serum can be explained as being due to increased intracranial pressure. There was, however, a marked contrast between the effects noted during the injection of the serum by means of a syringe as compared with the gravity method. With the gravity method the blood pressure rose some 10 to 20 mm. and returned to normal soon after the injection was completed. In a few cases stimulation of the cardio-inhibitory center, as a result of the increase in intracranial tension, caused a moderate fall in blood pressure, which could be relieved by section of both vagi or the injection of atropine. This vagus stimulation is explained by Eyster (**10**) as being due to anemia of the vagus center as a result of the increase in intracranial tension. The respiration was sometimes not affected, or it was only moderately depressed in rate and depth, but never stopped. On the other hand,

when injecting serum by means of the syringe it often happened that the respiration stopped temporarily and the blood pressure, after having risen very high, fell rapidly to a low level, with recovery after only a few minutes of artificial respiration. It was evident, however, that if the injection was made very slowly no untoward symptoms appeared. In other words, the appearance of the symptoms apparently depended entirely on the rate of injection of the serum. Using the syringe, one is apt to exceed the safe rate of injection.

### 3. ACTION OF TRIKRESOL SERUM.

The concentrations of trikresol in serum used in these experiments were 0.1, 0.25, 0.3, and 0.5 per cent.[1] The 0.25 per cent seems to be the concentration used by most manufacturers in the preparation of antimeningitis serum. One-tenth per cent trikresol serum (gravity method) in the quantities used did not affect the blood pressure and respiration. Twenty-five hundredths per cent trikresol serum had a decided toxic effect, lowering the blood pressure and causing a slowing of the rate and depth of respiration. Breathing, however, was never suspended for any length of time. With 0.3 and 0.5 per cent trikresol serum the toxicity increased, and very often the respiration stopped after a dose of from 3 to 5 c. c. and the blood pressure fell to a low level, and this was followed by the death of the animal in a few minutes unless artificial respiration was given. The toxicity of the serum was so much more apparent when the syringe method was used, thus again showing the relation between the rate of the injection and the toxicity. It was interesting to note that the blood pressure and respiration recovered very rapidly, so that several injections could be made in the same animal. After several injections of serum (0.3, 0.5 per cent trikresol) had been made, the medullary centers seemed to have suffered considerably, and a dose of serum which had previously not caused death was fatal later on in the experiment. In animals under curare (without ether) and artificial respiration the fall in blood pressure after 0.3 to 0.5 per cent trikresol serum was not so rapid as in the case of spontaneously breathing animals. This is probably explained by the fact that the high degree of ventilation of the blood and central nervous system kept the vasomotor center in better condition than during ether anesthesia. In this instance the depressing effect of trikresol was not added to that of the anesthetic, consequently the action was not so pronounced. The fact that the depression of the respiration sets in rather soon after the administration of the trikresol serum (0.3 to 0.5 per cent) under ordinary conditions would furthermore enhance the depressing action of the trikresol on the vasomotor center.

[1] The trikresol is added in the desired proportion to normal horse serum and is thoroughly mixed. After an hour's standing the floccular precipitate which consists in coagulated protein is filtered off through a cotton filter.

#### 4. THE EFFECT OF PHENOL SERUM.

The action of phenol serum (0.1, 0.25, and 0.5 per cent) was in all particulars analogous to that of trikresol serum. It is generally assumed that trikresol is less toxic than phenol. In our experiments there was no difference in the toxicity of phenol and trikresol serum, provided that they were of the same concentration. Using the gravity method, we were never able to produce a preliminary slight rise in blood pressure with a concentration of 0.3 to 0.5 per cent of phenol or trikresol, respectively. This preliminary rise, if it occurs, may be due to the vaso-constriction following a moderate increase in intracranial tension. As soon as the phenol has had time to reach the medullary centers, the depressing action sets in. With a higher concentration in phenols in the serum, more phenol in absolute amount reaches the medulla in the time unit and, therefore, the depression appears more rapidly. This fact is also well illustrated in Hale's tracings, where a 0.3 per cent trikresol serum produced a slight preliminary rise, whereas a 0.5 per cent trikresol serum injected in about the same length of time caused at once a fall in blood pressure. *The fact that an increase in the concentration of the phenol or trikresol in the serum increased also the fall in blood pressure in the same proportion, clearly proves that the fall in blood pressure in these cases is not due primarily to increased intracranial tension, but to the inherent toxic action of the preservative on the medullary centers.*

#### 5. ACTION OF FORMALDEHYDE SERUM.

This serum was tested only in a few instances as it was at once obvious that even concentrations of 1 to 1,000 in serum rendered the serum very toxic. The symptoms observed were the same as those arising after phenol or trikresol serum (0.25 and 0.5 per cent).

#### 6. ACTION OF CHLOROFORM SERUM.

Normal horse serum was saturated with chloroform and used for injections into the subarachnoid space of dogs and monkeys. Even with as great a volume of serum as 9 c. c. injected into a dog weighing 7.7 kilos by the gravity method, no appreciable effect on the blood pressure or respiration was observed. This, however, at a first glance, seems rather unexpected as the depressing effect of chloroform on the nervous system is well known. If one considers, however, that the nervous system of an animal under ether anæsthesia is already more or less saturated with a volatile anæsthetic, the addition of a small amount of chloroform introduced into the subarachnoid space of the lumbar region will not materially change the degree of anæsthesia, blood pressure, and respiration. As is well known the volatile anæsthetics are readily removed from the central nervous system by the lipoids of the blood (Meyer and Overton). The small quantity of chloroform injected with the serum would, therefore, be

easily removed from the higher centers. The injection of the same amount (9 c. c.) of chloroform serum by means of a syringe and under high pressure resulted in a considerable temporary increase in blood pressure, the respiration being not at all affected. The rise in blood pressure under these conditions is due to the increased intracranial tension. These results were confirmed in animals under curare in which the complicating action of the ether was ruled out. The only difference observed in this case was that the blood pressure, after a preliminary rise following the injection, did fall below the normal, but recovered soon after. Using a very high injection pressure (syringe) we noted several times the appearance of vagus stimulation with its accompanying fall in blood pressure. Section of both vagi or injection of atropine was followed in this instance immediately by a rise of the blood pressure to normal. It may be emphasized that such extreme conditions would hardly be comparable with the careful administration of serum to patients.

## DISCUSSION OF EXPERIMENTAL RESULTS.

### 1. RELATION OF SYRINGE METHOD TO GRAVITY METHOD.

The evidence obtained in these experiments is highly in favor of the gravity method and supports the views of Sophian and others that this method should be given the preference over others in the administration of serum to patients. It was found, however, that even the gravity method should be used with the greatest precaution inasmuch as toxic symptoms are apt to appear with trikresol serum, especially if a large volume of spinal fluid has been withdrawn before the injection of the serum. In this instance the serum will flow into the subarachnoid space much faster than if no spinal fluid is withdrawn. A larger amount of trikresol serum will reach the medullary centers in this case and untoward symptoms are apt to follow.

### 2. TOXIC ACTION OF SERUM IN ITS RELATION TO THE PERCENTAGE OF THE PRESERVATIVE AND THE RATE OF INJECTION.

Sophian, in his monograph on "Epidemic Cerebrospinal Meningitis," speaking of the administration of the serum to patients by the gravity method, says on page 207:

> As a rule, as soon as the injection of the serum is begun, the blood pressure drops and continues to drop steadily. Reasoning by the old method of injecting serum, one would expect a rise in blood pressure; this, however, is rarely the case. As stated, in the great majority of cases when the injection of serum into the subarachnoid space is begun, the blood pressure drops and continues dropping steadily as the larger quantity of serum is injected. After there has been a material drop, for example, of 20 to 30 mm. of mercury, the blood pressure begins to drop relatively much faster if more serum is injected. Thus, if an injection of 15 c. c. of serum causes a drop of 20 mm. of mercury in blood pressure, injecting only a few more cubic centimeters of serum may cause a sudden drop of 40 more mm. of mercury, making a total drop of 60 mm. or more.

On page 208 he says:

> As a rule, very rapid injection under considerable force will cause a greater drop in blood pressure. By the gravity method the serum is allowed to run in slowly by gravity, the funnel being raised or lowered to regulate the flow. Ten minutes is a good average time to allow for the fluid to run in, though I have frequently taken 20 minutes or longer, especially in cases beginning with a low blood pressure, or when the blood pressure dropped very quickly.

It is interesting to note that Sophian very rarely observed a rise in blood pressure after the administration of the serum had begun. This, however, in itself speaks against the hypothesis that the untoward symptoms are due to an increase in intracranial tension, as undoubtedly a very considerable preliminary rise in the blood pressure would have preceded the fall. The experiments included in this bulletin agree with the blood-pressure curves obtained by Sophian in the administration of the serum to patients. As a general rule, it may be stated that the effect on blood pressure increases in proportion to (*a*) the rate of injection and (*b*) the concentration of the phenol preservative. With a low concentration (0.1 per cent) of trikresol no appreciable effect is obtained if the rate of injection is low. If the injection is made faster, pressure symptoms appear which have to be attributed to increased intracranial tension. With higher concentrations of the trikresol, the toxic effect and the depressing action of the preservative become more evident and mask or antagonize the symptoms due to increased intracranial pressure. Under these conditions the vasomotor and respiratory centers are depressed by the trikresol from the beginning of the injection, and the vasomotor center does not respond to the increased intracranial tension in the manner as described by Cushing and Eyster. It should not be forgotten that phenol administered in fatal doses to animals exhibits a marked depressing effect on the central nervous system. Both formaldehyde and phenols are strong protoplasmic poisons.

### 3. MOST SUITABLE SERUM PRESERVATIVE.

From the experiments described in this bulletin it would seem that chloroform when added to serum even to the point of saturation, is not capable of imparting to the serum a degree of toxicity which could be compared with that obtained on mixing serum with phenol and trikresol (0.25 and 0.5 per cent). Practically the only effect which could be produced by the subdural injection of chloroform serum is the result of an increase in intracranial tension. With the use of the gravity method such results are not very apt to follow and it seems very doubtful that they occur at all. We, therefore, strongly suggest that chloroform be used as a preservative for antimeningitis serum. It is well recognized that serum preserved with chloroform after long standing will show a cloudiness which is probably due to

the partial precipitation of the serum proteins. This fact, however, does not alter the efficiency of such a serum in the treatment of the disease.

#### 4. SAFETY MEASURES TO BE OBSERVED IN THE ADMINISTRATION OF ANTIMENINGITIS SERUM.

The results obtained in our work on animals regarding the rules to be followed in order to avoid untoward symptoms from the subarachnoid injection of serum with or without preservative fully corroborate those suggested by Sophian and other clinicians, who have had a good deal of experience in the administration of antimeningitis serum to patients.

1. The gravity method is far superior to the syringe method.

2. Blood pressure estimations during the injections will furnish a good index of the margin of safety.

In concluding it may be once more strongly emphasized that the free and early administration of serum in cerebrospinal meningitis is of the utmost importance and benefit to the patients.

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(4) Litterer. Idem, 1912, vol. 59, p. 531.
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(8) Cushing. Am. Journ. Med. Sci., 1902, vol. 124, p. 375.
(9) Eyster. Journ. Exper. Med., 1909, vol. 11, p. 489.
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ADDENDUM.—Since this article went to press a short paper on the same subject by John Auer appeared in the Journal of the American Medical Association on June 6. Auer arrives at somewhat similar conclusions as those presented in the present communication. He also gives preference to chloroform or ether over the phenol preservatives. He does not take into consideration, however, the rate of injection or the change in the concentration of the preservative contained in the serum, factors of great importance for the correct interpretation of the results of the serum injections. This fact probably explains his statement that "the effects obtained on the blood pressure and respiration by trikresol seem partly dependent on an increased intraspinal pressure." I have shown conclusively in this paper that if a low rate of injection is used (gravity) no pressure effects can be obtained with 0.3 per cent trikresol serum (the concentration used by Auer).

## HYGIENIC LABORATORY BULLETINS OF THE PUBLIC HEALTH SERVICE.

The Hygienic Laboratory was established in New York, at the Marine Hospital on Staten Island, August, 1887. It was transferred to Washington, with quarters in the Butler Building, June 11, 1891, and a new laboratory building, located in Washington, was authorized by act of Congress March 3, 1901.

The following *bulletins* [Bulls. Nos. 1–7, 1900 to 1902, Hyg. Lab., U. S. Mar.-Hosp. Serv., Wash.] have been issued:

*No. 1.—Preliminary note on the viability of the *Bacillus pestis*. By M. J. Rosenau.

No. 2.—Formalin disinfection of baggage without apparatus. By M. J. Rosenau.

*No. 3.—Sulphur dioxid as a germicidal agent. By H. D. Geddings.

*No. 4.—Viability of the *Bacillus pestis*. By M. J. Rosenau.

No. 5.—An investigation of a pathogenic microbe (*B. typhi murium* Danyz) applied to the destruction of rats. By M. J. Rosenau.

*No. 6.—Disinfection against mosquitoes with formaldehyde and sulphur dioxid. By M. J. Rosenau.

†No. 7.—Laboratory technique: Ring test for indol, by S. B. Grubbs and Edward Francis; Collodium sacs, by S. B. Grubbs and Edward Francis; Microphotography with simple apparatus, by H. B. Parker.

By act of Congress approved July 1, 1902, the name of the "United States Marine-Hospital Service" was changed to the "Public Health and Marine-Hospital Service of the United States," and three new divisions were added to the Hygienic Laboratory.

Since the change of name of the service the bulletins of the Hygienic Laboratory have been continued in the same numerical order, as follows:

*No. 8.—Laboratory course in pathology and bacteriology. By M. J. Rosenau. (Revised edition, March, 1904.)

†No. 9.—Presence of tetanus in commercial gelatin. By John F. Anderson.

*No. 10.—Report upon the prevalence and geographic distribution of hookworm disease (uncinariasis or anchylostomiasis) in the United States. By Ch. Wardell Stiles.

*No. 11.—An experimental investigation of *Trypanosoma lewisi*. By Edward Francis.

*No. 12.—The bacteriological impurities of vaccine virus; an experimental study. By M. J. Rosenau.

*No. 13.—A statistical study of the intestinal parasites of 500 white male patients at the United States Government Hospital for the Insane; by Philip E. Garrison, Brayton H. Ransom, and Earle C. Stevenson. A parasitic roundworm (*Agamomermis culicis* n. g., n. sp.) in American mosquitoes (*Culex sollicitans*); by Ch. Wardell Stiles. The type species of the cestode genus *Hymenolepis;* by Ch. Wardell Stiles.

*No. 14.—Spotted fever (tick fever) of the Rocky Mountains; a new disease. By John F. Anderson.

*No. 15.—Inefficiency of ferrous sulphate as an antiseptic and germicide. By Allen J. McLaughlin.

*No. 16.—The antiseptic and germicidal properties of glycerin. By M. J. Rosenau.

*No. 17.—Illustrated key to the trematode parasites of man. By Ch. Wardell Stiles.

*No. 18.—An account of the tapeworms of the genus *Hymenolepis* parasitic in man, including reports of several new cases of the dwarf tapeworm (*H. nana*) in the United States. By Brayton H. Ransom.

*No. 19.—A method for inoculating animals with precise amounts. By M. J. Rosenau.

*No. 20.—A zoological investigation into the cause, transmission, and source of Rocky Mountain "spotted fever." By Ch. Wardell Stiles.

*No. 21.—The immunity unit for standardizing diphtheria antitoxin (based on Ehrlich's normal serum). Official standard prepared under the act approved July 1, 1902. By M. J. Rosenau.

*No. 22.—Chloride of zinc as a deodorant, antiseptic, and germicide. By T. B. McClintic.

*No. 23.—Changes in the Pharmacopœia of the United States of America. Eighth Decennial Revision. By Reid Hunt and Murray Galt Motter.

No. 24.—The International Code of Zoological Nomenclature as applied to medicine. By Ch. Wardell Stiles.

*No. 25.—Illustrated key to the cestode parasites of man. By Ch. Wardell Stiles.

*No. 26.—On the stability of the oxidases and their conduct toward various reagents. The conduct of phenolphthalein in the animal organism. A test for saccharin, and a simple method of distinguishing between cumarin and vanillin. The toxicity of ozone and other oxidizing agents to lipase. The influence of chemical constitution on the lipolytic hydrolysis of ethereal salts. By J. H. Kastle.

*No. 27.—The limitations of formaldehyde gas as a disinfectant with special reference to car sanitation. By Thomas B. McClintic.

*No. 28.—A statistical study of the prevalence of intestinal worms in man. By Ch. Wardell Stiles and Philip E. Garrison.

*No. 29.—A study of the cause of sudden death following the injection of horse serum. By M. J. Rosenau and John F. Anderson.

†No. 30.—I. Maternal transmission of immunity to diphtheria toxine. II. Maternal transmission of immunity to diphtheria toxine and hypersusceptibility to horse serum in the same animal. By John F. Anderson.

†No. 31.—Variations in the peroxidase activity of the blood in health and disease. By Joseph H. Kastle and Harold L. Amoss.

†No. 32.—A stomach lesion in guinea pigs caused by diphtheria toxine and its bearing upon experimental gastric ulcer. By M. J. Rosenau and John F. Anderson.

*No. 33.—Studies in experimental alcoholism. By Reid Hunt.

