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CHEMICAL TESTS FOR BLOOD

By

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CHEMICAL TESTS FOR BLOOD.[a]

By Joseph Hoeing Kastle, Ph. D.,
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The greater number of strictly chemical tests for blood in use at the present time depend on the power of blood to induce the oxidation of various chromogenic substances by means of various oxidizing agents of the type of hydrogen peroxide. In such tests the blood plays the part of an oxygen carrier. In the clinical examination for blood and in forensic investigations a considerable number of chromogenic substances have been employed, such as guaiacum, aloin, the leuco base of malachite green, benzidin, phenolphthalin, etc., as well as various oxidizing agents, such as ozonized oil of turpentine, hydrogen peroxide, etc. When small amounts of any one of these chromogenic substances are brought together with water and hydrogen peroxide or ozonized oil of turpentine and a minute quantity of blood, a colored substance is produced the nature and color of which depend, of course, on the nature of the chromogenic substance employed. Properly controlled, the development of such a color under these conditions indicates blood, and certainly if no color develops it indicates the absence of blood.

The historical development of this subject forms one of the most interesting chapters in the history of clinical chemistry.

The fact that blood has the power to blue guaiacum in the presence of ozonized turpentine or hydrogen peroxide was first pointed out by Schoenbein in 1856. In a communication on "Chemical Contact Actions" (149) he describes the remarkable effect of certain substances on such compounds as hydrogen peroxide, old oil of turpentine, and ozonized ether, as a result of which the inactive oxygen which they contain is either set free or converted into ozonized (active) oxygen. He had previously shown that ozone and the so-called ozonids have the power of bluing the tincture of guaiacum, whereas pure hydrogen peroxide and other antozonids can not effect

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this change. If, however, to the mixture of hydrogen peroxide and tincture of guaiacum one adds a small amount of platinum black, the mixture at once develops a blue color, for the reason that, according to Schoenbein, the antozone of the hydrogen peroxide is converted into ozone or an ozonid through the action of the platinum, and this at once blues the guaiacum in the manner already described. He observed, further, that certain organic substances act upon the mixture of hydrogen peroxide and guaiacum in precisely the same way as platinum black. This action is shown by blood corpuscles and the gluten of wheat in a remarkable manner. In describing these experiments he goes on to say that if one separates the blood corpuscles as completely as possible from fibrin and serum and dissolves them in water one obtains a strong red solution. If one now adds a small amount of such a solution to hydrogen peroxide containing guaiacum it causes the mixture to take on a deep blue color after a few seconds, and this property of blood is not influenced by drying or boiling. That the red blood corpuscles and flour do not blue guaiacum alone needs scarcely to be mentioned. Animal albumin, fibrin, and casein are without action on the mixture of hydrogen peroxide and guaiacum tincture, and creatinin acts only slowly. He then showed that a mixture of guaiacum tincture containing a few drops of strongly ozonized oil of turpentine is colored a deep blue after a few seconds by platinum black and also by a solution of blood corpuscles. So, in the same way, ozonized ether, which alone does not blue guaiacum tincture, does so with the help of platinum black or blood corpuscles. He also observed that an indigo solution containing hydrogen peroxide, which, alone, required six hours for its bleaching, was bleached under the influence of blood corpuscles in a few seconds, and also that a mixture of indigo tincture and ozonized turpentine was bleached by blood corpuscles. He also pointed out that blood corpuscles instantly effect the liberation of iodine with the formation of the blue iodide of starch in solutions containing potassium iodide, starch, and hydrogen peroxide. It is evident from these facts that certain materials act upon oxygen and certain oxygen compounds (antozonids) in such a way as to convert the oxygen into an active form and thereby oxidize many substances which neither ordinary oxygen nor the antozonids can attack.

In a second communication (150) on this subject, Schoenbein calls attention to the fact that ferrous sulfate acts toward guaiacum and hydrogen peroxide or any antozonid, in precisely the same way as a dilute solution of blood corpuscles; and in this connection his colleague, Hiss, showed that the chemical activity of red blood corpuscles in this regard is proportional to the amount of iron which they contain. He therefore concluded that blood corpuscles owe their power to increase the chemical activity of oxygen to the iron which they contain, and

in this connection he cites the fact that neither putrefaction nor boiling destroy the activity of solutions of blood corpuscles, which would indicate that their power to activate oxygen depends less on their organization than on their iron content. He is careful to point out, however, that certain iron-free organic substances, among them benzaldehyde, also possess the power of converting inactive oxygen into the active form and thereby accomplish oxidations which can not be brought about by ordinary oxygen.

In a still later communication (151) he considers these phenomena in their relation to oxidation processes in the animal organism, and points out the importance, to the respiratory process, of the oxygen-carrying power of the blood corpuscles and their correlated property of actively decomposing hydrogen peroxide, as a means whereby the inactive oxygen of the atmosphere is rendered available for the requirements of the organism. He also calls attention to the delicacy of the guaiacum-hydrogen peroxide test for blood and to its value in forensic investigations, and later he observed that dried blood corpuscles are more active than the fresh material, so far as their power of inducing oxidation is concerned.

Our knowledge of the guaiacum test for blood has been considerably extended by the investigations of van Deen, Day, Taylor, Liman, and others; and in medical writings, despite the earlier work of Schoenbein, the test has generally come to be known as the van Deen test, or among certain English authors, as the Day test.

According to van Deen (193) the smallest quantity of blood, no matter how old it may be or how mixed with other substances, is colored blue by tincture of guaiacum and an ozone-carrier, such as oil of turpentine. That such is the case is indicated by the following: First, a very small quantity of old, stinking blood, which had stood eight or nine months, was greatly diluted with distilled water so that the liquid was nearly colorless. Oil of turpentine and then tincture of guaiacum were added to this, and an intense blue color soon developed. This blue color also develops on allowing the blood to stand for twenty-four hours with oil of turpentine and then adding the guaiacum. On the other hand, if the mixture is filtered, the guaiacum is then without action on the filtrate, for the reason that the turpentine does not pass through the filter and the blood does not take up the ozone contained therein, which it is necesssary to add to the guaiacum. Second, blood which had stood for two years with glacial acetic acid was diluted with distilled water until the solution was nearly colorless. To this there were then added 2 drops of oil of turpentine and tincture of guaiacum; the mixture took on a blue color. Third, still more glacial acetic acid was added to the blood used in Experiment No. 2, and the solution filtered. A small amount of the filtrate still gave a blue color with oil of turpentine

and guaiacum tincture, which, however, disappeared on standing. Blood does not lose this reaction on cooking. Fourth, blood which had stood in contact with alcohol for two years gave in very small quantity, with oil of turpentine and tincture of guaiacum, a blue color; the alcohol itself, however, did not give a blue color. Fifth, dried blood cakes from a calf, 3 years old, were rubbed up to a fine powder. One gram of this powder was then mixed with 400 grams of water and shaken a number of times. A few drops of this gave, with old oil of turpentine and guaiacum tincture, a blue color. Sixth, 1 drop of solution No. 5 with 5 drops of water gave the reaction. Seventh, if one doubles the dilution of the last solution so that 1 drop contains not more than 1 part of blood in 40,000, the reaction still persists. With very small quantities of blood at extreme dilution it is necessary to wait for a few minutes before the reaction takes place.