†No. 34.—I. *Agamofilaria georgiana* n. sp., an apparently new roundworm parasite from the ankle of a negress. II. The zoological characters of the roundworm genus *Filaria* Mueller, 1787. III. Three new American cases of infection of man with horsehair worms (species *Paragordius varius*), with summary of all cases reported to date. By Ch. Wardell Stiles.

†No. 35.—Report on the origin and prevalence of typhoid fever in the District of Columbia. By M. J. Rosenau, L. L. Lumsden, and Joseph H. Kastle. (Including articles contributed by Ch. Wardell Stiles, Joseph Goldberger, and A. M. Stimson.)

†No. 36.—Further studies upon hypersusceptibility and immunity. By M. J. Rosenau and John F. Anderson.

†No. 37.—Index-catalogue of medical and veterinary zoology. Subjects: Trematoda and trematode diseases. By Ch. Wardell Stiles and Albert Hassall.

No. 38.—The influence of antitoxin upon post-diphtheritic paralysis. By M. J. Rosenau and John F. Anderson.

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†No. 40.—1. The occurrence of a proliferating cestode larva (*Sparganum proliferum*) in man in Florida, by Ch. Wardell Stiles. 2. A reexamination of the type specimen of *Filaria restiformis* Leidy, 1880=*Agamomermis restiformis*, by Ch. Wardell Stiles. 3. Observations on two new parasitic trematode worms: *Homalogaster philippinensis*

n. sp., *Agamodistomum nanus* n. sp., by Ch. Wardell Stiles and Joseph Goldberger. 4. A reexamination of the original specimen of *Tœnia saginata abietina* (Weinland, 1858), by Ch. Wardell Stiles and Joseph Goldberger.

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†No. 42.—The thermal death points of pathogenic microorganisms in milk. By M. J. Rosenau.

†No. 43.—The standardization of tetanus antitoxin (an American unit established under authority of the act of July 1, 1902). By M. J. Rosenau and John F. Anderson.

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TREASURY DEPARTMENT

UNITED STATES PUBLIC HEALTH SERVICE

HYGIENIC LABORATORY—BULLETIN No. 97

OCTOBER, 1914

# I. SOME NEW SIPHONAPTERA

# II. A FURTHER REPORT ON THE IDENTIFICATION OF SOME SIPHONAPTERA FROM THE PHILIPPINE ISLANDS

# III. THE TAXONOMIC VALUE OF THE COPULATORY ORGANS OF THE FEMALES IN THE ORDER SIPHONAPTERA

BY

CARROLL FOX

WASHINGTON
GOVERNMENT PRINTING OFFICE
1914

# ORGANIZATION OF HYGIENIC LABORATORY.

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# 1. SOME NEW SIPHONAPTERA.[1]

(Pls. I, II, III, IV, V.)

## ROOSEVELTIELLA gen. nov.

A noncombed, noneyed, genus. The third joint of the antenna is short and distinctly segmented only on the posterior side. The antennal groove is partly overlapped by the gena. A row of stout hairs is to be found along the posterior border of the antennal groove more distinct in the male than in the female. The labial palpi are four jointed, including the basal unpaired segment. The tip of the rostrum is asymmetrical.

The first abdominal tergite is almost as long as the three thoracic nota taken together. The single antipygidial bristle in the male is placed on a cone, while in the female it is separate from the apical edge of the seventh tergite. The clasping organs conform to the type found in *Xenopsylla.*

The hind coxa is pyriform in shape with a shallow notch posteriorly at the apex. On the inner side of the hind coxa, there is a row of small teeth. The last tarsal joint of all the legs has 4 spines and an apical hair on either side with 2 subapical spines on the ventral surface.

### ROOSEVELTIELLA GEORYCHI sp. n.

(Pl. I, figs. 1–6.)

*Head.*—The only bristle to be found on the anterior portion of the head is near the lower border of the gena. The gena is sharply pointed behind. The antennal groove in the male reaches to the top of the head, in the female to within one-third of the top. The second joint of the antenna has a row of slender hairs, which in the female are about as long as the third joint. There is 1 bristle back of the antennal groove and a subapical row of about 10 bristles on the hind margin of the occiput. The rostrum reaches to the apex of the fore coxa.

[1] Manuscript submitted for publication July 7, 1914.

*Thorax.*—Each notum contains a single row of bristles (6 to 8, 12, 12). On the mesothorax there is 1 bristle. On the sternum of the metathorax there are 3 bristles with 1 on the episternum. On the epimerum are two rows of 5 or 6 bristles each. The pleura are normally divided.

*Abdomen.*—The first abdominal tergite contains an anterior row of 4 or 5 bristles and a posterior row of about 8. The other tergites from the second to the seventh have a single row of about 14 bristles each. The abdominal sternites from the third to the seventh have each a single row of about 10 bristles.

*Legs.*—On the inner side of the hind femur there is a row of 4 or 5 bristles, while apically on the outer surface there are 2. The hind tibia has on its dorsal border five pairs of bristles. On the inner surface of this segment close to the posterior border there is a row of about 8 bristles. The apical bristle of the second hind tarsal segment reaches well to the fifth.

*Modified segments—Male.*—The modified segments in both male and female resemble the *Xenopsylla scopulifer*. The second process in the male claspers, however, is broader toward the tip and is more curved on both sides. There are more bristles on the first process and they are heavier. The ninth sternite is more slender, decidedly so at the apex.

*Female.*—In the female, on the apical border of the eighth tergite, there is an external row of about 18 bristles and an internal row of 8 or 10. On the lateral surface from the stigma downward there is a row of about 8 bristles extending almost to the apical edge. In front of the 3 lowest bristles of this row there are 3 bristles in line.

Length of male, 1.7 mm.; length of female, 1.6 mm.

Twelve specimens of this flea, six males and six females, are contained in the collection at the United States National Museum. They were collected by the Misses M. and H. J. Melville about 300 miles inland from Benguela, Africa, off *Georychus*.

Type No. 18452, U. S. N. M.

### XENOPSYLLA PROCAVIAE sp. n.

(Pl. II, figs. 1–5.)

*Head.*—The rostrum extends a little beyond the middle of the fore coxa. The antennal groove is closed behind and partly overlapped by the gena. There are but 2 bristles on the front of the head, 1 placed on the lower general edge and 1 near the edge. On the occiput there is a bristle back of the antennal groove and a subapical row of about 8.

*Thorax.*—The tergites have each a single row of bristles containing on the pronotum 10 bristles and on the meso and metanotum 7

bristles each. The pleura are normally divided. The mesothorax bears 3 bristles in the male and 4 in the female. The episternum of the metathorax has 1 bristle, the stermum of this segment 1 bristle, while the epimerum bears anteriorly 1 bristle and an apical row of 4.

*Abdomen.*—The abdominal tergites contain each a single row of bristles, the first having 6 bristles in the row and anteriorly a few scattered hairs. The others from the second to the sixth have about 10 bristles each. On the seventh tergite is a row of about 6 bristles, the lowest widely separated from the others. There is 1 antipygidial bristle on each side placed on the apical edge of the seventh tergite. The sternites from the third to the seventh bear in the male a bristle on each side, while in the female there is a row of 4 or 5 on the third, fourth, fifth, and sixth, and about 10 on the seventh, on the two sides taken together.

*Legs.*—The fore coxa has about 11 bristles on its outer surface. The hind coxa is sinuate behind and has a patch of about 10 teeth on its inner surface. The hind femur has but 2 bristles on its outer surface, located ventrally near the apex, and a row of 5 bristles on its inner surface. There is no tooth ventrally at its widest portion. The hind tibia bears a row of about 7 bristles on its outer surface and none on its inner surface. On the posterior border are 5 pairs of bristles and an apical group of 4 bristles. One of the apical bristles on the second hind tarsal segment extends beyond the base of the fifth segment. The fourth segment is short and triangular, and in the female the longest of its apical bristles extends to the apex of the fifth joint. The fifth segment contains on each side 4 spines and a subapical pair ventrally.

*Modified segments—Male.*—The clasper has two free processes with a third much smaller containing about 3 or 4 hairs, and not separated from the body. The first free process, the superior of the three, is longer than the others, narrow and regular in outline, and has from its tip downward a row of about 6 bristles. The second free process is shorter than the first, about as broad, and asymmetrical at its tip, where there are two groups of very small bristles or hairs. The manubrium is short and narrow. The ninth sternite, narrow and bluntly rounded at its tip, expands below to again become narrow at its angle.

*Female.*—There are two widely separated bristles beneath the pygidium on the lateral portion of the eighth segment. The apical margin is rounded, with an outside row of about 14 bristles and an inside row of about 6. The style is about twice as long as wide at the base.

Length of male 1.5 mm.; length of female, 1.7 mm.

Ten specimens, three males and seven females, are contained in the collection at the United States National Museum from British East

Africa, off *Procavia*, collected during the Smithsonian African Expedition.

Type No. 18453, U. S. N. M.

### RHOPALOPSYLLUS GWYNI sp. n.

(Plate III, figs. 1–6.)

*Head.*—The frons bears an anterior row of 4 or 5 bristles and an eye row of 3 longer and stouter bristles. There are also 2 stout bristles on the gena, one located below and one behind the eye. The rostrum extends to the apex of the fore coxa. On the occiput there are three rows of bristles, an anterior of about 8, a middle of about 10, and a posterior subapical row of 10 or 12 bristles.

*Thorax.*—In the anterior row of the pronotum there are about 10 bristles and in the posterior row about 14. The mesonotum contains two rows of bristles with about 14 in the anterior and the same number in the posterior. The metanotum has three rows of bristles, an anterior of 7 or 8, a middle of about 16 and a posterior of about 14. On the apical edge of the metanotum there are 7 or 8 teeth. The mesothorax bears 4 bristles. The episternum of the metathorax bears 2 bristles and 2 hairs. There is a single bristle on the sterum, while the epimerum contains two rows of bristles of 5 or 6, and 4.

*Abdomen.*—Each abdominal tergite has two rows of bristles, first, 12 and 12; second, 12 and 14; third, 12 and 14; fourth, 10 and 14; fifth, 5 and 14; sixth, 3 or 4 and 14; seventh, 4 and 10. On the apical edge of the seventh tergite there is 1 long antipygidial bristle on each side, and a row of about 6 very fine bristles or hairs. The apical edge is not sinuate. The second abdominal segment contains laterally 4 fine bristles. Along the apical edge of the first tergite are about 12 small teeth.

*Legs.*—On the outer surface of the mid and fore femora are scattered 7 or 8 fine bristles and on the hind femur but 2 located basally. On the inner surface of the mid and hind femora are about 2 small bristles only. The hind tibia has on its outer surface arranged in three irregular rows about 18 bristles with a similar arrangement of a less number of bristles on the outer surface of the fore and mid tibia. On the inner surface of the fore and hind tibia are about 2 fine bristles. The last tarsal segment of all of the legs has 4 lateral spines with a supernumary pair apically. There is also a long hair on each side apically and a hair at the location of the third lateral bristle.

*Modified segments—Male.*—The claspers resemble somewhat those of *R. bohlsi.* The finger is heavier and the ninth sternite is slimmer and contains more hairs. The shape of the base of this sternite is also different.

*Female.*—From the stigma downward there is on the eighth abdominal segment a regular row of about 9 large and 8 smaller bristles, and anterior to this row are about 4 small bristles. There is also an apical outside row of about 7 or 8 and an inside row of 6 or 7 bristles.

Length of male, 2.4 mm.; length of female, 3.4 mm.

There are deposited in the Hygienic Laboratory four males and one female, collected by Surg. M. K. Gwyn, United States Public Health Service, at the quarantine station located at Brunswick, Ga., 1904, off "rats."

From the brief description of these rats given by Dr. Gwyn, it is likely that they were *Epimys norvegicus.* The genus *Rhopalopsyllus* being practically confined to South America, it is probable that the rats were brought from that continent by vessels stopping at the quarantine station.

Type No. 18454, U. S. N. M.

### DORATOPSYLLA BLARINAE sp. n.

(Pl. IV, figs. 1–3.)

*Head.*—There are two rows of bristles extending almost vertically on the frons, an anterior of smaller bristles comprised of 5 and a posterior comprised of 3 large bristles. The gena along its lower edge bears 4 stout spines extending from the root of the maxillary pulpus to the margin of the antennal groove. On the occiput are three rows of bristles, an anterior of 3, a middle of 4, and the usual subapical row of about 6 on each side. Both the first and second joints of the antenna contained a row of short hairs. The rostrum extends to three-fourths the length of the fore coxa and consists of 5 joints including the unpaired basal joint. Its tip is rounded and symmetrical.

*Thorax.*—On the pronotum there is an anterior row of about 10 bristles and posterior to this a row of 14 or 15 spines, on the two sides together. The mesonotum bears a row of about 10 bristles, and the metanotum two rows of bristles, both the anterior and posterior row having about 6 bristles each. The pleura are normally divided. The mesothorax bears 6 bristles, one of them being situated anteriorly, and two at and near the posterior inferior angle, with a middle row of 3. On the metathorax there is 1 bristle on the episternum, 1 on the sternum, and two rows of 2 bristles each on the epimerum.

*Abdomen.*—The first abdominal tergite bears two rows of bristles, an anterior of 4 and a posterior of 7 or 8. The tergites from the second to the fourth bear likewise two rows of bristles, an anterior of about 10 and a posterior of 11 or 12. The fifth, sixth, and seventh

tergites have the posterior row of 10 or 11 bristles, the anterior row being represented by 2 bristles each. On the apical edge of the seventh tergite are 3 antipygidial bristles on each side, the middle being the longest. The sternites from the second to seventh bear each a single row of about 4 bristles, with an additional long bristle on the seventh sternite in line with and above the others.

*Legs.*—The fore coxa is clothed on its outer side with about 24 bristles. The midfemur is without a row of bristles. The hind coxa has no teeth on its inner side. The hind femur is without a row of bristles. The hind tibia has on its outer side about 10 bristles arranged in general in two rows, while on the posterior border are 5 pairs of bristles, every other pair being larger. Apically there is a group of 3 bristles, and situated between the second and third pair is a single small bristle. None of the bristles on the second tarsal joints of all the legs extend farther than the middle of the next succeeding joint. On the fifth joint of all the legs there are 4 pairs of lateral spines with a supernumerary pair ventrally at the base and a pair at the apex.

*Modified segments.*—The claspers contain two processes and a long, thin, slightly curved movable finger. The anterior process is short and blunt and bears three bristles, one quite heavy. The posterior process is much longer and has a single hair at its apex and a few on the posterior margin. On the posterior border of the finger there are 3 widely separated fine bristles. Arising from the clasper at the root of the finger is a single long, strong bristle. The ninth sternite is broad, narrowing at the apex, and from the posterior border at the widest part is a short, thick bristle, while above this is another finer one, and near the apex two or three hairs.

Length, 1.7 mm.

There is one specimen of this flea (male) in the collection at the United States National Museum, collected by Mr. G. S. Miller, in Washington, D. C., off *Blarina brevicauda.*

Type No. 18455, U. S. N. M.

### CERATOPHYLLUS STIMSONI sp. n.

(Pl. IV, figs. 4–6.)

*Head.*—The frontal notch is distinct, the eye is absent. The maxillary palpi are not as long as the labial palpi. The rostrum extends about three-fourths the length of the fore coxa. The antennal groove reaches to the top of head. There are 6 bristles in the upper or anterior genal row, one being placed at the anterior edge of the antennal groove slightly above the others, and four stouter bristles in the lower or posterior genal row. One bristle is located back of the antennal groove. There are numerous fine hairs along the pos-

terior margin of the groove. There are about 10 subapical bristles along the posterior border of the occiput; a few short hairs can be seen on the margin of the second joint of the antenna.

*Thorax.*—The pronotum bears a ctenedium of 18 spines, while anterior to this comb there is a row of about 10 bristles. The meso- and meta-notum have each two rows of bristles. The anterior and posterior rows on the mesonotum are comprised of about 10 bristles each, while on the metanotum the bristles in both rows are more numerous. The mesothorax contains 7 bristles, 5 of which are found posterior to its dividing internal incrassation, while there are 1 large and 2 small bristles or hairs anterior to this division. The episternum of the metathorax bears 5 bristles, while just below, springing from the sternum, are 2 large bristles. The epimerum has three somewhat irregular rows of bristles consisting of 4, 5, and 3, the latter being located close to or at the posterior margin.

*Abdomen.*—The first abdominal tergite has an anterior row of about 10 and a posterior row of about the same number, but heavier, bristles. The other tergites have each two rows of bristles, an anterior row of about 16 small, and a posterior row of from 20–24 larger bristles. The second, third, fourth, and fifth tergites have each a small tooth on each side. There is 1 apical bristle on the seventh segment, extending well beyond the sensory plate. The abdominal sternites from the third to the seventh have each two rows of bristles, consisting of from 3 to 4 bristles in each row, the posterior being the heaviest.

*Legs.*—The hind femur has a row of about 10 bristles on the outer surface, and a row of 7 or 8 bristles on the inner surface. There are two irregular rows of bristles on the outer surface of the hind tibia close to the posterior border. One of the bristles on the posterior border of the hind tibia is unusually long. The longest apical bristle of the first hind tarsus reaches to beyond the base of the second tarsal segment. The longest apical bristle of the second tarsal segment reaches to the middle of the fifth. The last tarsal joints of all the legs have five pairs of lateral spines, the first pair being slightly dislocated toward the median line.