Since Schoenbein has found it probable that the iron in the blood carries the ozone of the ozone carrier to the tincture of guaiacum, control experiments must be made with iron preparations. Accordingly, ferric oxide and hydroxide, caput mortuum, and the basic carbonate of iron were tried with negative results; also limatura ferri (iron filings), ferrous oxide, hydroferrocyanic acid, potassium ferrocyanide, and phosphate of iron did not carry ozone. On the other hand, ferrous sulfate, ferric lactate, ferrous iodide, and ferrous sulfide did blue guaiacum, but not to a very high degree; and ferric acetate, citrate, and chloride did blue it to a very high degree. These, however, did not approach old putrid blood in activity. Subacetate of copper and copper sulfate were also recognized as ozone carriers, but not in very high degree. On the other hand, red lead and antimony preparations gave negative results, as did also the red coloring matters, logwood, brazil wood, santal wood, and carmine. Of the iron preparations which are ozone carriers, none have a color similar to blood, so that no confusion could arise; ferric acetate is indeed red, but it is much brighter in color than blood. It also happens that by means of ammonia water we can very easily distinguish between liquids containing blood and those containing iron preparations. The former give a greenish-yellow liquid, while the latter give a strong red turbidity and soon produce a red precipitate. A very small amount of iron also gives a yellow-colored solution, which, however, is bright and clear, while the blood gives a fluid which is never transparent. One must not employ too much ammonia water in making this test. Only by sulphuratum ferri (ferrous sulfide) was this distinction between fresh blood and iron salts not so evident. After a few hours, however, and especially after a few days, the difference became observable. The confusion of the blood reaction with the copper reaction is prevented by potassium ferrocyanide. Finally, old blood

which has become fluid and foul is more active as an ozone carrier than fresh blood.

A critical study of the van Deen test was made by Liman (105) in 1863. According to this author when the spot or substance under examination gives a negative result with tincture or guaiacum and oil of turpentine, it can be said with certainty that it is not blood. When, however, a positive reaction is obtained—that is, when the spot or substance develops a blue color on the addition of these reagents—one can not be sure that it is blood without further investigation, for the reason that many substances, such as iron salts and various organic materials, the vegetable gums, fresh roots, and the casein of milk, and tanned sheep leather, also give a blue color with guaiacum and oil of turpentine.

So far as I have been able to discover, Day's work (44, 45) consisted mainly in the repetition and verification of the earlier observations of Schoenbein on the oxygen-carrying power of blood. His method of carrying out Schoenbein's blood test consisted in brushing over the spot with tincture of guaiacum and then pouring on the spot so treated an ethereal solution of hydrogen peroxide; a beautiful blue color showed itself at once. On dark cloth he proceeded in the same manner, but after adding the reagents he pressed down on the cloth a sheet of white blotting paper, when each spot so treated gave a blue impression on the paper. In this way he succeeded in obtaining distinct tests for blood in certain cases when the microscopic examination had failed.

The guaiacum test for blood was also thoroughly investigated by Taylor (184, 185, 186), his attention having been called to the test through the work of Day, who had succeeded in detecting blood on the clothes of a Chinaman under circumstances of great difficulty. In the course of his investigations Taylor pointed out the disadvantages of oil of turpentine as a reagent for the test, and employed in its stead first ozonized ether (a solution of hydrogen peroxide in ether) and later aqueous solutions of hydrogen peroxide. In this connection he found that hydrogen peroxide or a similar oxidizing agent is absolutely essential to the test, since the red coloring matter of blood, whether dissolved in water or alcohol, whether recent or of many years' standing, whether from bird, fish, or reptile, does not render blue (oxidize) freshly precipitated guaiacum. In the presence of hydrogen peroxide, however, blood effects this change at once. He also called attention to the great solubility of the red-blood coloring matter as one of its most distinguishing characteristics, and to the fact that blood may be distinguished from rust spots, from red dyes that have been fixed by mordants, and from red paint and from other red animal colors, by its great solubility. He found further that blood so dilute as to barely give a stain on white blotting paper

admits of detection by the guaiacum test. He was also able to detect it in from 1 to 2 dram portions of a solution containing 1 drop of blood in 8 ounces (240 c. c.) of water. These small amounts, he says, are far beyond recognition by the ordinary chemical tests. He was also able to detect blood on a towel two years after the spot was produced. He observed further that hemin crystals also acquire a blue color when treated with hydrogen peroxide and guaiacum.

He also investigated the conduct of a large number of substances toward guaiacum alone and toward guaiacum containing hydrogen peroxide. Among the inorganic substances which he found to blue guaiacum directly are potassium manganate and permanganate, lead and manganese peroxides, and, in the presence of water, solutions of chlorine, bromine, iodine, hyponitrous acid, hypochlorites, the persalts of iron, potassium ferro- and ferri-cyanides, and platinum black, and among organic substances, gums—such as gum acacia—gl ten, unboiled milk, raw potato, the juices of many fresh roots, certain inks, and certain kinds of white leather. On the other hand, starch, fibrin, and boiled milk have no effect upon it. He observed further that heat destroys the power of these vegetable substances to blue guaiacum, and that with ordinary fruit stains, cochineal, and iron rust, guaiacum gives no test. He points out that with some solutions of hydrogen peroxide guaiacum gives a blue color in the absence of blood and similar carriers, probably for the reason that such solutions contain hydrochloric acid containing traces of ferric salts, and that certain old tinctures of guaiacum give a blue color with blood without the addition of hydrogen peroxide. He therefore calls attention to the necessity of selecting unoxidized pieces of guaiacum from the central portions of the lumps of resin for medicolegal work.

Finally, he calls attention to the injustice which has been done to the Van Deen test by medicolegal writers, some of whom have pronounced the guaiacum test untrustworthy for the reason that other substances impart the blue color to guaiacum. He points out that while this statement is true, it does not convey the whole truth; and he himself is of the opinion that the use of guaiacum adds another and valuable chemical test to those hitherto employed for the detection of blood. It enables the chemist to detect it with reasonable certainty when it is present in very small quantities, or to trace it in those cases in which an attempt has been made to remove it by washing; and when the result of the test is negative, it enables him to say that the suspected stain was not caused by blood, a fact of considerable importance in medicolegal inquiries.