*Modified segments.*—The manubrium is broad at its base but contracted at its apex. The process is small, slightly expanding at its apex where there are 3 long slender bristles. The movable finger is long and narrow, slightly curved throughout its length, and ending above in a more or less recurved tip. At its insertion it is markedly curved. In general it has the shape of the blade of a scythe. The bristles frequently seen at the insertion of the finger are in this case absent. The ninth sternite can be better understood

from the drawing than from a description. The eighth sternite is prominent and contains several small hairs along the posterior border below its apex.

Length, 2.4 mm.

There are in the personal collection of the author two specimens of this flea (males) collected by Passed Asst. Surg. Arthur M. Stimson, United States Public Health Service, in Los Angeles, Cal., 1908, off "Gophers" (*Thomomys*).

Type No. 18456, U. S. N. M.

### CERATOPHYLLUS APACHINUS sp. n.

(Pl. V, figs. 1–3.)

The specimen, a female, has been mutilated during its collection or preservation and therefore the following description is lacking in certain details.

*Head.*—The rostrum extends to the apex of the trochanter, or possibly a little beyond. The antenna is relatively small. Its second joint bears a row of hairs which are longer than the third joint. The eye is absent.

*Thorax.*—On the pronotum there is a ctenedium of about 22 spines and anteriorly a row of about 16 bristles. The bristles on the other thoracic nota can not be determined accurately, but it appears that the mesonotum has two rows of numerous bristles and the metanotum three rows.

*Abdomen.*—Like the other prairie dog fleas the abdomen is markedly hairy. Each tergite bears two rows of bristles of about 24 bristles each. On the apical edge of the seventh tergite are 3 long, strong, antipygidial bristles on each side. The second sternite bears about 2 bristles. The sternites from the third to the sixth, have each three rows of bristles, the third row much reduced in numbers. The posterior row has about 14 and the anterior row about 8 smaller bristles. On the seventh sternite ventrally there is a group of about 7 bristles and above this group on each side may be seen 3 more in line. Anteriorly, this segment also contains the row of small bristles common to the others.

*Legs.*—The mid-femur has on its inner surface a row of about 10 bristles. The hind coxa is broad, about as broad as it is long. The hind femur contains on its outer surface a row consisting of about 10 bristles and on its inner surface a row of about the same number. On the posterior border of the hind tibia are 7 pairs of bristles and an apical group of 4. The inner surface of this segment bears a row of about 7 bristles, and the outer surface about 16 arranged in two rows.

*Modified segments.*—The terminal segments of the abdomen are quite hairy. There are about 15 small bristles or hairs on the eighth tergite anterior to the sensory plate, while below this plate there are 4 large and 3 small bristles. The lateral surface of the eighth segment contains a patch of about 20 large and small bristles extending to the apical edge. The style is stout and terminates in a bristle. Subapically there is another bristle and several hairs. The tenth sternite is hairy and bears along its margin 5 or 6 stout bristles on each side.

Length, 3.3 mm.

A single male specimen is contained in the collection at the United States National Museum. It came from Apache County, N. Mex., off "Prairie dog" (probably *Cynomys arizonensis*).

Type No. 18458 U. S. N. M.

### CERATOPHYLLUS GIBSONI sp. n.

(Pl. V, figs. 4–5.)

The specimens have been permitted to remain too long in caustic solution and are somewhat distorted and certain details obscured. This flea partakes of the general appearance of other bird fleas.

*Head.*—The rostrum reaches almost to the apex of the fore coxa. Eyes are present.

*Thorax.*—The pronotum bears a ctenedium of 25 spines with a row of bristles anteriorly. The other thoracic nota bear two rows of bristles each, containing about 10 bristles in each row. There is a small tooth on each side on the apical margin of the metanotum.

*Abdomen.*—The first abdominal tergite bears three rows of bristles, a posterior and middle of about 12 each and an anterior row of about 8 bristles. On the other tergites is a posterior row of about 12 and an anterior row of 8 or 10 smaller bristles. On the apical edge of each of the first 4 tergites are 2 teeth on each side. There is 1 antipygidial bristle on each side. On each of the sternites from the second to the seventh is a single row of about 6 bristles.

*Modified segments—Male.*—The clasping organs resemble somewhat those of the C. niger. The manubrium is broader and the movable finger notched on its dorsal edge.

*Female.*—The receptaculum seminis has an entirely different shape from that of C. niger.

Length of male, 2.2 mm.; length of female, 1.9 mm.

Two specimens (male and female) are in the collection at the United States National Museum. Collected by Mr. Arthur Gibson in a hen house, Point Lepreux, Ottawa, Canada, July 13, 1909.

Type No. 18457, U. S. N. M.

### ISCHNOPSYLLUS, TEXANUS sp. n.

(Pl. V, figs. 6–8.)

*Head.*—The cephalic processes are rather long and slender. The eye is absent but is represented by a thickening of the chitin. The antennal groove extends to the top of the head and from its extension reaching to the anterior border of the frons is a row of about 12 small bristles. Just below the row, 1 located near the antennal groove, and 1 near the anterior border of the head, are 2 larger bristles, while scattered on the lateral surface are numerous fine hairs. Along the posterior margin of the antennal groove are two rows of bristles, one close to the margin and comprised of about 12 smaller bristles, and immediately above a row of about 4 larger. Running obliquely across the occiput are four more or less regular rows of bristles of 4, 3, 5, and 5 or 6 on each side, with the usual row along the posterior margin. At the inferior posterior angle of the occiput there are 3 bristles, the uppermost the longest and the lowest the shortest. From the anterior edge of the antennal groove springs a long bristle, the largest to be found on the head. The first joint of the antenna has a patch of about 8 stout hairs, while along the margin of the second joint is a row of 7 or 8 thinner but longer hairs—not as long as the third joint.

*Thorax.*—The pronotum contains a ctenedium of about 26 spines, and three rows of bristles of 4 to 6 in the anterior, 12 to 14 in the middle, and about 10 in the posterior. In addition to these there are several hairs at the posterior inferior angle. The mesonotum is the longest of the nota and has a number of bristles more or less regularly disposed in five or six rows, the anterior rows being very irregular and the bristles smaller. The metanotum has numerous bristles rather irregularly placed. The apical edge of this notum bears a small tooth on each side. The mesothorax has about 4 bristles posteriorily, and a patch of about 12 anteriorly. The episternum of the metathorax is small and contains 3 bristles, the sternum 1 bristle, and the epimerum three rows of bristles (2, 4, 3).

*Abdomen.*—The first, second, and third abdominal tergites contain each a single tooth on each side. Each tergite has three rows of about 10 or 12 bristles, the bristles in the anterior and middle rows being somewhat irregularly placed. There is 1 long apical bristle springing from the seventh tergite. The sternites from the third to the sixth have each two rows of bristles, an anterior of 10, and a posterior of less numerous smaller ones. The seventh has two rows of about 12 bristles each.

*Legs.*—The hind femur is without a row of bristles on either surface. There are 3 bristles situated on the outer surface basally and 3 apically, and on the inner surface basally a few small ones.

The anterior border carries a row of 3 bristles toward the apex. On the posterior border of the hind tibia there are 4 single and 1 pair of long bristles including the apical bristles, and in addition several much smaller bristles singly, or in pairs. The outer surface contains numerous bristles arranged more or less regularly in three rows. On the inner surface is a row of 6 bristles close to the anterior border. The tarsi are very hairy. The first tarsal joint in both the hind and middle legs is about as long as its respective tibia. The first pair of lateral spines on the fifth tarsal joints is situated between the second pair.

*Modified segments.*—On the lateral surface of the eighth abdominal segment below the sensory plate there is a group of 4 bristles, while toward the inferior surface there are 5 regularly placed bristles (2, 3). On the apical margin are 8 thick-set bristles. Two bristles are situated on the edge of the eighth sternite.

Length, 2.6 mm.

There is one specimen of this flea (female) in the collection at the United States National Museum, collected in Pecos, Tex., March 21, 1902, off a "bat" (*Nyctinomus mexicanus*).

Type No. 18459, U. S. N. M.

## II. A FURTHER REPORT ON THE IDENTIFICATION OF SOME SIPHONAPTERA FROM THE PHILIPPINE ISLANDS.

Since the first report on this subject[1] the writer has had an opportunity to study several other series of fleas taken off different hosts in the Philippine Islands. The results have been similar to those previously reported, namely, that the *Xenopsylla cheopis* Roths. was the only rat flea found and that the *Ctenocephallus canis* Dugés seems to be absent as in India and Panama. It is apparently a flea which does not thrive in the Tropics.

In the article previously mentioned there was a statement made which is erroneous and will therefore be corrected here. It was said that the *Ctenocephallus felis* Bouché of the Philippine Islands differed from that of the United States and Europe. As a matter of fact the differences pointed out at that time were not differences at all, but common characteristics.

In addition to the specimens previously reported upon the following have been collected and identified:

TABLE 1.—*Specimens of Xenopsylla cheopis Rothschild examined.*

| Host. | Males. | Females. | Total. | Locality. | Date. |
|---|---|---|---|---|---|
| *Epimys norvegicus* | 1 | 6 | 7 | Manila | July, 1912. |
| *Epimys alexandrinus* | ..... | 2 | 2 | ...do | Do. |
| *Epimys querceti* | 7 | 10 | 17 | ...do | Do. |
| *Mus commissarius* | 1 | 3 | 4 | ...do | Do. |
| *Epimys norvegicus* | 67 | 68 | 135 | Iloilo | August, September, and October, 1912. |
| *Epimys alexandrinus* | 16 | 11 | 27 | ...do | Do. |
| *Epimys rattus* | 24 | 28 | 52 | ...do | Do. |
| *Pachyura occultidens* | 11 | 8 | 19 | ...do | Do. |
| | 127 | 136 | 263 | | |

TABLE 2.—*Specimens of Ctenocephallus felis Bouché examined.*

| Host. | Males. | Females. | Total. | Locality. | Date. |
|---|---|---|---|---|---|
| *Epimys norvegicus* | 1 | 1 | 2 | Iloilo | October, 1912. |
| *Epimys rattus* | 1 | ..... | 1 | ...do | Do. |
| *Canis familiaris* | 18 | 24 | 42 | ...do | November, 1912. |
| *Felis domestica* | 7 | 17 | 24 | ...do | Do. |
| Wild cat sp | 10 | 21 | 31 | Palawan | January, 1909. |
| *Homo sapiens* | 1 | ..... | 1 | Iloilo | September, 1912. |
| Do | ..... | 1 | 1 | Manila | October, 1912. |
| | 38 | 64 | 102 | | |

TABLE 3.—*Specimens of Pulex irritans Linn. examined.*

| Host. | Males. | Females. | Total. | Locality. | Date. |
|---|---|---|---|---|---|
| *Canis familiaris* | ..... | 1 | 1 | Iloilo | November, 1912. |

[1] Philippine Journal of Science, Vol. VII, No. 2, sec. B. The Philippine Journal of Tropical Medicine, April, 1912.

## III. THE TAXONOMIC VALUE OF THE COPULATORY ORGANS OF THE FEMALES IN THE ORDER SIPHONAPTERA.

(Pl. VI–XXII.)

It is strange that students of the Siphonaptera, who depend to such a large extent upon the characters of the clasping organs of the males in determining the species, have not also taken more advantage of the almost equally characteristic copulatory organs of the females, especially of that organ known as the receptaculum seminis or spermatheca.

Until the appearance of Lass's article on the anatomy of the dog flea no attention had been paid to any part but the receptaculum seminis, and to that organ but few had given more than a superficial or more or less inaccurate description. In 1900 Rothschild mentions it as the abdominal gland and pictures it in differentiating between the *Ceratophyllus gallinae* and the *Ceratophyllus hirundinis*. Later he drew specific attention to it in differentiating between the *Xenopsylla nubicus*, the *X. cheopis*, the *X. aequisetosus*, and the *X. brasiliensis*, and in all of his recent writings he pictures the *spermatheca*. Wagner has described it for the *Hystrichopsyllus talpae*, a flea belonging to a genus the members of which have a double receptaculum seminis. Baker mentions the fact that it "possesses a very characteristic form in many of the species." He says, however, that "it rests in various positions, and this makes its comparative study very difficult."

The writer, after having studied thousands of specimens of *Xenopsylla cheopis* Roths., *Ceratophyllus fasciatus* Bosc., *Ctenocephallus canis* Duges, *Ctenocephallus felis* Curtis, *Pulex irritans* Linn., as well as numerous specimens of other species, was impressed with the marked differences in the shape of the spermatheca and in its other general characteristics in the different species, and with the fact that the characteristics remain constant for the species; and that, while it may assume different positions in the abdomen, in the majority of instances its position is such that it may be easily studied. In fact, that it is almost as valuable in the determination of species in the female as the claspers are in the male, except, perhaps, in some very closely allied species. He started to write an article on this subject in 1908, but was interrupted and has only recently been in position to continue. In the meantime Oudemans published a valuable article,

not only pointing out the value of the receptaculum seminis as an aid to identification, but also differences in the bursa copulatrix and the accessory ducts.

In the following description of the anatomy of the female copulatory organs, Lass is followed more or less closely.

The vagina has its origin at the vulva, which is formed by the eighth and ninth abdominal segments and is located close to the ventral wall of the abdomen, following its contour and ending in the uterus, which in turn becomes continuous with the oviducts. Just within the vulva (anterior) is seen an arborescent mass with a varying number of branches, depending on the species. This is known as the glandula vaginalis and empties by a very short duct into the roof of the vagina. It is probably the colleterial gland. Still anterior is the opening of the bursa copulatrix, while immediately in front of this is the duplicatura vaginalis, an invagination of the roof of the vagina which probably acts as a valve or stop, preventing the passage of the penis into the uterus, at the same time directing the passage of this organ into the bursa. Lass says that its lining membrane is ciliated and that it may act as a tickling organ.

The bursa copulatrix is shaped somewhat like the letter S. It receives the penis during copulation. It may be divided into three parts, a lower portion or duct which is surrounded by tissue possibly of a glandular nature; a middle portion, usually more chitinous than the rest, more or less rigid, and which in the passive condition has its walls more or less collapsed; and an upper portion which is dilated, forming a sac or pouch, as in the *Xenopsylla cheopis* or an elongated tube as in the *Ceratophyllus fasciatus*. These are probably extreme examples and between them many variations exist. At times, as in the *Ctenopsylla musculi*, the upper portion of the bursa is more highly chitinized than the middle.

Into the upper or dilated portion of the bursa there open two ducts. One, the longer, more tortuous, is known as the ductus receptaculi seminis, which connects the bursa with the receptaculum seminis. This duct varies greatly in diameter, length, and tortuosity in the different species and connects with the body of the spermatheca at the external os. Its first or distal part is, in some species, dilated, and to this portion Oudemans has suggested the name of pars dilatata. The other duct is a shorter, blind duct known as the ductus obturatorius. It is probably a vestigial organ representing the second ductus receptaculi seminis in those fleas having a double receptaculum seminis, an adherence to the original type. The ductus obturatorius expands slightly at its blind termination. It varies in length and diameter in the different species and is sometimes apparently absent. It may be very short, as in the *C. fasciatus*, or it may be almost as long as the ductus receptaculi seminis, as in the *C.*

*bruneri*, and at times, as in the *C. niger*, is very prominent by reason of the high degree of chitinization in a part of its length. At least in three species, namely the *Xenopsylla cheopis*, the *X. brasiliensis* and the *Ceratophyllus wagneri*, it opens into the pars dilatata of the ductus receptaculi seminis.

The receptaculum seminis is the reservoir for the spermatozoa. In the fertilized female a section of the spermatheca will show both the body and the appendix filled with spermatozoa. This organ, according to Mitzmain, pulsates rhythmically during copulation. He says that it is faintly discernible during the act, as it swells and collapses. It is probable that by this action the spermatozoa are drawn up into its interior to be stored until needed to fertilize the egg. During the passage of the egg through the egg canal, preparatory to oviposition the spermatozoa, aided by the secretion from the gland cells which surround the external os and portions of the duct, make their way back to the mouth of the bursa where they enter the egg through the microphyles.

The receptaculum seminis may be divided into a dilated portion or body and a contracted portion or appendix. There is an opening in the body which may be called the external os, to the margins of which there is attached the ductus receptaculi seminis. The opening between the interior of the body and the interior of the appendix may be called the internal os. The appendix is more or less bent on the body, forming a hollow or concavity. Spanning this concavity and connecting the body with the appendix are a number of muscle fibers. These muscle fibers, by their contraction, probably cause the rhythmical pulsations mentioned above, the spermatheca returning naturally to its original shape by reason of the elastic properties of the chitin which enters to a great extent into the composition of the organ.

In the interior of the spermatheca the chitin forms a series of ridges or rugae, most pronounced in the body and toward the blind end of the appendix. The area of the body immediately surrounding the external os is perforated with numerous minute holes and will therefore be called the cribiform area. Built up around the external os and surrounding the beginning of the ductus receptaculi seminis are numerous gland cells which empty into the interior of the body through the minute perforations in the cribiform area. These cells probably furnish a secretion which keeps the contents of the spermatheca fluid and a suitable medium in which the spermatozoa can remain alive for long periods.

The external os may be located concentrically or eccentrically within the cribiform area, depending on the species.

The spermatheca is usually found in an upright position with the external os directed in general caudo-ventrad. The appendix points

in various directions, depending upon the degree of curvature in the particular species under study. It may point caudad, ventrad, cephelad, or in intermediate positions.

The most important for purposes of identification is the spermatheca because of its striking and characteristic appearance, and because it is unusual not to see it plainly in all specimens properly prepared. Next in importance is the bursa copulatrix which is often dark, at least in part, and usually to be made out with ease. On the other hand, the ducts are more difficult to see and study, and to be certain of their arrangement and character a series of specimens is frequently necessary. It should be mentioned that while the general relation, diameter, length, and tortuosity of the ducts remain constant in the same species, the loops formed by the tortuous ducts do not necessarily lie in the same position, nor do the tubes and bends necessarily take the same direction.

Oudemans has arranged his material into three groups—a *sciurorum*, a *fringillae*, and a *fasciatus* group. It is thought that such a division is hardly helpful, as specific variations are so numerous that a grouping based on general characteristics would be of little value.

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# DESCRIPTION OF PLATES.

## Plate I.

Fig. 1. Head of Rooseveltiella georychi, male.
2. Apex of labial palpus of Rooseveltiella georychi.
3. Genitalia of Rooseveltiella georychi, male.
4. Terminal segments of abdomen Rooseveltiella georychi, female.
5. Hind tarsi of Rooseveltiella georychi.
6. Receptaculum seminis of Rooseveltiella georychi.

## Plate II.

Fig. 1. Head of Xenopsylla procaviæ, male.
2. Genitalia of Xenopsylla procaviæ, male.
3. Terminal segments of abdomen of Xenopsylla procaviæ, female.
4. Hind tarsi of Xenopsylla procaviæ.
5. Receptaculum seminis of Xenopsylla procaviæ.

## Plate III.

Fig. 1. Head of Rhopalopsyllus gwyni, male.
2. Terminal segments of abdomen of Rhopalopsyllus gwyni, female.
3. Apex of labial palpus of Rhopalopsyllus gwyni.
4. Receptaculum seminis of Rhopalopsyllus gwyni.
5. Hind tibia and tarsi of Rhopalopsyllus gwyni.
6. Genitalia of Rhopalopsyllus gwyni, male.

## Plate IV.

Fig. 1. Head of Doratopsylla blarinæ, male.
2. Genitalia of Doratopsylla blarinæ, male..
3. Second, third, fourth, and fifth hind tarsal segments of Doratopsylla blarinæ.
4. Head of Ceratophyllus stimsoni, male.
5. Genitalia of Ceratophyllus stimsoni, male.
6. Second, third, fourth, and fifth hind tarsal segments of Ceratophyllus stimsoni.

## Plate V.

Fig. 1. Head of Ceratophyllus apachinus, female.
2. Terminal segments of abdomen of Ceratophyllus apachinus, female.
3. Receptaculum seminis of Ceratophyllus apachinus.
4. Receptaculum seminis of Ceratophyllus gibsoni.
5. Genitalia of Ceratophyllus gibsoni, male.
6. Head of Ischnopsyllus texanus, female.
7. Terminal segments of abdomen of Ischnopsyllus texanus, female.
8. Receptaculum seminis of Ischnopsyllus texanus.

## Plate VI.

Fig. 1. Copulatory organs of Ceratophyllus fasciatus, female.

## Plate VII.

Fig. 2. Copulatory organs of Ceratophyllus niger, female.
3. Copulatory organs of Ceratophyllus acutus, female.

PLATE VIII.

FIG. 4. Copulatory organs of Ceratophyllus californicus, female.
5. Copulatory organs of Ceratophyllus telchinum, female.

PLATE IX.

FIG. 6. Copulatory organs of Ceratophyllus multidentatus, female.
7. Copulatory organs of Ceratophyllus sexdentatus, female.
8. Copulatory organs of Ceratophyllus tuberculatus, female.

PLATE X.

FIG. 9. Copulatory organs of Ceratophyllus bruneri, female.
10. Copulatory organs of Ceratophyllus labiatus, female.
11. Copulatory organs of Ceratophyllus wagneri, female.

PLATE XI.

FIG. 12. Copulatory organs of Ceratophyllus idahoensis, female.
13. Copulatory organs of Ceratophyllus wickhami, female.
14. Copulatory organs of Ceratophyllus eremicus, female.

PLATE XII.

FIG. 15. Copulatory organs of Ceratophyllus franciscanus, female.
16. Copulatory organs of Ceratophyllus ignotus, female.
17. Copulatory organs of Ceratophyllus hirsutus, female.

PLATE XIII.

FIG. 18. Copulatory organs of Dasypsyllus perpinnatus, female.
19. Copulatory organs of Odontopsyllus charlottensis, female.
20. Copulatory organs of Odontopsyllus wymani, female.

PLATE XIV.

FIG. 21. Copulatory organs of Pulex irritans, female.
22. Copulatory organs of Xenopsylla cheopis, female.
23. Copulatory organs of Xenopsylla brasiliensis, female.

PLATE XV.

FIG. 24. Copulatory organs of Spilopsyllus inaequalis, female.
25. Copulatory organs of Ctenopsylla genalis, female.
26. Copulatory organs of Ctenopsylla musculi, female.

PLATE XVI.

FIG. 27. Copulatory organs of Ctenophthalmus heiseri, female.
28. Copulatory organs of Ctenophthalmus pseudagyrtes, female.
29. Copulatory organs of Echidnophaga gallinacea, female.

PLATE XVII.

FIG. 30. Receptaculum seminis of Ceratophyllus fasciatus.
31. Receptaculum seminus of Ceratophyllus idahoensis.
32. Receptaculum seminis of Ceratophyllus ignotus.
33. Receptaculum seminis of Ceratophyllus franciscanus.

34. Receptaculum seminis of Ceratophyllus montanus.
35. Receptaculum seminis of Ceratophyllus bruneri.
36. Receptaculum seminis of Ceratophyllus acutus.
37. Receptaculum seminis of Ceratophyllus tuberculatus.

PLATE XVIII.

FIG. 38. Receptaculum seminis of Ceratophyllus californicus.
39. Receptaculum seminis of Ceratophyllus divisus.
40. Receptaculum seminis of Ceratophyllus proximus.
41. Receptaculum seminis of Ceratophyllus ciliatus.
42. Receptaculum seminis of Ceratophyllus sexdentatus
43. Receptaculum seminis of Ceratophyllus telchinum.
44. Receptaculum seminis of Ceratophyllus wickhami.
45. Receptaculum seminis of Ceratophyllus leucopus.

PLATE XIX.

FIG. 46. Receptaculum seminis of Ceratophyllus niger.
47. Receptaculum seminis of Ceratophyllus stylosus.
48. Receptaculum seminis of Ceratophyllus wagneri.
49. Receptaculum seminis of Ceratophyllus keeni.
50. Receptaculum seminis of Ceratophyllus labiatus.
51. Receptaculum seminis of Ceratophyllus multidentatus.
52. Receptaculum seminis of Hystrichopsylla dippiei.
53. Receptaculum seminis of Anomiopsyllus nudatus.

PLATE XX.

FIG. 54. Receptaculum seminis of Ctenopsylla musculi.
55. Receptaculum seminis of Ctenopsylla hesperomys.
56. Receptaculum seminis of Ctenopsylla genalis.
57. Receptaculum seminis of Dasypsylla perpinnatus.
58. Receptaculum seminis of Odontopsyllus wymani.
59. Receptaculum seminis of Odontopsyllus charlottensis.
60. Receptaculum seminis of Ctenophthalmus heiseri.
61. Receptaculum seminis of Ctenophthalmus pseudagyrtes.

PLATE XXI.

FIG. 62. Receptaculum seminis of Xenopsylla cheopis.
63. Receptaculum seminis of Xenopsylla brasiliensis.
64. Receptaculum seminis of Pulex irritans.
65. Receptaculum seminis of Pulex dugesii.
66. Receptaculum seminis of Hoplopsyllus lynx.
67. Receptaculum seminis of Hoplopsyllus anomalus.
68. Receptaculum seminis of Rhopalopsyllus australis.
69. Receptaculum seminis of Rhopalopsyllus lutzii.

PLATE XXII.

FIG. 70. Receptaculum seminis of Spilopsyllus inaequalis.
71. Receptaculum seminis of Hectopsylla pulex.
72. Receptaculum seminis of Echidnophaga gallinacea.

## HYGIENIC LABORATORY BULLETINS OF THE PUBLIC HEALTH SERVICE.

The Hygienic Laboratory was established in New York, at the Marine Hospital on Staten Island, August, 1887. It was transferred to Washington, with quarters in the Butler Building, June 11, 1891, and a new laboratory building, located in Washington, was authorized by act of Congress March 3, 1901.

The following *bulletins* [Bulls. Nos. 1–7, 1900 to 1902, Hyg. Lab., U. S. Mar.-Hosp. Serv., Wash.] have been issued:

*No. 1.—Preliminary note on the viability of the *Bacillus pestis*. By M. J. Rosenau.

No. 2.—Formalin disinfection of baggage without apparatus. By M. J. Rosenau.

*No. 3.—Sulphur dioxid as a germicidal agent. By H. D. Geddings.

*No. 4.—Viability of the *Bacillus pestis*. By M. J. Rosenau.

No. 5.—An investigation of a pathogenic microbe (*B. typhi murium* Danyz) applied to the destruction of rats. By M. J. Rosenau.

*No. 6.—Disinfection against mosquitoes with formaldehyde and sulphur dioxid. By M. J. Rosenau.

†No. 7.—Laboratory technique: Ring test for indol, by S. B. Grubbs and Edward Francis; Collodium sacs, by S. B. Grubbs and Edward Francis; Microphotography with simple apparatus, by H. B. Parker.

By act of Congress approved July 1, 1902, the name of the "United States Marine-Hospital Service" was changed to the "Public Health and Marine-Hospital Service of the United States," and three new divisions were added to the Hygienic Laboratory.

Since the change of name of the service the bulletins of the Hygienic Laboratory have been continued in the same numerical order, as follows:

*No. 8.—Laboratory course in pathology and bacteriology. By M. J. Rosenau. (Revised edition, March, 1904.)

†No. 9.—Presence of tetanus in commercial gelatin. By John F. Anderson.

*No. 10.—Report upon the prevalence and geographic distribution of hookworm disease (uncinariasis or anchylostomiasis) in the United States. By Ch. Wardell Stiles.

*No. 11.—An experimental investigation of *Trypanosoma lewisi*. By Edward Francis.

*No. 12.—The bacteriological impurities of vaccine virus; an experimental study. By M. J. Rosenau.

*No. 13.—A statistical study of the intestinal parasites of 500 white male patients at the United States Government Hospital for the Insane; by Philip E. Garrison, Brayton H. Ransom, and Earle C. Stevenson. A parasitic roundworm (*Agamomermis culicis* n. g., n. sp.) in American mosquitoes (*Culex sollicitans*); by Ch. Wardell Stiles. The type species of the cestode genus *Hymenolepis*, by Ch. Wardell Stiles.

*No. 14.—Spotted fever (tick fever) of the Rocky Mountains; a new disease. By John F. Anderson.

*No. 15.—Inefficiency of ferrous sulphate as an antiseptic and germicide. By Allan J. McLaughlin.

*No. 16.—The antiseptic and germicidal properties of glycerin. By M. J. Rosenau.

*No. 17.—Illustrated key to the trematode parasites of man. By Ch. Wardell Stiles.

*No. 18.—An account of the tapeworms of the genus *Hymenolepis* parasitic in man, including reports of several new cases of the dwarf tapeworm (*H. nana*) in the United States. By Brayton H. Ransom.

*No. 19.—A method for inoculating animals with precise amounts. By M. J. Rosenau.

*No. 20.—A zoological investigation into the cause, transmission, and source of Rocky Mountain "spotted fever." By Ch. Wardell Stiles.

*No. 21.—The immunity unit for standardizing diphtheria antitoxin (based on Ehrlich's normal serum). Official standard prepared under the act approved July 1, 1902. By M. J. Rosenau.

*No. 22.—Chloride of zinc as a deodorant, antiseptic, and germicide. By T. B. McClintic.

*No. 23.—Changes in the pharmacopœia of the United States of America. Eighth Decennial Revision. By Reid Hunt and Murray Galt Motter.

No. 24.—The international code of zoological nomenclature as applied to medicine. By Ch. Wardell Stiles.

*No. 25.—Illustrated key to the cestode parasites of man. By Ch. Wardell Stiles.

*No. 26.—On the stability of the oxidases and their conduct toward various reagents. The conduct of phenolphthalein in the animal organism. A test for saccharin, and a simple method of distinguishing between cumarin and vanillin. The toxicity of ozone and other oxidizing agents to lipase. The influence of chemical constitution on the lipolytic hydrolysis of ethereal salts. By J. H. Kastle.

*No. 27.—The limitations of formaldehyde gas as a disinfectant with special reference to car sanitation. By Thomas B. McClintic.

*No. 28.—A statistical study of the prevalence of intestinal worms in man. By Ch. Wardell Stiles and Philip E. Garrison.

*No. 29.—A study of the cause of sudden death following the injection of horse serum. By M. J. Rosenau and John F. Anderson.

†No. 30.—I. Maternal transmission of immunity to diphtheria toxine. II. Maternal transmission of immunity to diphtheria toxine and hypersusceptibility to horse serum in the same animal. By John F. Anderson.

†No. 31.—Variations in the peroxidase activity of the blood in health and disease. By Joseph H. Kastle and Harold L. Amoss.

†No. 32.—A stomach lesion in guinea pigs caused by diphtheria toxine and its bearing upon experimental gastric ulcer. By M. J. Rosenau and John F. Anderson.

*No. 33.—Studies in experimental alcoholism. By Reid Hunt.

†No. 34.—I. *Agamofilaria georgiana* n. sp., an apparently new roundworm parasite from the ankle of a negress. II. The zoological characters of the roundworm genus *Filaria* Mueller, 1787. III. Three new American cases of infection of man with horsehair worms (species *Paragordius varius*), with summary of all cases reported to date. By Ch. Wardell Stiles.

†No. 35.—Report on the origin and prevalence of typhoid fever in the District of Columbia. By M. J. Rosenau, L. L. Lumsden, and Joseph H. Kastle. (Including articles contributed by Ch. Wardell Stiles, Joseph Goldberger, and A. M. Stimson.)

†No. 36.—Further studies upon hypersusceptibility and immunity. By M. J. Rosenau and John F. Anderson.

†No. 37.—Index-catalogue of medical and veterinary zoology. Subjects: Trematoda and trematode diseases. By Ch. Wardell Stiles and Albert Hassall.

No. 38.—The influence of antitoxin upon post-diphtheritic paralysis. By M. J. Rosenau and John F. Anderson.

†No. 39.—The antiseptic and germicidal properties of solutions of formaldehyde and their action upon toxines. By John F. Anderson.

†No. 40.—1. The occurrence of a proliferating cestode larva (*Sparganum proliferum*) in man in Florida, by Ch. Wardell Stiles. 2. A reexamination of the type specimen of *Filaria restiformis* Leidy, 1880=*Agamomermis restiformis*, by Ch. Wardell Stiles. 3. Observations of two new parasitic trematode worms: *Homalogaster philippinensis* n. sp., *Agamodistomum nanus* n. sp., by Ch. Wardell Stiles and Joseph Goldberger. 4. A reexamination of the original specimen of *Tœnia saginata abietina* (Weinland, 1858), by Ch. Wardell Stiles and Joseph Goldberger.

†No. 41.—Milk and its relation to the public health. By various authors.

†No. 42.—The thermal death points of pathogenic micro-organisms in milk. By M. J. Rosenau.

†No. 43.—The standardization of tetanus antitoxin (an American unit established under authority of the act of July 1, 1902). By M. J. Rosenau and John F. Anderson.

No. 44.—Report No. 2 on the origin and prevalence of typhoid fever in the District of Columbia, 1907. By M. J. Rosenau, L. L. Lumsden, and Joseph H. Kastle.

†No. 45.—Further studies upon anaphylaxis. By M. J. Rosenau and John F. Anderson.

†No. 46.—*Hepatozoon perniciosum* (n. g., n. sp.); a hæmogregarine pathogenic for white rats; with a description of the sexual cycle in the intermediate host, a mite (*lelaps echidnimus*). By W. W. Miller.

No. 47.—Studies on Thyroid: I. The relation of iodine to the physiological activity of thyroid preparations. By Reid Hunt and Atherton Seidell.

†No. 48.—The physiological standardization of digitalis. By Charles Wallis Edmunds and Worth Hale.

No. 49.—Digest of comments on the United States pharmacopœia. Eighth decennial revision for the period ending December 31, 1905. By Murray Galt Motter and Martin I. Wilbert.

No. 50.—Further studies upon the phenomenon of anaphylaxis. By M. J. Rosenau and John F. Anderson.

No. 51.—Chemical tests for blood. By Joseph H. Kastle.

No. 52.—Report No. 3 on the origin and prevalence of typhoid fever in the District of Columbia (1908). By M. J. Rosenau, Leslie L. Lumsden, and Joseph H. Kastle.