As may be seen from the bibliography given at the end of this article, these and similar reactions for blood have formed the subject of a large number of investigations. It is impossible to include

abstracts of all of these within the scope of this article. In general it may be said that these investigations have been concerned with improvements in the general technique of the test, with the use of various chromogenic substances in the place of guaiacum, with the study of the conduct of various substances other than blood toward such reagents, and with the delicacy of such tests. Finally, a number of these communications are devoted in whole or in part to a criticism of the guaiacum reaction and similar tests.

In what follows, therefore, an attempt has been made to condense and arrange this vast amount of material under several heads: First, "The nature and general theory of the color tests for blood;" second, "Substances other than blood which show the guaiacum reaction and similar tests;" third, "Substances which do not give the guaiacum reaction and similar tests;" fourth, "The delicacy of the chemical tests for blood;" and fifth, "The value of the guaiacum reaction and similar tests;" and to give under each topic considered all references obtainable to the original literature on the subject for the benefit of those seeking further information on any of the subjects herein presented.

THE NATURE AND GENERAL THEORY OF THE COLOR TESTS FOR BLOOD.

The guaiacum test for blood and all similar reactions depends upon the oxidation of guaiacum or some other chromogenic substance to guaiacum blue or some other colored derivative, by ozonized oil of turpentine, hydrogen peroxide, or some other oxidizing agent having the properties of a peroxide, under the influence of blood, or more especially of the iron-containing blood pigments, as oxygen carriers. Among all of the chromogenic substances thus far employed in testing for blood, guaiacum has been the most extensively used. No fewer than 107 out of the 212 references to the literature of blood testing given at the close of this article have to do with the use of guaiacum in this connection.

Among the other chromogenic substances which have been employed for this purpose may be mentioned the following:

Guaiaconic acid: Doebner, 46; Schaer, 140.

Aloin: Buckmaster, 29; Einhorn, 49; Heuberger, 69; Jaworski and Korolewicz 75; Klunge, 88; Koziczkowski, 91; Rossel, 137, 138; Schaer, 141, 142; Zuelger, 212.

Benzidin: Adler, 2; Ascarelli, 7; Einhorn, 49; Jaworski and Korolewicz, 75; Löb and Mulzer, 108; Messerschmidt, 113a; Schlessinger and Holst, 143; Schumm, 159, 161, 162, 163; Schumm and Westphal, 164; Utz, 192.

The leuco-base of malachite green: Adler, 2; Buckmaster, 30; Czyhlarz and Von Fürth, 43.

Phenolphthalin: Deléarde and A. Benoit, 16; Kastle and Amoss, 80; Meyer, 114; Pozzi-Escot, 128; Utz, 191.

In addition to these, a large number of aromatic amines, mono-, di-, and tri-acid phenols and aromatic acids have been studied by Adler (2).

Similarly a considerable number of oxidizing agents of the general type of hydrogen peroxide have been studied with regard to their general applicability to the Schoenbein-Van Deen blood test. Chief among these are old oil of turpentine (the so-called ozonized oil of turpentine) and hydrogen peroxide itself. Other observers have employed ozonized ether and other ozonized ethers and oils. Battelli and Stern (13) have used ethyl peroxide, and Ladendorf (96) has recommended oil of eucalyptus as preferable to oil of turpentine.

In a private communication Dr. A. S. Loevenhart informs me that iodoso-benzoic acid oxidizes phenolphthalin under the influence of blood.

GENERAL THEORY OF THE REACTION.

According to Schoenbein (151), blood acts upon hydrogen peroxide and other antozonids after the manner of platinum, in that it converts the antozonid into an ozonid, or into a compound in which the oxygen exists in the active condition, viz, in the form of ozone, and that the ozone of the ozonid then converts the guaiacum into a similar ozonid compound which is blue in color. Schmidt (144) even went so far as to believe that he had shown that blood itself contains ozone, for the reason that it blues guaiacum in the absence of ozonized oil of turpentine. Schuster (165), however, was unable to confirm Schmidt's observation that blood alone blues guaiacum, and it is now known that old tinctures of guaiacum, in contradistinction to the fresh tinctures of the resin, contain peroxide compounds, which, under the influence of oxygen-carriers, like blood, produce guaiacum blue. Therefore, in all such cases, it is the old guaiacum and not the blood itself which supplies the oxygen necessary for this reaction. Schuster (165) was also of the opinion that the bluing of guaiacum by various substances was perhaps partly the result of a mechanical action, since he was able to blue it by shaking together a mixture containing the tincture, turpentine, and fine glass fragments. On the other hand, he found a simple oil emulsion to be inactive. He was of the opinion, therefore, that the reaction was not sufficiently well understood to justify the complete acceptance of Schoenbein's theory of the process.

It has been held by some observers that the activity of blood as an oxygen carrier depends upon the presence of an oxidase or peroxidase. Thus, in their recent study of the oxidation of phenolphthalin by blood, Deléarde and A. Benoit (16) arrived at the conclusion that the blood plays the part of an indirect oxidizing ferment (peroxidase). Buckmaster (30) regards the oxidation as due to a pseudo-peroxi-

dase, and, for lack of a better term, Kastle and Amoss (80) have used the expression "peroxidase activity" of the blood as descriptive of the oxidation of phenolphthalin by hydrogen peroxide under the influence of blood as an oxygen carrier. They were careful to point out, however, that in its capacity as an oxygen carrier, blood exhibits certain important differences from the true peroxidases, among which may be mentioned the fact that it does not lose its activity as an oxygen carrier on boiling, and that it reacts in alkaline solutions of sufficient concentration to destroy many, if not all, of the true peroxidases, and further, that the activity was found to be proportional to the hemoglobin content.

Lumiére and Chevrotier (109) claim to have shown that an aqueous extract of frozen and washed blood corpuscles possesses the properties of an oxidase to a remarkable degree.

According to Carlson (33) the action of blood on hydrogen peroxide and guaiacum is due to some organic constituent of the blood.

It has gradually come to be recognized, however, that the oxygen-carrying power of blood can not be due entirely to the presence of oxidases and peroxidases, for the reason that the oxygen-carrying power persists after boiling and treatment with acids and alkalis. Indeed, it seems to persist as long as the blood pigments are not deprived of their iron. Thus, according to Moitessier (117) the so-called peroxidase reaction of the blood is not really due to a peroxidase but to hemoglobin and hematin, nor do the nonferruginous blood pigments, such as hematoporphyrin, exhibit such reactions. Czyhlarz and von Fürth (43) also arrived at the conclusion that the oxidizing reaction is due to hematin and not to a peroxidase. Lesser (101) has also observed that blood gives the guaiacum reaction after it has been boiled, and that the reaction is due to the blood pigment, and further that the iron-free derivatives of hemoglobin do not give it. Whitney (203) also concludes that it is the iron of the hemoglobin and its iron-containing derivatives which are responsible for the guaiacum reaction. As already pointed out, the importance of the iron of the blood pigments in such reactions was recognized by Schoenbein and his colleague, Hiss, according to whom the oxidizing power of the blood is proportional to its iron content.