No. 53.—The influence of certain drugs upon the toxicity of acetanilide and antipyrine. By Worth Hale.

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No. 55.—Quantitative pharmacological studies; adrenalin and adrenalin-like bodies. By W. H. Schultz.

No. 56.—Milk and its relation to the public health. (Revised edition of Bulletin No. 41.) By various authors.

No. 57.—I. The presence of tubercle bacilli in the circulating blood in clinical and experimental tuberculosis. By John F. Anderson. II. The viability of the tubercle bacillus. By M. J. Rosenau.

No. 58.—Digest of comments on the pharmacopœia of the United States of America (eighth decennial revision) and the national formulary for the period ending December 31, 1906. By Murray Galt Motter and Martin I. Wilbert.

No. 59.—The oxidases and other oxygen catalysts concerned in biological oxidations. By Joseph Hoeing Kastle.

No. 60.—A study of the anatomy of Watsonius (n. g.) Watsoni of man and of 19 allied species of mammalian trematode worms of the superfamily Paramphistomoidea. By Ch. Wardell Stiles and Joseph Goldberger.

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†No. 63.—Digest of comments on the pharmacopœia of the United States of America (eighth decennial revision) and the national formulary (third edition) for the calendar year ending December 31, 1907. By Murray Galt Motter and Martin I. Wilbert.

†No. 64.—Studies upon anaphylaxis with special reference to the antibodies concerned. By John F. Anderson and W. H. Frost.

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†No. 67.—The solubilities of the pharmacopœial organic acids and their salts. By Atherton Seidell.

†No. 68.—The bleaching of flour and the effect of nitrites on certain medicinal substances. By Worth Hale.

No. 69.—The effects of restricted diet and of various diets upon the resistance of animals to certain poisons. By Reid Hunt.

No. 70.—A study of melting-point determinations with special reference to the melting-point requirements of the United States pharmacopœia. By George A. Menge.

No. 71.—1. Some known and three new endoparasitic trematodes from American fresh-water fish. By Joseph Goldberger. 2. On some new parasitic trematode worms of the genus *Telorchis*. By Joseph Goldberger. 3. A new species of Athesmia from a monkey. By Joseph Goldberger and Charles G. Crane.

†No. 72.—I. Report on an outbreak of typhoid fever at Omaha, Nebr. (1909–1910). By L. L. Lumsden. II. The water supply of Williamson, W. Va., and its relation to an epidemic of typhoid fever. By W. H. Frost.

No. 73.—The effect of a number of derivatives of choline and analogous compounds on the blood pressure. By Reid Hunt and R. de M. Taveau.

†No. 74.—Digitalis standardization and the variability of crude and of medicinal preparations. By Worth Hale.

No. 75.—Digest of comments on the pharmacopœia of the United States of America (eighth decennial revision) and the national formulary (third edition) for the calendar year ending December 31, 1908. By Murray Galt Motter and Martin I. Wilbert.

No. 76.—The physiological standardization of ergot. By Charles Wallis Edmunds and Worth Hale.

No. 77.—Sewage pollution of interstate and international waters, with special reference to the spread of typhoid fever. By Allan J. McLaughlin.

No. 78.—Report No. 4 on the origin and prevalence of typhoid fever in the District of Columbia (1909). By L. L. Lumsden and John F. Anderson. (Including articles contributed by Thomas B. McClintic and Wade H. Frost.)

†No. 79.—Digest of comments on the pharmacopœia of the United States of America (eighth decennial revision) and the national formulary (third edition) for the calendar year ending December 31, 1909. By Murray Galt Motter and Martin I. Wilbert.

†No. 80.—Physiological studies in anaphylaxis. Reaction of smooth muscle from various organs of different animals to proteins. (Including reaction of muscle from nonsensitized, sensitized, tolerant, and immunized guinea pigs.) By William H. Schultz.

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†No. 83.—I. Sewage pollution of interstate and international waters with special reference to the spread of typhoid fever. II. Lake Superior and St. Marys River. III. Lake Michigan and the Straits of Mackinac. IV. Lake Huron, St. Clair River, Lake St. Clair, and the Detroit River. V. Lake Ontario and St. Lawrence River. By Allan J. McLaughlin.

†No. 84. Digest of comments on the pharmacopœia of the United States of America (eighth decennial revision) and the national formulary (third edition) for the calendar year ending December 31, 1910. By Murray Galt Motter and Martin I. Wilbert.

†No. 85.—Index catalogue of medical and veterinary zoology. Subjects: Cestoda and cestodaria. By Ch. Wardell Stiles and Albert Hassall.

By act of Congress approved August 14, 1912, the name of the "Public Health and Marine-Hospital Service of the United States" was changed to the "Public Health Service." Since the change in name the bulletins of the Hygienic Laboratory have been issued without break in their numerical order.

†No. 86.—Studies on typhus. By John F. Anderson and Joseph Goldberger.

No. 87.—Digest of comments on the pharmacopœia of the United States of America (eighth decennial revision) and on the national formulary (third edition) for the calendar year ending December 31, 1911. By Murray Galt Motter and Martin I. Wilbert.

No. 88.—Method for determining the toxicity of coal-tar disinfectants, together with a report on the relative toxicity of some commercial disinfectants. By Worth Hale.

No. 89.—Sewage pollution of interstate and international waters with special reference to the spread of typhoid fever. VI. The Missouri River from Sioux City to its mouth. By Allan J. McLaughlin.

No. 90.—Epidemiologic studies of acute anterior poliomyelitis. I. Poliomyelitis in Iowa, 1910. II. Poliomyelitis in Cincinnati, 1911. III. Poliomyelitis in Buffalo and Batavia, N. Y., 1912. By Wade H. Frost.

No. 91.—I. The cause of death from subdural injections of antimeningitis serum. By Worth Hale. II. Some noncholera selective media. By Joseph Goldberger.

No. 92.—Gaseous impurities in the air of railway tunnels. By Atherton Seidell and Philip W. Meserve.

No. 93. Digest of comments on the pharmacopœia of the United States of America (eighth decennial revision) and on the national formulary (third edition) for the calendar year ending December 31, 1912. By Murray Galt Motter and Martin I. Wilbert.

No. 94.—I. Collected studies on the insect transmission of *Trypanosoma evansi*. By M. Bruin Mitzmain. II. Summary of experiments in the transmission of anthrax by biting flies. By M. Bruin Mitzmain.

No. 95. Laboratory studies on tetanus. By Edward Francis.

No. 96.—1. Report of investigation of coastal waters in the vicinity of Gulfport and Biloxi, Miss., with special reference to the pollution of shellfish. By R. H. Creel. 2. A comparison of methods for the determination of oxygen in waters in presence of nitrite. By Elias Elvove. 3. Some new compounds of the choline type. III. Including preparation of monacetate of $\alpha$, $\beta$ dioxy- $\beta$-methyl butane. By G. A. Menge. 4. The detection of white phosphorus in matches. By Earle B. Phelps. 5. The chemical composition of rubber used in nursing nipples and in some rubber toys. By Earle B. Phelps and Albert F. Stevenson. 6. The analysis of thymol capsules. By Atherton Seidell. 7. Seasonal variation in the composition of the thyroid gland. By Atherton Seidell and Frederic Fenger. 8. Note on a new apparatus for use with the Winkler method for dissolved oxygen in water. By Hyman Shoub. 9. The pharmacological action of some serum preservatives. By Carl Voegtlin.

No. 97.—1. Some new Siphonaptera. 2. A further report on the identification of some siphonaptera from the Philippine Islands. 3. The taxonomic value of the copulatory organs of the females in the order Siphonaptera. By Carroll Fox.

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The editions of the publications marked (*) are no longer available for distribution by the Surgeon General of the Public Health Service. Copies of those marked (†) may, however, be obtained from the Superintendent of Documents, Government Printing Office, Washington, D. C., who sells publications at cost, and to whom requests for publications thus marked should be made.

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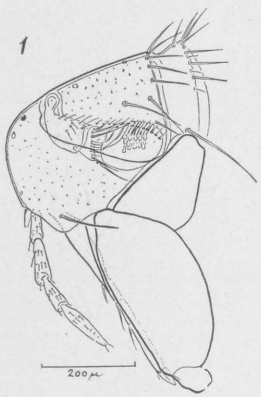
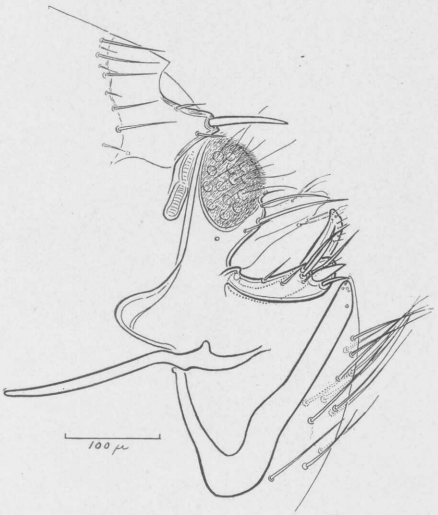
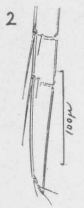
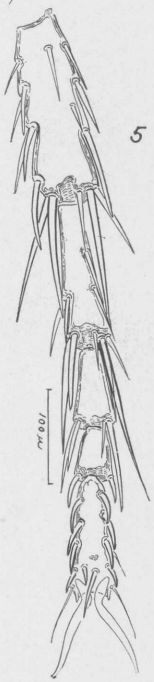
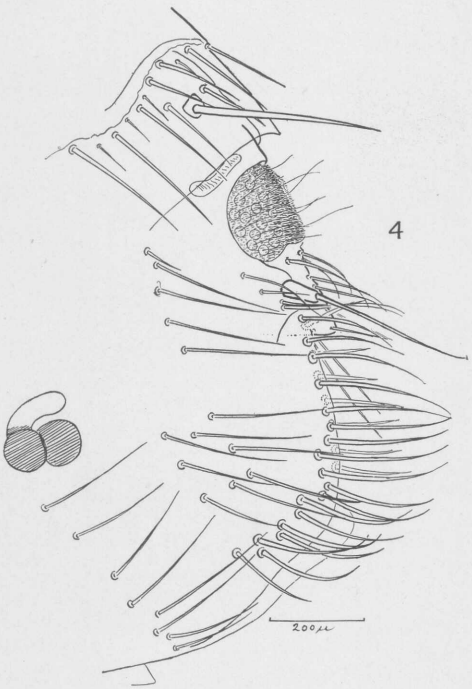
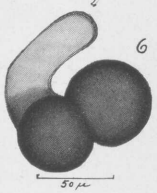


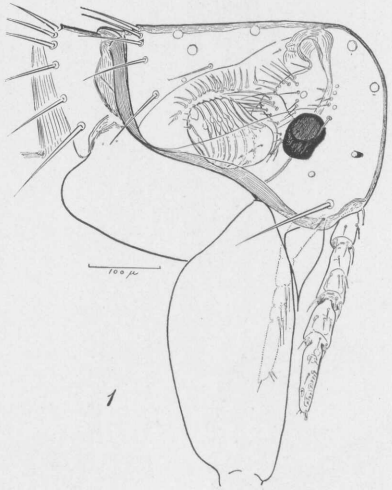



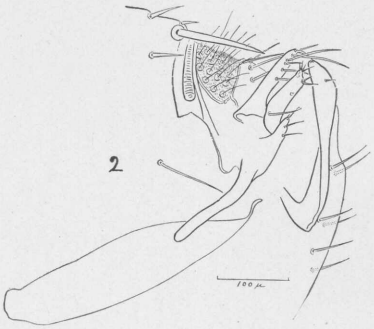



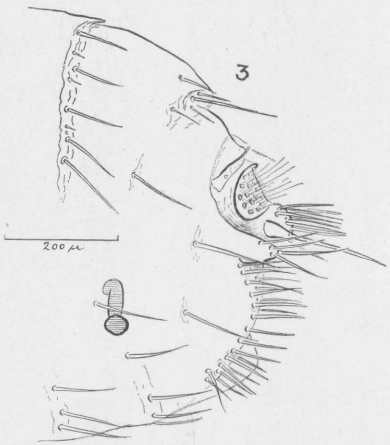

ROOSEVELTIELLA GEORYCHI.

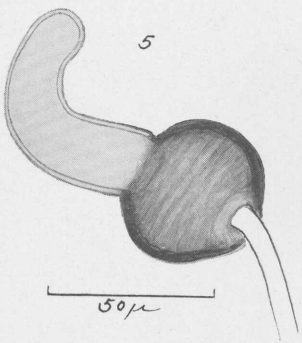
L. H. Wilder.