Buckmaster (29) also concludes that this test depends on some part played by the iron in the hemoglobin molecule. According to this author, however, the precise way in which the iron acts is still obscure. It can not be due merely to a ferric salt; the iron must act while the change is taking place. He suggests that the moment the iron is split off from the hemoglobin molecule it acts toward guaiacum, not as a carrier of oxygen, but as a direct oxidizing agent, causing the oxidation of the guaiacum to guaiacum blue in a manner similar to the action of iodine.

Tarugi (183), on the other hand, is of the opinion that the reaction is accomplished by the hemoglobin, and that the sulphur in this compound is first converted into Caro's acid by the action of the hydrogen peroxide or ozonized oil of turpentine, and that it is this acid which accomplishes the bluing of the guaiacum.

According to Liebermann (103) the peroxide contained in old oil of turpentine first gives up its oxygen to the hemoglobin, forming a compound of hemoglobin rich in oxygen, probably a peroxide of hemoglobin, and that the oxygen of this compound is appropriated by the guaiacum with the formation of guaiacum blue, and that methemoglobin is also a carrier of oxygen.

The whole subject of induced oxidations by means of hydrogen peroxide has been extensively studied by Loevenhart and Kastle (107), and in the second part of their article on this subject the view has been advanced that irrespective of the composition of the carrier the latter acts by first combining with the hydrogen peroxide to form complex unstable holoxide or moloxide derivatives, which give up their oxygen to the reducing substances more easily than does the hydrogen peroxide itself. This theory of oxygen-carrying is in harmony with the more recent work of Kastle (79) on the subject of peroxidase accelerators. According to this hypothesis, therefore, the function of blood as an oxygen carrier in reactions of the Schoenbein-Van Deen type could probably be explained most simply and easily on the supposition that the iron-containing blood pigment combines with the peroxide to form a moloxide derivative, which in turn easily gives up the whole or a part of its oxygen to the chromogenic substance with the production of a dye of characteristic color. Thus if the carrier in blood be represented by *B* and the chromogenic substance by *Chr*, we would have—

$$B + H_2O_2 = B.H_2O_2,$$

and

$$B.H_2O_2 + Chr = B + H_2O + ChrO,$$

or

$$2B.H_2O_2 + Chr = B + 2H_2O + ChrO_2,$$

or to take the specific case of phenolphthalin, in which the precise nature of the oxidation product, phenolphthalein, is known, we would have—

$$B + H_2O_2 = B.H_2O_2,$$

and

$$B.H_2O_2 + C_{20}H_{16}O_4 = B + 2H_2O + C_{20}H_{14}O_4.$$

This, of course, presupposes that the carrier is a perfect carrier, and that therefore it is completely regenerated at the end of any complete cycle of the reaction. If it is not thus completely regenerated in

unaltered condition, it is probable that the carrier itself has suffered complete and final oxidation in greater or less amount and that the resulting oxide or oxidation product can no longer combine with the hydrogen peroxide, in which case we would have—

$$2B.H_2O_2 + C_{20}H_{16}O_4 = B.O + 3H_2O + C_{20}H_{14}O_4.$$

SUBSTANCES OTHER THAN BLOOD WHICH REACT WITH GUAIACUM OR WITH OTHER CHROMOGENIC SUBSTANCES EMPLOYED IN HEMATOLOGIC INVESTIGATIONS, EITHER ALONE OR WITH HYDROGEN PEROXIDE.

The chief objection which has been urged against the guaiacum test for blood and all tests of a similar character is that these reactions are given by a large number of substances other than blood. Various observers working in this field have found the following substances to give the guaiacum or similar reactions (the numbers refer to the bibliography at the end of this article):

All oxidizing agents, 10.
The halogens, 170.
- Chlorine, 10, 24, 37, 124, 139, 147, 184.
- Bromine, 37, 124, 139, 147, 184.
- Iodine, 90, 124, 147, 184.

Ozone, 24, 90, 139, 146, 147, 170.
- Ozonized air, 25, 146.
- Hydrogen peroxide, 76, 170, 184.

Ammonia, 37.
Finely divided metals, especially the noble metals, 170.
- Copper, 164.
- Iron, 164.
- Platinum black, 10, 141, 146, 147, 149, 164, 184.
- Steel, 203.

Compounds of certain heavy metals:
- Cobalt salts, 128.
  - Cobalt chloride, 137, 169.
- Chromium salts, 111, 170.
  - Chromic acid, 24, 137, 139, 161.
  - Potassium chromate, 24, 73, 147.
- Copper sulfate and other copper compounds, 3, 7, 50, 73, 129, 137, 139, 147, 165, 170, 171, 197, 203.
  - Copper nitroprusside, 137.
  - Fehling's solution, 10, 161.
- Gold salts, 203.
  - Gold chloride, 137.
- Iron salts, 2, 21, 37, 76, 111, 128, 139, 165, 169, 170, 171, 184, 193, 197, 201, 203.
  - Ferrous sulfate, 4, 155, 165.
  - Ferrous sulfide, 165.
  - Ferrous chloride, 147, 165.
  - Ferric chloride, 10, 24, 76, 105, 156, 161, 165.
  - Ferric citrate, 105.
  - Ferric hydroxide, 73.
  - Ferric lactate, 165.

Various miscellaneous substances:
- Certain medicinal preparations, 201.
- Certain phosphorizing substances, 25.
- Flannel, 73, 203.
- Ink stains, 45, 184.
- Leather, 10, 203.
  - Tanned sheep leather, 45, 73, 184.
- Papers:
  - Certain kinds of, 25.
  - Filter papers, 203.
  - Glazed papers containing alum, 76.
- Soap, 37.

SUBSTANCES WHICH DO NOT REACT WITH GUAIACUM OR WITH THE OTHER CHROMOGENIC SUBSTANCES EMPLOYED IN HEMATOLOGIC INVESTIGATIONS EITHER ALONE OR WITH HYDROGEN PEROXIDE.

In the course of these investigations a number of substances have been found by various observers which do not show the guaiacum or similar reactions. These are as follows (the numbers refer to the bibliography at the end of this article):

Metallic compounds:
- Alumina, old and fresh, 73.
- Bismuth salts, 73.
- Chromium chloride, 73.
- Cobalt salts, 73.
- Cuprous chloride, 73.
- Iron—
  - Bog iron, 73.
  - Ferrous oxide, 73.
  - Ferric hydroxide, heated, 73.
  - Hematite, 73.
  - Rust, 7, 73, 110, 184.
  - Traces of iron, 192.
- Lead salts, 73.
- Manganese sulfate and carbonate, 73.
- Mercuric chloride and nitrate, 73.
- Nickel salts, 73.
- Palladium chloride, 73.
- Platinum chloride, 73.
- Zinc chloride, 73.

Substances of animal origin:
- Albumin, 25, 73, 105.
- Bile, 73, 105.
  - Coloring matter of bile, 203.
- Bilirubin, 29, 165.
- Blood serum, 73, 105, 165.
- Cochineal, 59, 73, 184.
- Clear exudate of ascites, 165.
- Fibrin, 73, 184.
- Gelatin, 25.
- Hematoidin, 29.

DELICACY OF THE CHEMICAL TESTS FOR BLOOD.

One of the principal advantages of the peroxide color tests for blood is their delicacy. Obviously this varies with the nature of the chromogenic substance employed and with the coloring power and stability of the colored product resulting from its oxidation. It may also depend

upon the nature of the oxidizing substance employed, and certainly to a great extent on the general technique of the operation and the skill and experience of the operator. In the following paragraphs I have summed up the principal facts concerning the delicacy of the chemical tests for blood with each of the several chromogenic substances which have been most extensively employed for this purpose.

GUAIACUM.

Aschern (8) was able to recognize 1 part of blood to 2,000; Adler (2), 1 part to 5,000; Bullard (31), 1 drop of blood in 6 ounces of water; Fahrner (52), 1 c. c. of blood to 20 c. c. of water; Huhnefeld (73), 1 part of blood to 6,000. According to this last-named author, the blood of even a flea spot which is very old can be recognized, and he was also able to prove the presence of hemoglobin in the 3-day-old embryo of the chick by means of his method of carrying out the guaiacum test. Breteau (25) observed that a solution of hemoglobin in tartaric acid so dilute as to be entirely colorless gave a blue color with guaiacum and old oil of turpentine, even after boiling; and Liman (105) was able to detect blood at a dilution of 1 to 40,000 by means of guaiacum. Schaer (140) describes the guaiacum test as remarkably delicate, and according to Siefert (170) a few milligrams of blood are sufficient for the test. Bertolet (18) has adapted the guaiacum test to the microscopic examination for blood, and Schuster (165) cites Vitali to the effect that blood gives the guaiacum test at the extraordinary dilution of 1 part to 100,000 million; this, if true, refers probably to a microscopic modification of the test. Schumm (161) was able to recognize blood by means of the guaiacum test at a dilution of 1 to 40,000 to 100,000, and in urine, 1 part of blood in 20,000 to 40,000 parts of urine.

Clement (37) was able to detect 1 drop of blood in 100 grams of water by means of guaiacum. Babcock (10) found the sensitiveness of the guaiacum reaction to be about 1 part of blood to 4,000. Jaworski and Korolewicz (75) detected 1 part of blood in 25,000 in feces, but failed to find it at a dilution of 1 to 100,000. Friedmann (58) detected 1 drop of blood in 100 c. c. of urine and 1 drop of blood in 500 c. c. of gastric contents; he detected 2 drops of blood in 50 grams of feces and 100 c. c. of water by filtering and testing the filtrate. In uncooked milk he detected 1 drop of blood in 430 c. c., and in cooked milk 2 drops in 100 c. c.; in pleuritic exudates, 3 drops of blood in 100 c. c. According to Fraenkel (57) 2.5 to 3 per cent of blood in feces can be recognized by Weber's test. He also obtained distinct reactions with blood at a dilution of 1 to 100,000.

ALOIN.

According to Buckmaster (29) aloin is less sensitive than guaiacum, but more stable. Schaer (141) has also found that the color produced with aloin is more stable than that obtained with guaiacum.

BENZIDIN.

According to Utz (192) benzidin is sharper than guaiacum, and Schumm (163, 164) describes benzidin as a delicate reagent. Adler (2) was able to detect blood at a dilution of 1 to 100,000 by means of benzidin, and Schumm (159) at a dilution of 1 to 200,000. I have also found benzidin to have nearly the same delicacy as phenolphthalin, especially as practically applied to the examination of animal fluids for blood, the difference between the two reagents being that phenolphthalin yields a more stable and permanent color (phenolphthalein) than benzidin. This has certain advantages.

Ascarelli (7) gives the delicacy of benzidin as a reagent for blood as 1 to 300,000, or in practice 1 to 250,000, and with benzidin paper, 1 part to 80,000; at a dilution of 1 to 120,000 the paper failed to give the test.

According to Schumm (163) the delicacy of the benzidin test for blood depends on the purity of the reagent. With 14 of Merck's preparations the limit of the reaction was about 1 part of blood to 200,000, and with two others about 1 part to 50,000. With two of Kahlbaum's preparations the limit of the reaction was about 1 part to 2,000,000 and with others about 1 part to 50,000.

THE LEUCO-BASE OF MALACHITE GREEN.

Adler (2) found this chromogenic substance to have about the same delicacy as benzidin, viz, 1 part of blood to 100,000. Czyhlarz and Von Fürth (43) also speak of it in the highest terms as a reagent for measuring the oxygen-carrying power of blood.

PHENOLPHTHALIN.

Utz (191) regards phenolphthalin as a more delicate and useful reagent than either guaiacum or aloin, and found that blood spots 2 square millimeters in area gave the reaction rapidly and with certainty. Deléarde and A. Benoit (16) found phenolphthalin to be more sensitive than guaiacum, and by means of it were able to detect blood at a dilution of 1 to 1,000,000. I have found it possible to recognize blood at a dilution of 1 part to 80,000,000 by means of phenolphthalin. (See page 30.)

THE VALUE OF THE GUAIACUM TEST AND SIMILAR REACTIONS.

It is evident from the foregoing that a considerable number of substances other than blood show the guaiacum reaction, or similar reactions with other chromogenic substances, either alone or in the presence of a peroxide. That such is the case has given rise to the most widely differing opinions as to the general utility of these tests in hematologic work and in forensic investigations. Thus, according to Alsberg (3) the guaiacum test for blood is unsatisfactory. According to Bell (15) the test is beautiful, but can not be relied on as positive proof of the presence of blood, and according to Breteau (25) the van Deen reaction should be handled with great circumspection. Woods (206) is of the opinion that the chief value of the guaiacum reaction is as a preliminary test, and Whitney (203) holds that the principal value of the guaiacum and aloin tests for blood is that if a specimen under examination gives no reaction with these substances then blood is absent. In other words, the principal value of such tests lies in the negative findings. On the other hand, according to Buckmaster (29), the guaiacum test, when properly carried out on boiled solutions, is a delicate reaction for blood, and according to Florence (54) also, it is a valuable reaction. Hemphill (67) also regards it as the most elegant and delicate of the chemical tests for blood. According to Jenne (76) the guaiacum and hematin tests are the most simple and generally employed. He calls attention to the well-known fallacies of the reaction, and recommends the proper control of the reaction by testing all of the reagents employed, and states that when these fallacies have been eliminated and the test cautiously applied and a blue color obtained the stain certainly contains blood. Fahrner (52) regards the test as specific for blood in spite of all objections which have been urged against it, and that so far as its practical significance is concerned, excluding certain possible errors, fresh tincture of guaiacum with old oil of turpentine is a reagent for blood. According to Weber (201) van Deen's reaction as applied to the acetic acid-ether extract of feces is a test of delicacy and reliability, and according to Schumm (160) with certain precautions the test is applicable to blood and is trustworthy. Maille, Mayet, Lefort, and Cornil (116) have arrived at the conclusion that the guaiacum test is always useful, especially if the findings are negative, and according to Palleske (123) if the guaiacum test fails it is absolutely certain that no blood can be present, whereas if it gives a positive result it indicates that blood is probably present, but the test must be confirmed. Siefert (170) is of the opinion that the guaiacum test enables one to arrive at a more than approximate conclusion, a negative result certainly indicating the absence of blood, and in case the substance under examination yields a blue