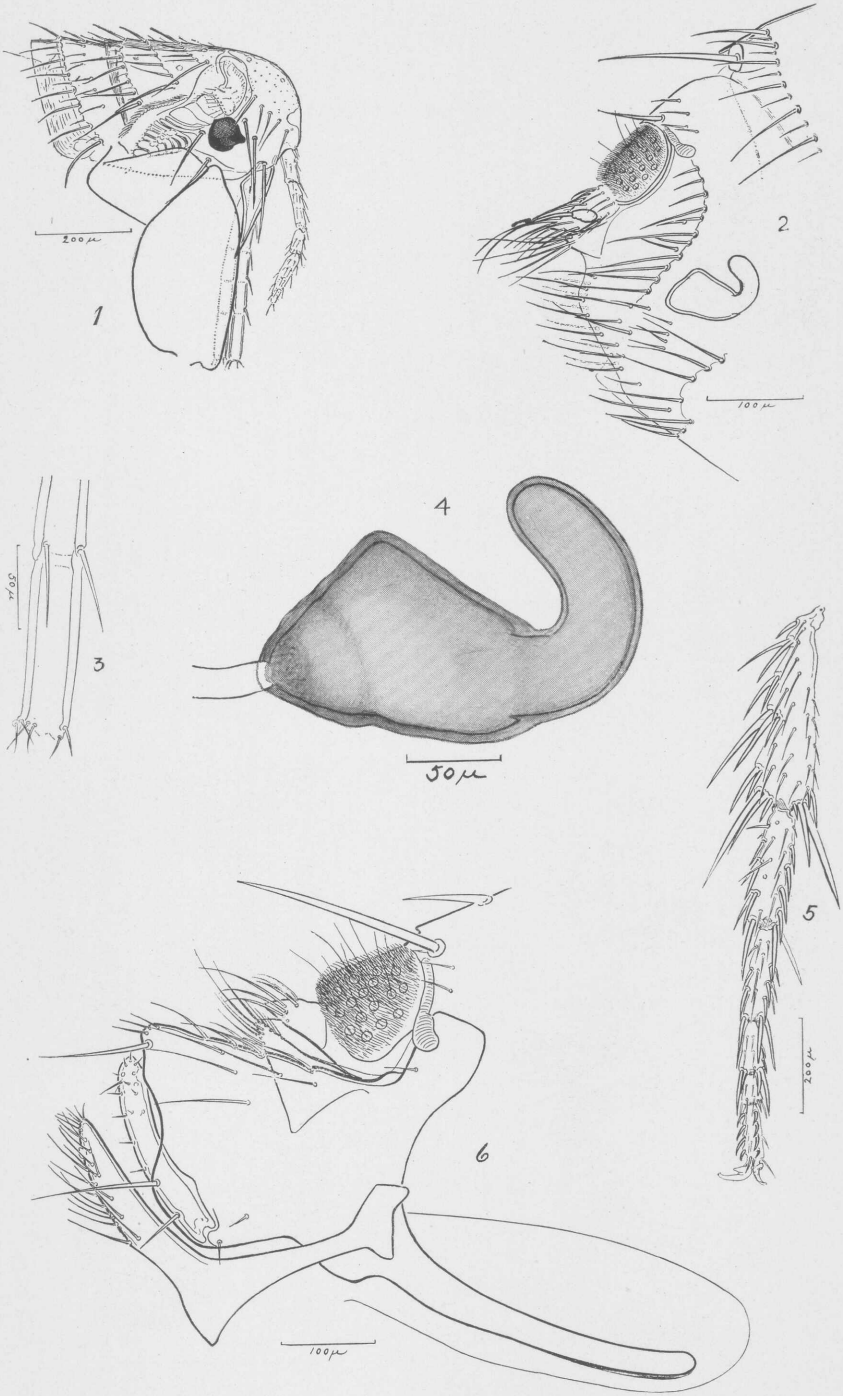




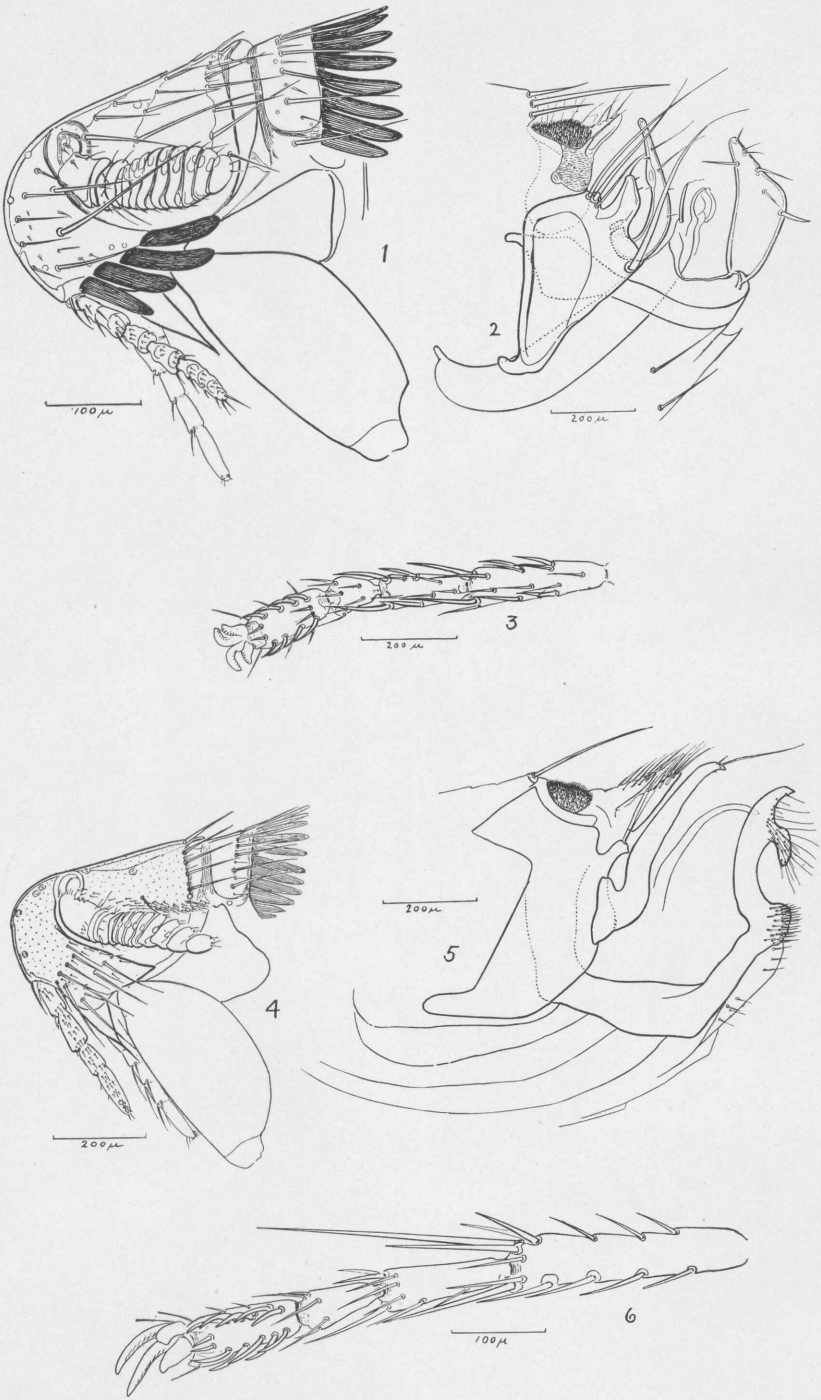

XENOPSYLLA PROCAVIAE.

L. H. Wilder.



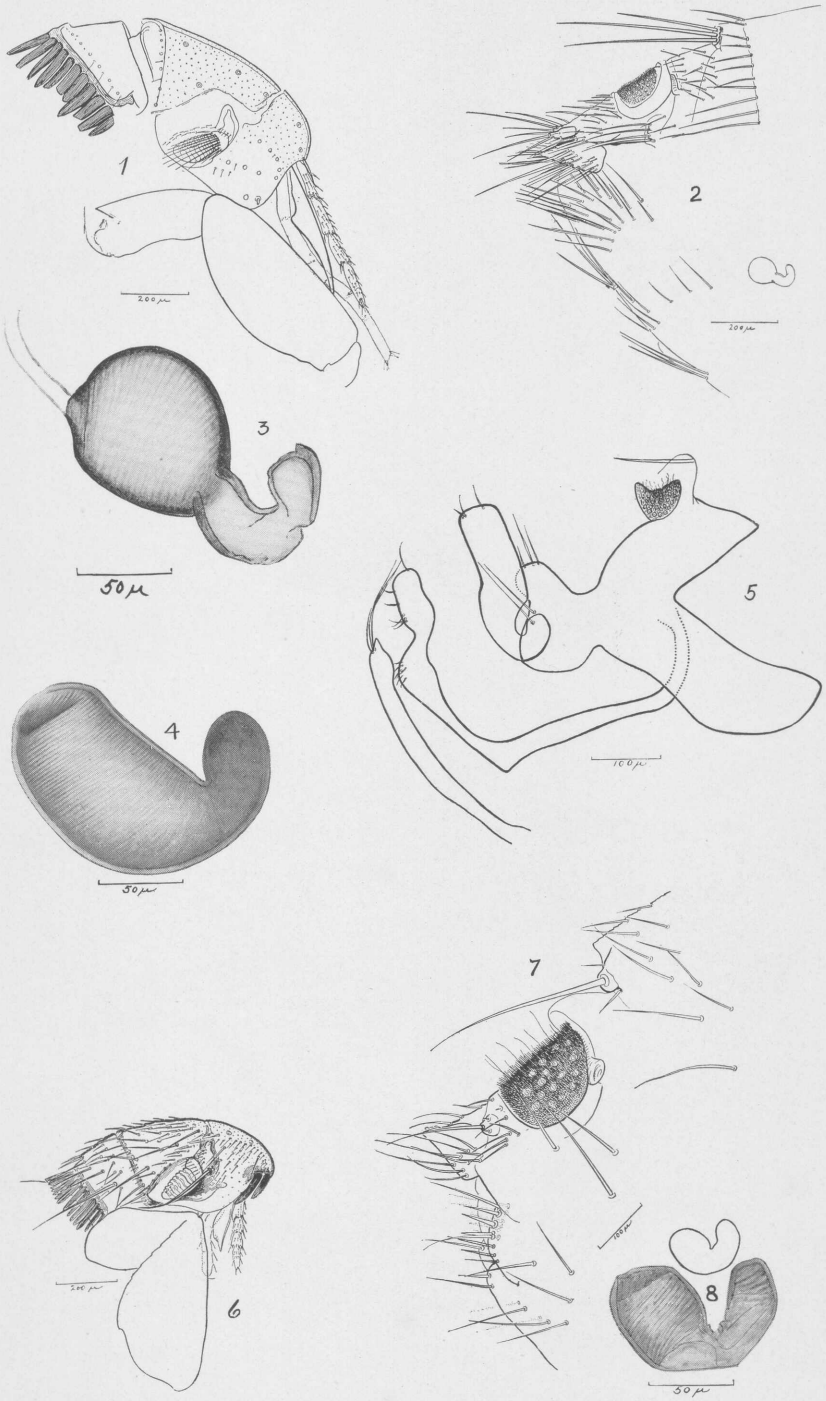

RHOPALOPSYLLUS GWYNI.

L. H. Wilder.



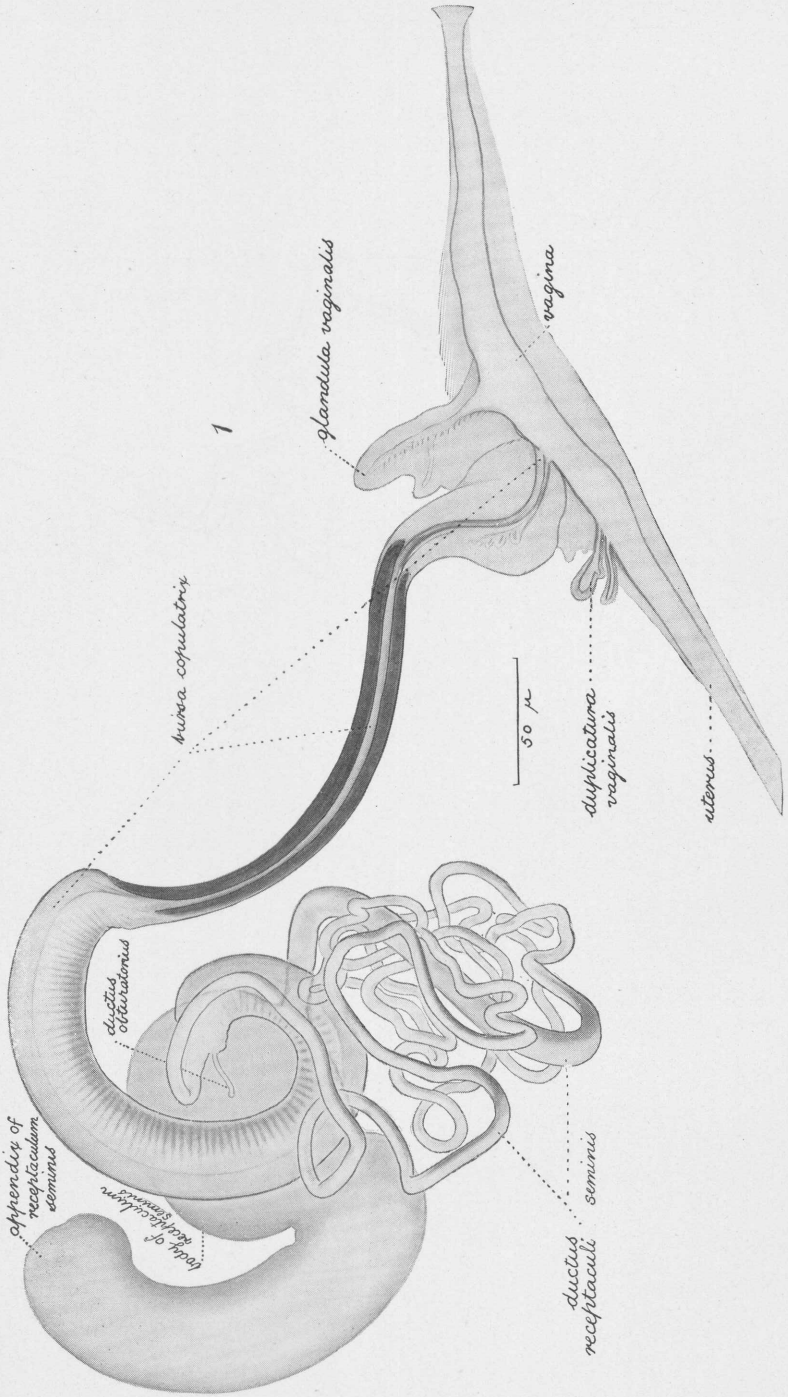

L. H. Wilder.

DORATOPSYLLA BLARINAE. CERATOPHYLLUS STIMSONI.




L. H. Wilder.

CERATOPHYLLUS APACHINUS. CERATOPHYLLUS GIBSONI. ISCHNOPSYLLUS TEXANUS.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glandula vaginalis
vagina
vulva copulatrix
50 μ
duplicatura vaginalis
uterus
appendix of receptaculum seminis
ductus obturatorius
body of receptaculum seminis
ductus receptaculi seminis

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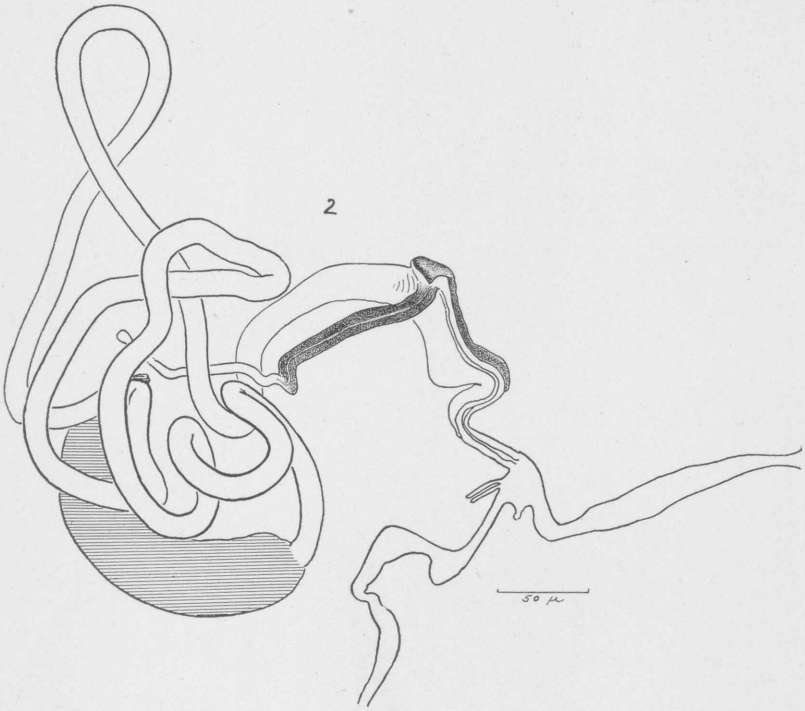


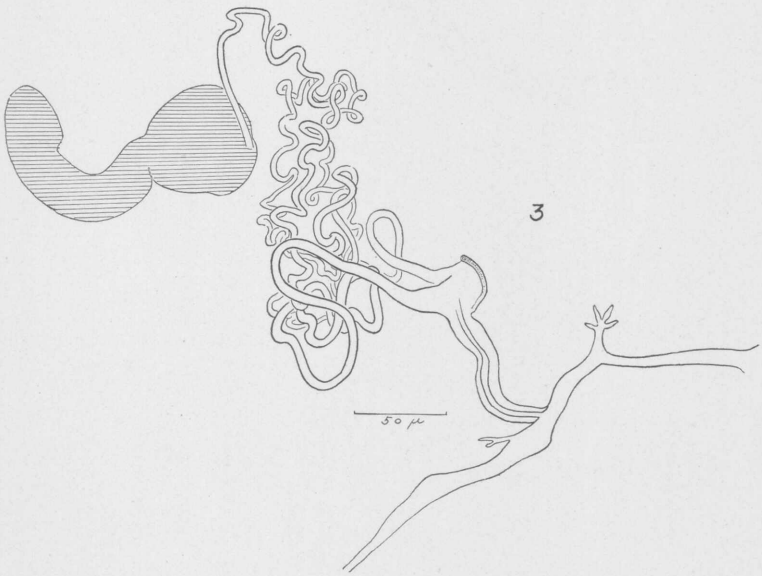


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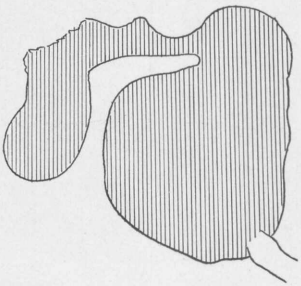

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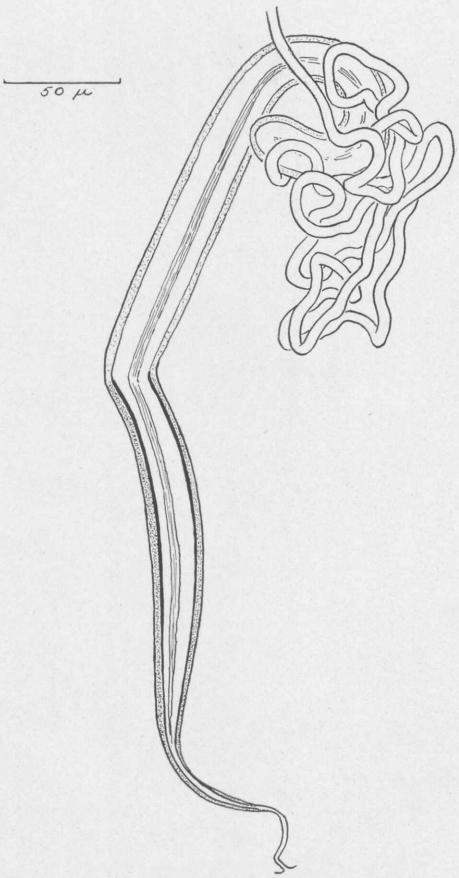


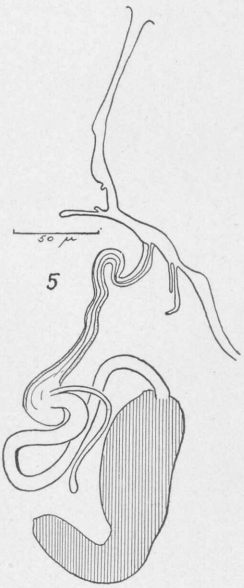


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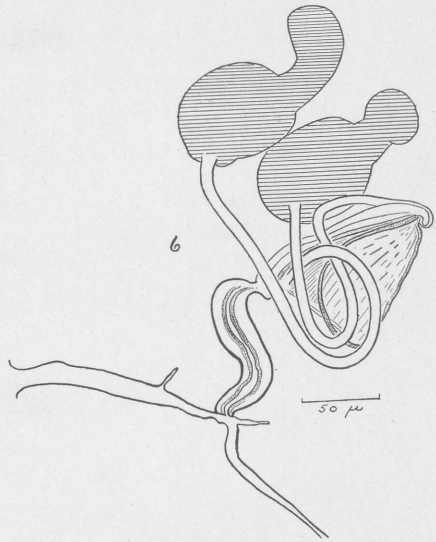


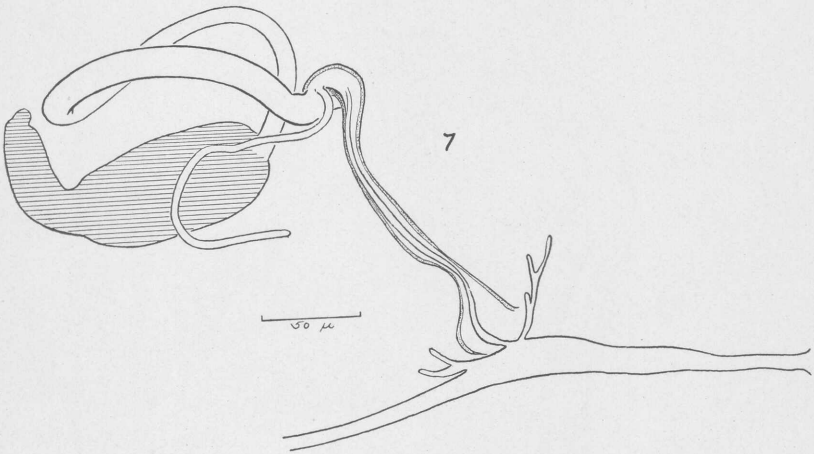


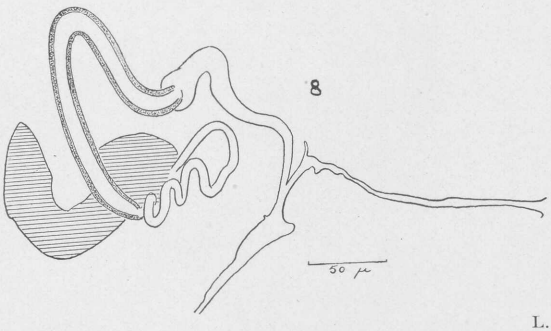


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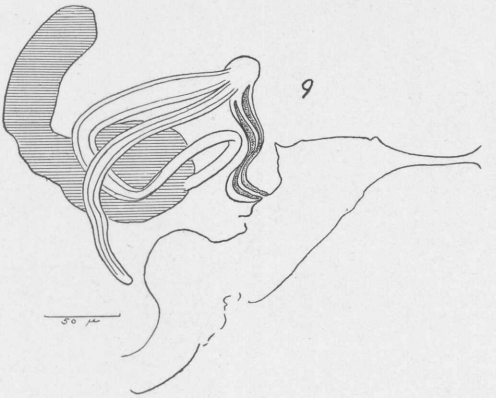


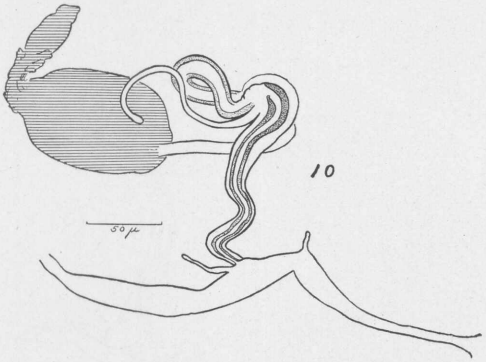


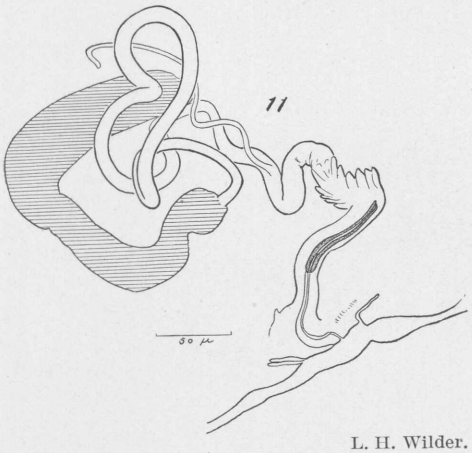


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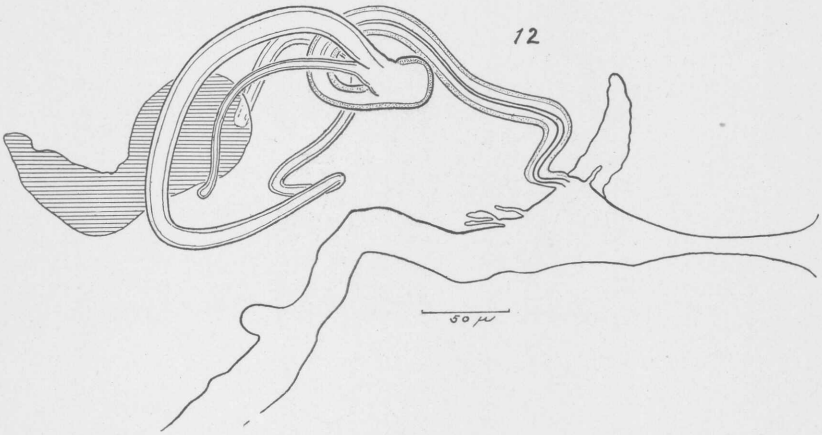


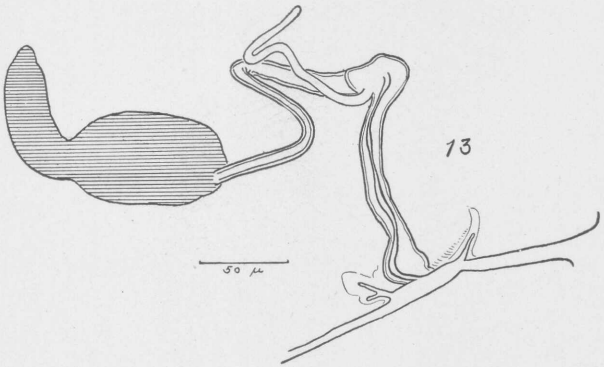


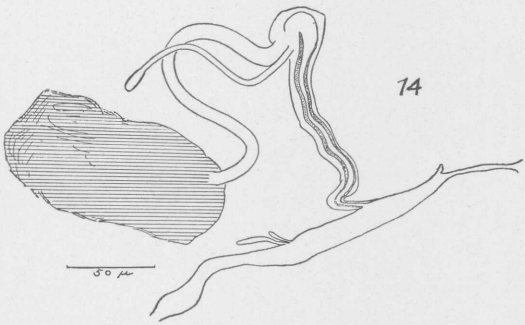


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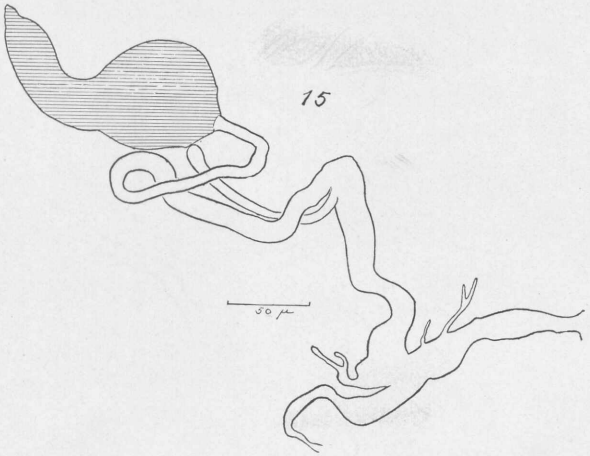


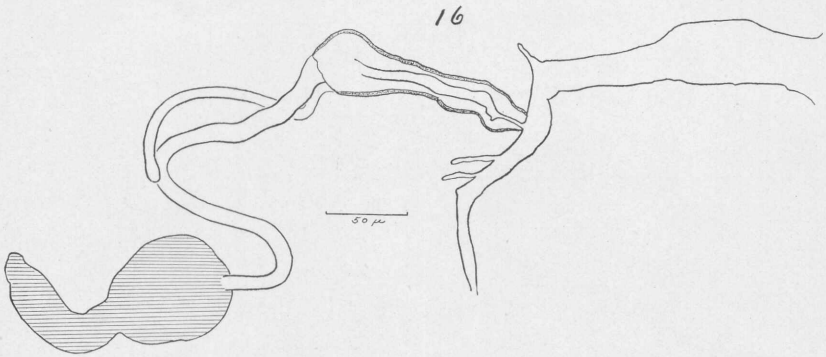


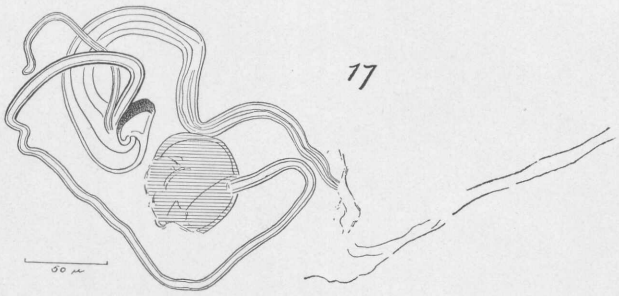


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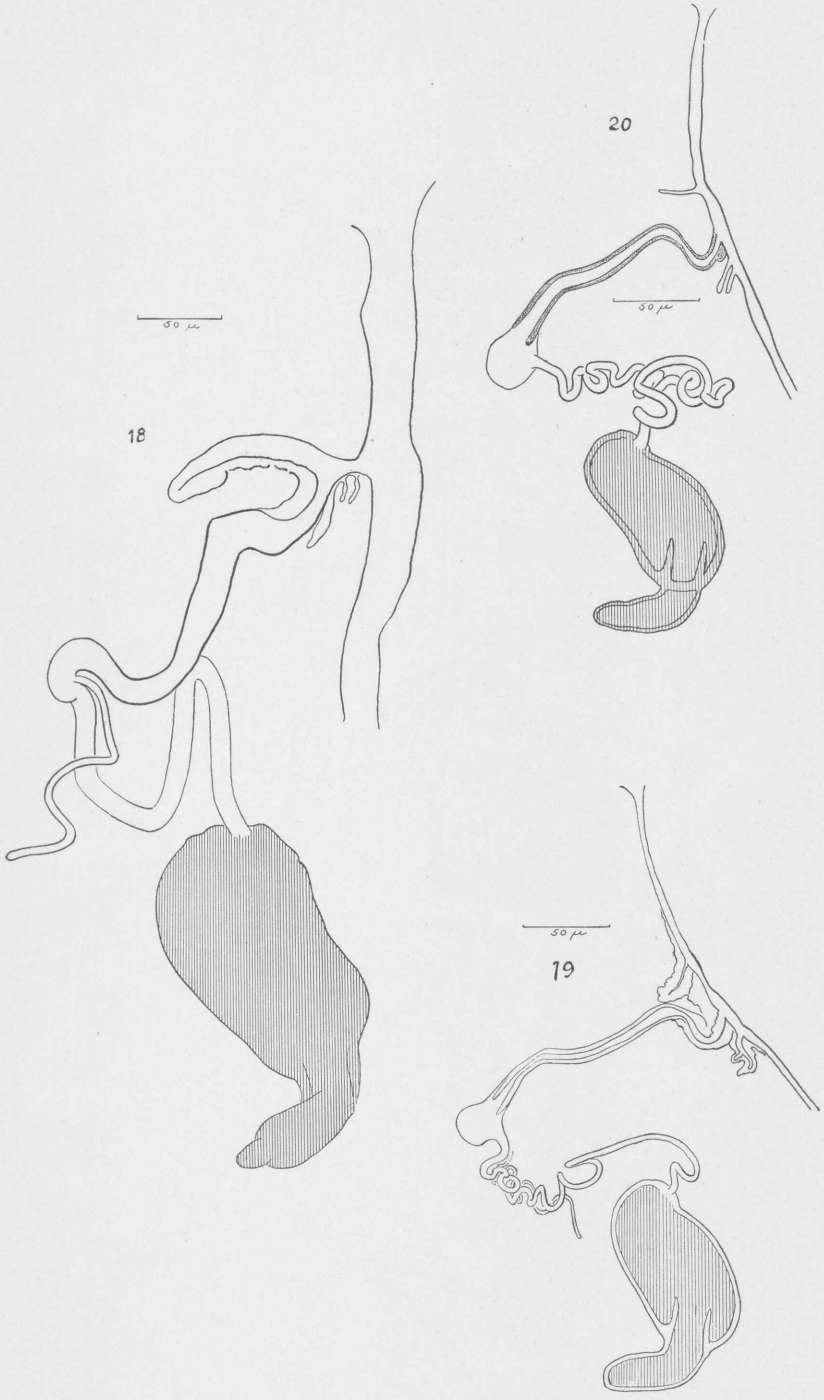
PLATE XIII.

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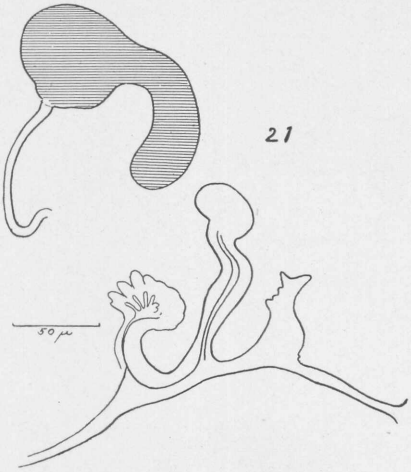


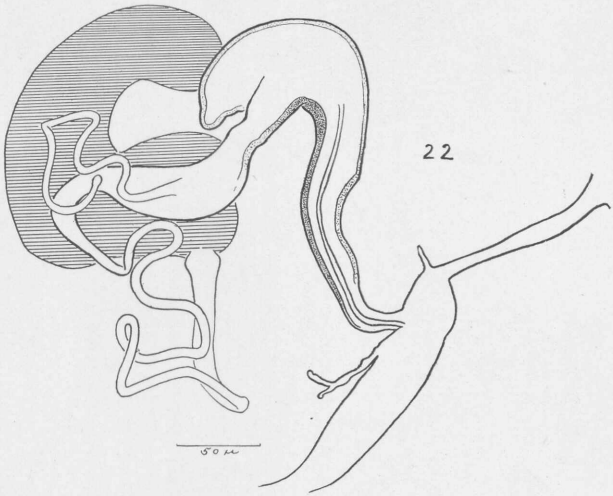


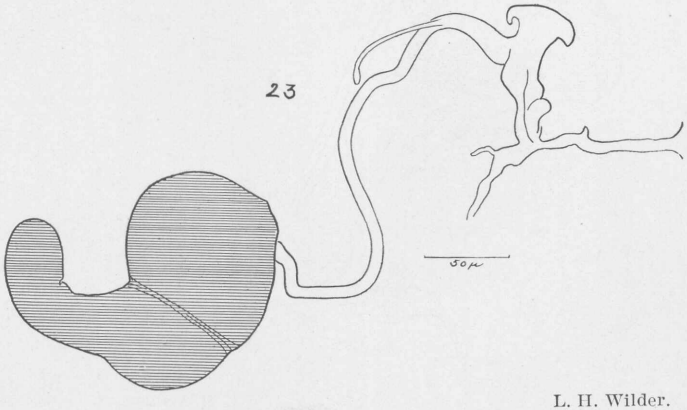


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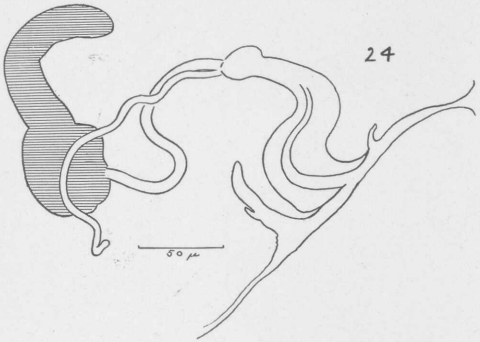


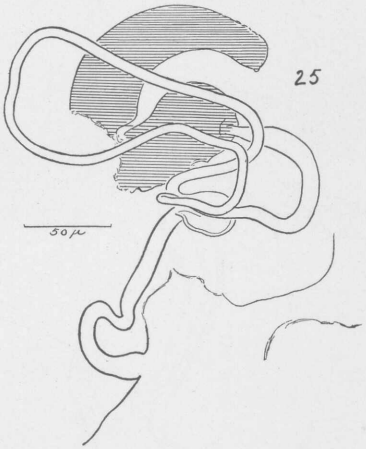


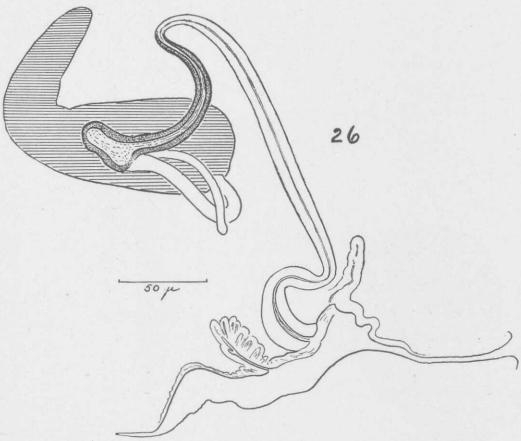


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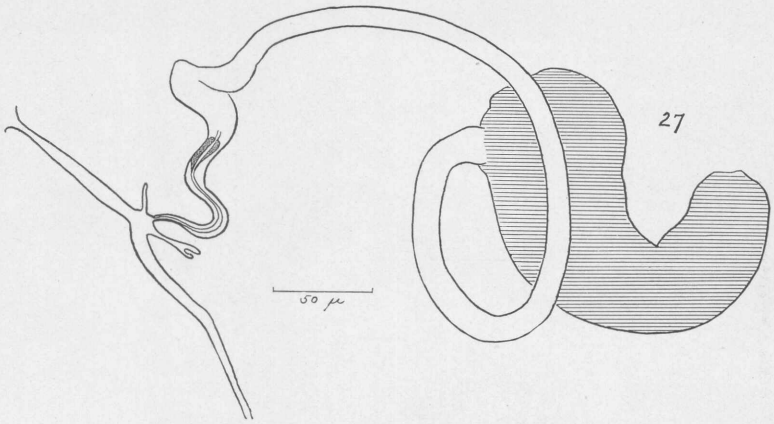