color with guaiacum, and iron and copper can be excluded, the probabilities are that it is blood. According to Klein (87) the guaiacum test is of great value, especially when it leads to a negative result. Liman (105) is also of the opinion that when a substance under examination gives a negative result with the van Deen test, it is not blood, but that a positive result does not prove the presence of blood without further investigation. Mecke and Wimmer (113) are also of the same opinion as Liman regarding the significance of this test. Marx (111) also calls attention to the negative aspect of the van Deen test as being the most valuable. According to Schaer (140) the guaiacum test is chiefly valuable as a control on the hematin test and as a control in the recognition of hemin crystals. Schwartz (167) agrees with Liman that if the result of the van Deen test is negative blood is absent, but that a positive result does not necessarily mean that blood is present. According to Foulis (56) the guaiacum test alone is not sufficient to prove the presence of blood in urine, but is useful if applied with other confirmatory tests. By others (4) it is looked upon as confirmative of the spectroscopic and hemin tests. Aschern (8) is of the opinion that the guaiacum test as applied to the examination of urine (Almen) is more delicate than the method of Bird (20) or of Struve (175); when the test is positive it should be confirmed with the microscope. According to Huhnefeld (73) one can conclude with certainty that if a spot or substance under examination does not show these color reactions it is not blood; a positive result, however, does not necessarily show blood, as a number of substances can give such a reaction. This author describes methods whereby such confusing substances can be gotten rid of. Schuster (165) has arrived at the conclusion that the chief distinguishing criterion for blood lies in the fact that the oxygen carrier present in blood is not destroyed by boiling. Siegel (171), from the results of his exhaustive studies on the detection of blood coloring matter in feces, concludes that if one obtains a negative guaiacum or benzidin test, it indicates that the substance contains no blood or at least only minimal traces; if the guaiacum test on feces is positive, it indicates the presence of blood originating in the organism, or at least very probably so, especially if meats and foodstuffs containing blood have been excluded from the diet, and provided further that the test has been carried out in an absolutely clean reagent glass. McNamara (110) says that the guaiacum test is not positive for blood, but is valuable if applied with other tests. According to Schumm (163) the benzidin test is of considerable practical significance. Lefort (100) comes to the conclusion that the guaiacum reaction is valuable if used with other corroborative tests. According to Babcock (10) the guaiacum reaction certainly indicates the absence of blood when negative, and that under the conditions which he enumerates it is reliable and certain.

Curtman (42) states that the guaiacum test is not sure for the presence of blood if positive, but that it is a favorite test with many authors. Gallaher (59) says that the guaiacum test is the best and surest, and that when the test is applied as directed and a blue color instantly obtained, the existence of blood in the liquid is demonstrated beyond a doubt. Ascarelli (7) finds the benzidin test to be more useful and sensitive than the guaiacum test. A negative test certainly means the absence of blood and even a positive test can be looked upon as undoubted evidence of the presence of blood. Einhorn (49) says finally:

> I do not hesitate to recommend benzidin paper as a useful method for the detection of blood in stomach contents, urine, and feces.

Deléarde and A. Benoit (16) regard phenolphthalin as a very delicate reagent for blood; on the other hand, Pozzi-Escot (128) regards it as altogether untrustworthy for the reason that similar reactions are obtained with a large number of substances.

The general consensus of opinion among those who have given this subject their attention would seem to be, therefore, that the guaiacum test for blood and similar color reactions are valuable especially if they lead to negative results, as proving beyond the peradventure of a doubt that blood is absent. On the other hand, if a positive test is obtained, care should be taken to exclude oxidases or peroxidases by boiling, and the salts of the heavy metals and other oxidizing agents by chemical methods, and, if possible, to subject the material under investigation to confirmative tests for blood before finally concluding that blood is present.

PHENOLPHTHALIN AS A REAGENT FOR BLOOD.

Phenolphthalin, or dioxy-triphenylmethane carbonic acid, is the leuco-compound of phenolphthalein, and on oxidation it is converted into the latter compound in the sense of the equation, $C_{20}H_{16}O_4 + O = C_{20}H_{14}O_4 + H_2O$. Pure phenolphthalin itself is colorless in alkaline solutions, but on adding alkali to the substance after oxidation, or if it be oxidized in the presence of alkali, there results the deep purple-red coloration characteristic of phenolphthalein in alkali.

Several years ago Kastle and Shedd (83) showed that a neutral solution of the sodium salt of phenolphthalin can be employed as a reagent for the oxidases. In 1903, Meyer (114) employed phenolphthalin as a reagent for the leucocytes. By means of this reagent he was able to determine not only the extent of the leucocytosis in certain pathological conditions, but also the character of the leucemia, and he proposed to employ it, instead of the leucocyte count, in arriving at the number of leucocytes in the blood. In the same year Utz (191) recommended a solution of phenolphthalin in sodium carbonate as a reagent for blood in forensic investigations, and

pointed out certain advantages which phenolphthalin possesses over guaiacum (van Deen's test) and aloin (Rossel's test). In 1906, Kastle and Amoss (80) employed an alkaline solution of phenolphthalin in measuring the oxidizing power of the blood in health and disease. They showed that both the oxidizing power of the blood and its peroxidase activity, viz. its power to induce the oxidation of phenolphthalin by hydrogen peroxide in alkaline solution, were directly proportional to its hemoglobin content. In 1908, Deléarde and A. Benoit (16) have also recommended an alkaline solution of phenolphthalin in conjunction with hydrogen peroxide as a reagent for blood, and have found it to be more sensitive than guaiacum or aloin. Still more recently, Pozzi-Escot (128) has made a few observations on the use of phenolphthalin as a reagent for blood. He criticises the work of Deléarde and A. Benoit (16) severely, and seems inclined to attach no particular value to the reagent as a test for blood for the reason that other substances, such as extract of malt, saliva, pus, etc., and a great number of the salts of the heavy metals, such as those of cobalt, manganese, iron, and lead, can accomplish the oxidation of phenolphthalin in the presence of hydrogen peroxide.

In attempting to repeat some of our earlier observations, considerable difficulty was experienced in the preparation of the peroxidase reagent, viz, an alkaline solution of phenolphthalin containing hydrogen peroxide. Similar difficulties have been experienced by other observers. (See Czyhlarz and von Fürth, 43.) It was therefore deemed expedient to make further studies along this line with the view of determining more exactly the most suitable means of preparing the reagent, and also with the view of determining more carefully than had hitherto been done the delicacy of the reagent and its applicability as a test for blood.

PREPARATION AND STABILITY OF THE REAGENTS.

In order to prepare phenolphthalin, phenolphthalein is dissolved in a considerable excess of 30 per cent sodium hydroxide solution and boiled with an excess of zinc dust until a few drops of the strongly alkaline liquid no longer give the color of phenolphthalein after neutralization with hydrochloric acid and the addition of sufficient alkali to give a slightly alkaline reaction. The solution is then decanted from the excess of zinc dust and the phenolphthalin precipitated by acidifying with hydrochloric acid. The substance is then collected on a filter and purified by several crystallizations from water and alcohol in the following manner: The phenolphthalin is dissolved in the smallest quantity of boiling alcohol in which it will dissolve, filtered if necessary, and cold water gradually added with constant stirring until the compound is precipitated out as a

white crystalline precipitate. From three to five crystallizations are carried out in precisely this manner, and in this way the phenolphthalin is finally obtained in the form of a white crystalline compound entirely free from all traces of phenolphthalein. It may then be dried in the air at ordinary temperature or on the hot plate or in the oven at temperatures ranging from 50° to 80° C. In handling and drying the compound it is necessary to keep it out of contact with all metallic surfaces and also to prevent access of dust and impurities from the laboratory. When first prepared, the compound is perfectly white. It dissolves in alkali to form solutions which are perfectly colorless and which remain colorless for some time even after the addition of small amounts of hydrogen peroxide. When preserved in glass-stoppered bottles, however, or even in sealed tubes, for some time, phenolphthalin gradually acquires a slight yellowish-pink color, and on dissolving in alkali, such specimens show the characteristic color of phenolphthalein in alkali, in greater or less intensity, depending on the extent of the oxidation of the compound by atmospheric air. If preserved in a glass-stoppered bottle in a dark closet the compound may be kept in good condition for a month or even longer; indeed I have prepared satisfactory reagents for blood testing from specimens of phenolphthalin which had been preserved in the dark in cork-stoppered specimen tubes for nine months. Such reagents, however, were never entirely colorless, although they were not sufficiently colored to interfere with their use in hematologic work. One of the great advantages of phenolphthalin in all work of this kind is that we know we are dealing with a perfectly definite compound the purity of which can be determined very simply and easily whenever desired, and which on oxidation passes into another equally definite compound the smallest amount of which can be readily detected by means of alkali, and which may be determined very accurately by colorimetric methods.

(1) *Alkaline phenolphthalin.*—This reagent is prepared by dissolving 0.032 gram of phenolphthalin in 21 c. c. of N/10 sodium hydroxide and adding sufficient water to make up the volume of the solution to 100 c. c. This reagent may also be prepared by bringing together 1 c. c. of N/10 sodium hydroxide with somewhat more phenolphthalin than will dissolve in this quantity of alkali, diluting with 10 to 20 c. c. of water, filtering, and adding to the filtrate 20 c. c. of N/10 sodium hydroxide and sufficient water to make up to 100 c. c. Since phenolphthalin itself is practically insoluble in water and since 1 c. c. of N/10 sodium hydroxide exactly neutralizes 0.032 gram of the compound, we obtain a solution by the latter mode of procedure of the same concentration as that prepared by the first method, no weighing being required.

When first prepared, the alkaline solution of phenolphthalin is perfectly colorless. On long standing, however, it gradually acquires a faint color, due to the oxidation of traces of the compound by atmospheric air. In no case, however, has this color proven of sufficient intensity to interfere with the use of the reagent in testing for blood, and if much work of this kind has to be done the solution would be used up long before it became unfit for use on account of this change. Utz (191) also found solutions of phenolphthalin in sodium carbonate to be stable.

(2) *Alkaline phenolphthalin containing hydrogen peroxide.*—The principal difficulty which has been encountered in the preparation of alkaline solutions of phenolphthalin containing small amounts of hydrogen peroxide is that it sometimes happens that the hydrogen peroxide alone oxidizes the alkaline solution of phenolphthalin, and, as already pointed out, it was this difficulty which caused me to undertake a reinvestigation of this subject in relation to the use of phenolphthalin in testing for blood. In our earlier work on the peroxidase activity of the blood in health and disease, however, Amoss and myself (80) had no special difficulty in preparing a colorless peroxidase reagent, but when I came to repeat some of this work later considerable difficulty was experienced in the preparation of a colorless peroxidase reagent. The cause of the trouble was found to be in the distilled water employed in the preparation of the reagent. With certain specimens of distilled water a pink to red coloration was produced in the reagent just as soon as the hydrogen peroxide was added. With other specimens of distilled water, however, the reagent remained colorless after the addition of the hydrogen peroxide. The spontaneous oxidation of certain solutions of alkaline phenolphthalin containing hydrogen peroxide is probably due to the presence of very small amounts of copper. Bourquelot and Bougault (23) have pointed out, for example, that it is not rare to encounter distilled water which blues guaiacum at 50° C. Similarly, Carnelly (35) found distilled water to dissolve small amounts of metallic copper, and in different samples of distilled water Barth (Nessler, 119) found amounts of copper varying from none to 0.015 gram of copper acetate per liter of water. Ascarelli (7) found copper sulphate to react with benzidin at a dilution of 1 to 1,000,000.

It therefore occurred to me that this difficulty could be overcome by redistilling the water used in the preparation of the reagent, in glass vessels. This was tried and found to give good results. As a matter of fact, since I began using water which had been once redistilled in glass vessels no difficulty has been experienced in the preparation of colorless alkaline solutions of phenolphthalin containing hydrogen peroxide in sufficient amount to accomplish its oxidation under proper conditions.

These solutions were prepared as follows: 0.032 gram of phenolphthalin was dissolved in 21 c. c. of N/10 sodium hydroxide solution and diluted with redistilled water to nearly 100 c. c. 0.1 c. c. of M/1 hydrogen peroxide was then added and the solution made up to exactly 100 c. c. with redistilled water. Or, phenolphthalin was mixed in slight excess with 1 c. c. of N/10 sodium hydroxide solution and a small quantity of redistilled water, and after shaking thoroughly was filtered and to the filtrate 20 c. c. of N/10 sodium hydroxide were then added, the solution made up to nearly 100 c. c. with redistilled water, 0.1 c. c. of M/1 hydrogen peroxide added, and the solution made up to exactly 100 c. c. with redistilled water. By this latter method weighings are obviated. It has been found further that for most blood work with this reagent it is sufficiently accurate to employ 0.1 c. c. of the 3 per cent commercial hydrogen peroxide solutions. It is scarcely necessary to state in this connection that only the purest forms of sodium hydroxide should be employed in a preparation of the N/10 sodium hydroxide used in making up the reagent, and also that redistilled water be used in the preparation of such solutions of sodium hydroxide.