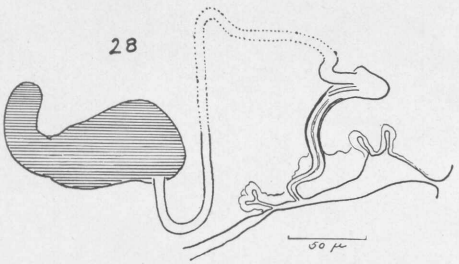


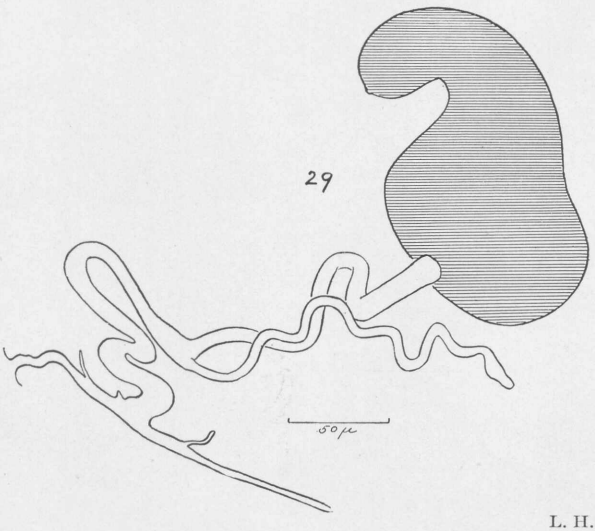


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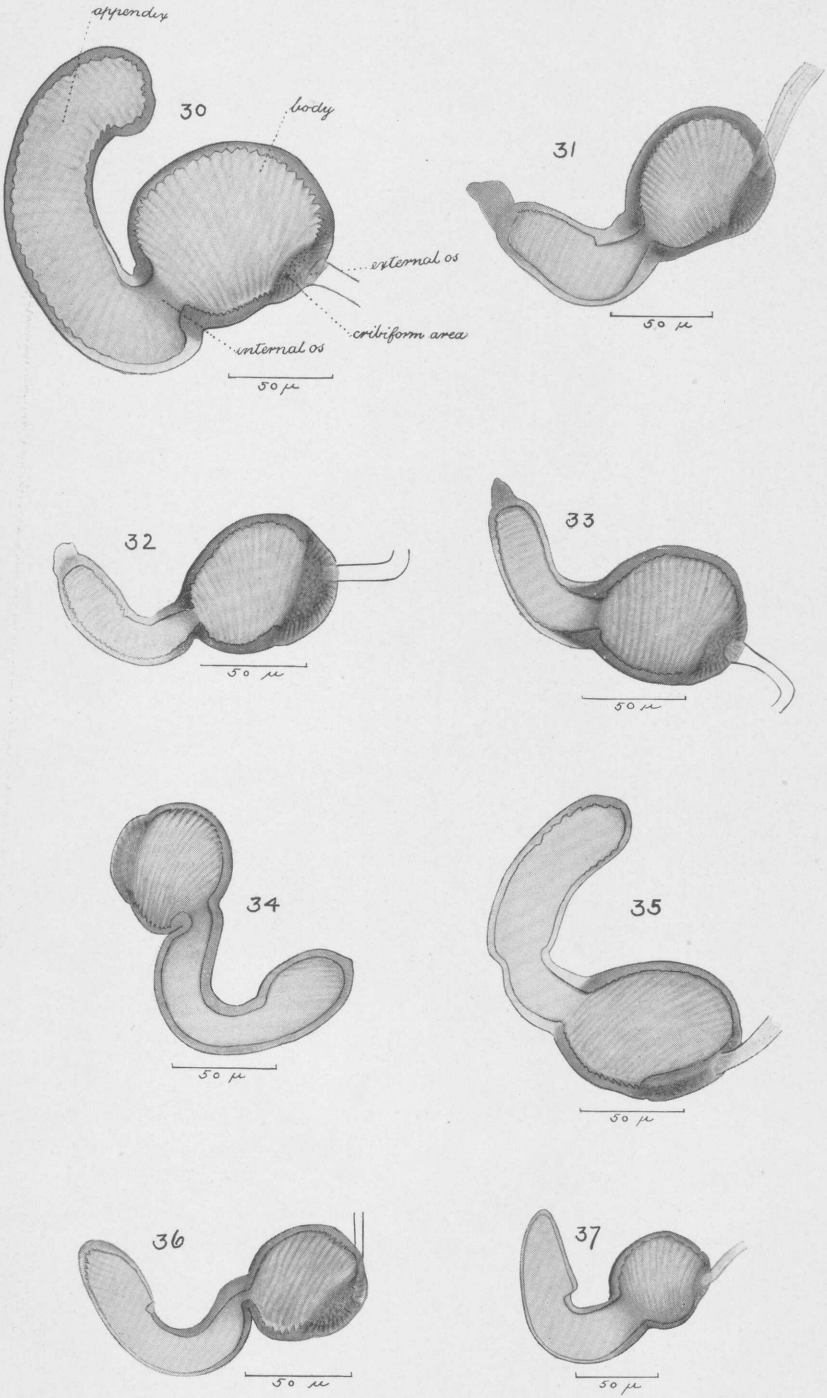
appendix
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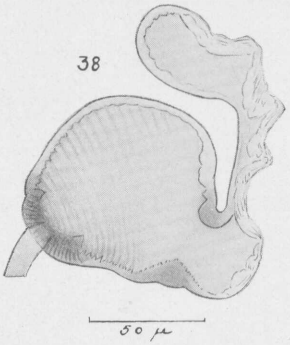


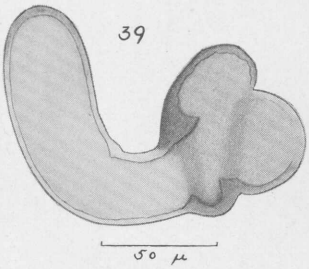


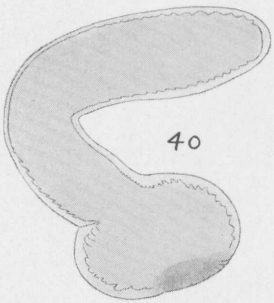


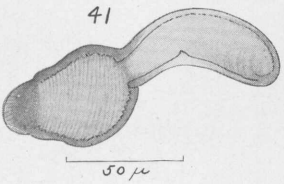


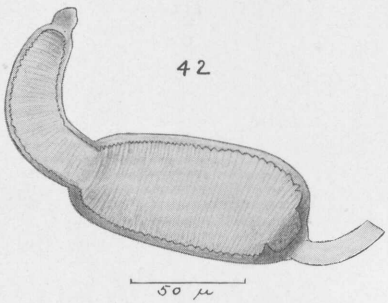


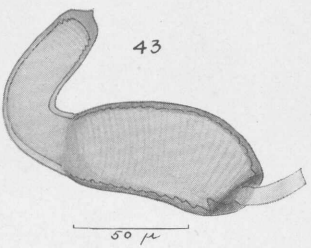


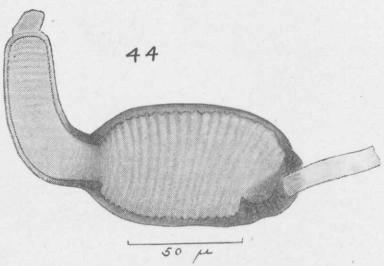


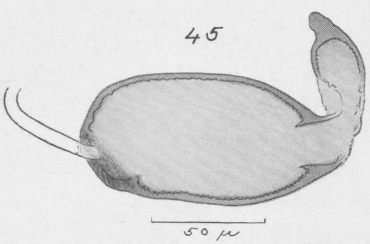


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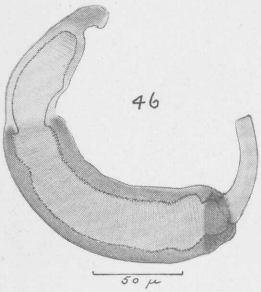


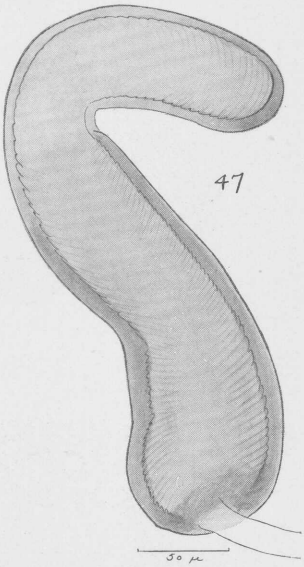


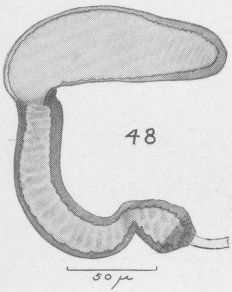


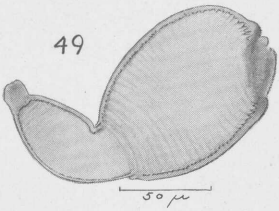


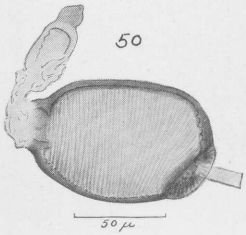


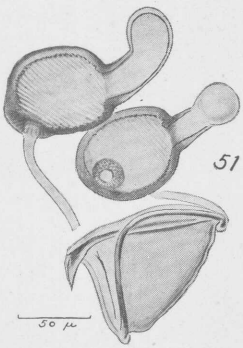


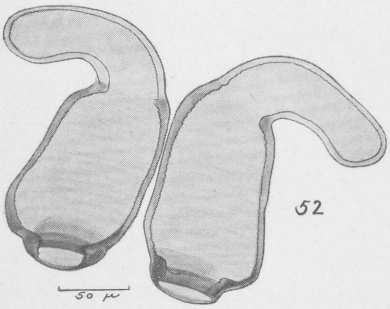


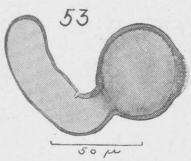


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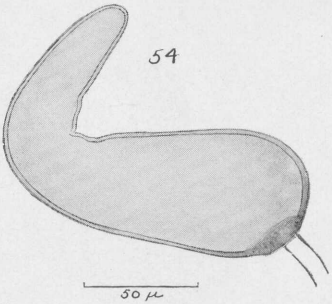


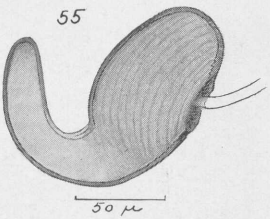


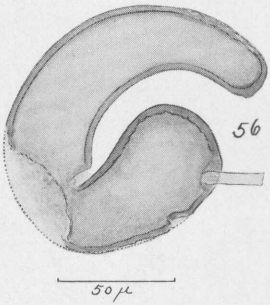


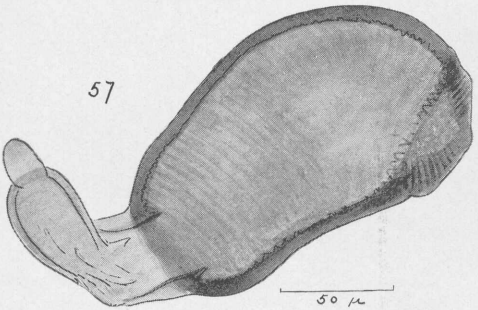


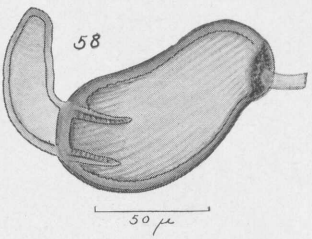


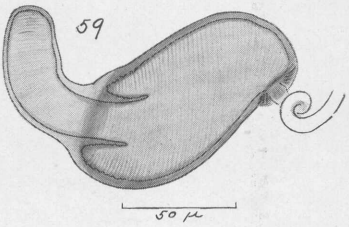


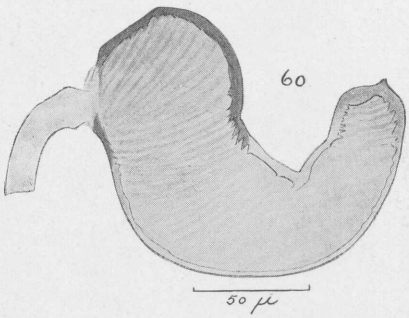


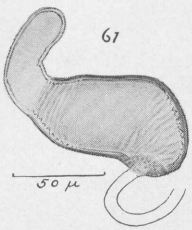


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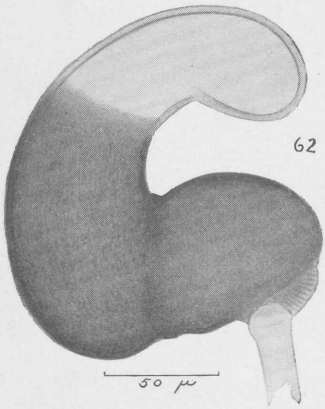


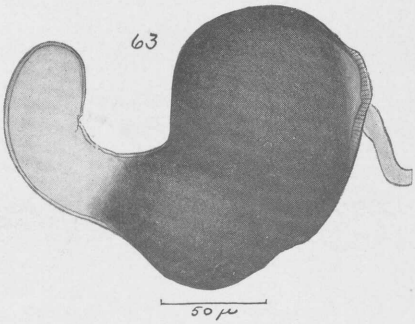


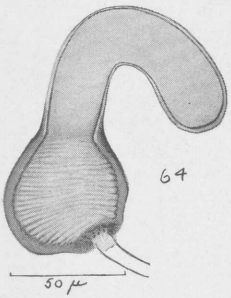


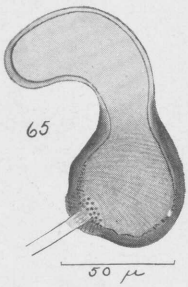


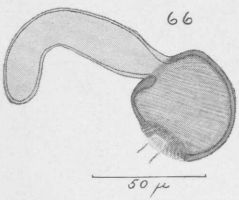


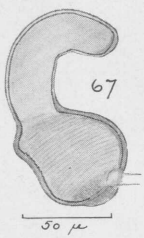


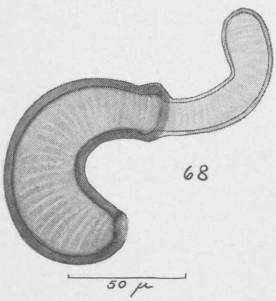


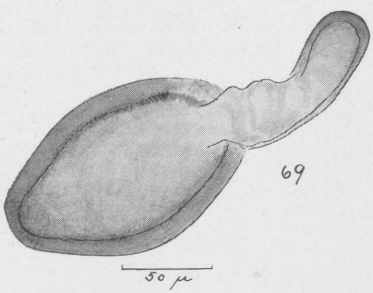


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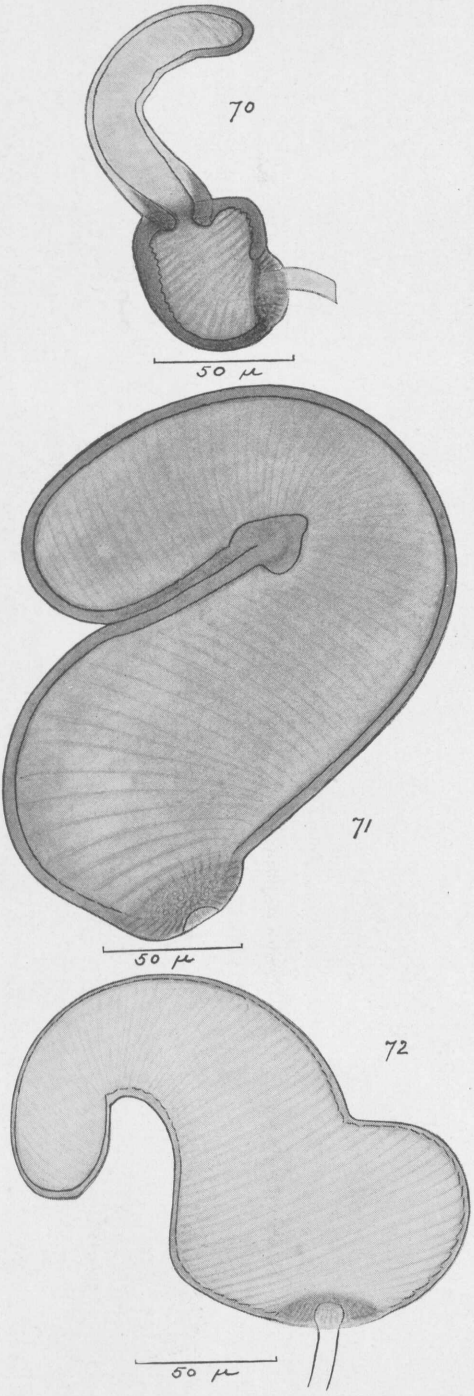


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