By closely following the above directions it has been found possible to prepare solutions of phenolphthalin in alkali containing the quantity of hydrogen peroxide required for the oxidation of the phenolphthalin, which were without the slightest trace of pink color at the time of their preparation, and which, when preserved in glass-stoppered bottles in a dark closet at ordinary temperature, showed only a faint trace of color, not measurable colorimetrically in the quantities of the solution employed in our observations, after forty-eight hours. In this way I have been able to overcome the difficulty encountered by Czyhlarz and Von Fürth (43) in their attempt to measure the peroxidase activity of the blood by means of phenolphthalin.

THE CONDUCT OF BLOOD TOWARD ALKALINE PHENOLPHTHALIN ALONE AND IN THE PRESENCE OF HYDROGEN PEROXIDE.

It has been observed by Meyer (114) and Utz (191) and independently by Kastle (80) that phenolphthalin in alkaline solution is oxidized by blood to phenolphthalein, and that the oxidation is greatly accelerated by hydrogen peroxide. Kastle and Amoss (80) found, further, that within certain limits the quantity of phenolphthalin oxidized by a given amount of blood and hydrogen peroxide is nearly proportional to the concentration of the sodium hydroxide. As to the precise function of the alkali in such oxidations nothing definite can be said at present. It should be borne in mind, however, that phenolphthalin contains two phenolic groups in its molecule, and that therefore, as is the case with other phenols, its oxidation is greatly facilitated by an excess of alkali. The fact that it is

oxidized so rapidly in alkaline solution by hydrogen peroxide under the influence of blood as a carrier, and that it is also oxidized in alkaline solution by blood alone, but more slowly, would seem to indicate either that in the alkali itself we have traces of hydrogen peroxide or that the blood pigment can function both as a peroxide and as an oxygen carrier. In support of the latter view it is worthy of note that the oxidation of phenolphthalin by blood occurs in solutions of N/10 sodium hydroxide which have been prepared by the action of sodium amalgam upon water and in which it seems fair to assume that no peroxide compounds would be present; and in no case did the N/10 solution of sodium hydroxide employed in this and the other investigations give any test for hydrogen peroxide with the titanic acid reagent.

In alkaline solutions of phenolphthalin containing hydrogen peroxide the principal function of the blood seems to be that of an oxygen carrier, similar in its action to finely divided platinum.

DELICACY OF THE PHENOLPHTHALIN BLOOD TEST.

As already pointed out, Utz (191) regards the phenolphthalin-hydrogen peroxide reagent as a more delicate reagent than either guaiacum (van Deen) or aloin (Rossel). Quite recently Deléarde and A. Benoit (16) have been able to recognize one part of blood diluted with 1,000,000 parts of water by means of Meyer's reagent and hydrogen peroxide. In my opinion, the delicacy of the phenolphthalin-hydrogen peroxide test is even greater than that observed by the latter authors, as may be seen from the following observations:

Fresh solutions of human blood were prepared of the following concentrations:

(1) 0.0038 gram of blood was dissolved in distilled water and made up to 100 c. c.
(2) 10 c. c. of solution (1) were made up to 100 c. c. with distilled water.
(3) 10 c. c. of solution (2) were made up to 100 c. c. with distilled water.
(4) 10 c. c. of solution (3) were made up to 100 c. c. with distilled water.

Hence, 1 c. c. of solutions (1), (2), (3), and (4) contained the following quantities of blood, respectively:

Solution.	Quantity of blood in 1 c. c., gram.
Number (1).....	0.000038
Number (2).....	.0000038
Number (3).....	.00000038
Number (4).....	.000000038

Tubes were prepared containing 1 c. c. of each of these solutions, and to each tube there was then added 2 c. c. of the reagent, and a control tube prepared containing 1 c. c. of distilled water and 2 c. c. of the reagent. These tubes were allowed to stand five minutes at room temperature, and at the end of this time the tubes showed the following colors:

Tube.	Quantity of blood. gram.	Color.
Control.....	None.	Trace of pink.
Number (1).....	0.000038	Deep purplish red.
Number (2).....	.0000038	Purplish red.
Number (3).....	.00000038	Pink.
Number (4).....	.000000038	Light pink.

The color shown by (3) was much deeper than that shown by the control tube, and while the color shown by tube (4) was only light pink it was decidedly more colored than the control, the difference in color being recognized independently by two persons. The colors of these solutions were then compared in the Duboscq colorimeter with a standard solution of phenolphthalein containing 0.000318 gram of phenolphthalein in 1 c. c. alcohol, 0.42 c. c. N/10 sodium hydroxide, and 1.58 c. c. water, when the following readings were obtained in divisions on the scale of the colorimeter, the test in all cases being put at 5 divisions on the scale:

Test.	Reading.	Standard.
Control.....	5	Not measurable.
Number (1).....	5	6.0.
Number (2).....	5	1.2.
Number (3).....	5	0.2.
Number (4).....	5	Not measurable.

It is evident from these results that, as actually measured in the colorimeter, we are able to detect the oxidizing power of 0.00000038 gram of blood acting at a total dilution of 3 c. c., or approximately 1 part of blood to 8,000,000 parts of water; and that by direct comparison of the colors produced, with the unaided eye we can detect the oxidizing effect of even one-tenth of this amount, viz, 0.000000038 gram at a total dilution of 3 c. c., or approximately 1 part of blood in 80,000,000 parts of water. The third dilution, or 1 part of blood to 8,000,000, closely corresponds to the minimum amount of blood found by Deléarde and A. Benoit (16) to produce a recognizable color with the reagent, provided that we take into account the total volume of fluid of all the reacting substances.

In order to test the conduct of these blood solutions towards an alkaline solution of phenolphthalin containing no hydrogen peroxide, tubes were prepared containing 2 c. c. of the alkaline phenolphthalin solution and 1 c. c. of blood solutions Nos. (1), (2), (3), and (4), and also a control containing 2 c. c. of alkaline phenolphthalin and 1 c. c. of distilled water. These were allowed to stand for eighteen hours at ordinary temperature; at the end of this time the tubes had developed colors as follows:

Test.	Quantity of blood, gram.	Color.
Control	None.	Trace of pink.
Number (1)	0.000038	Deep purplish red.
Number (2)	.0000038	Deep purplish pink.
Number (3)	.00000038	Light pink.
Number (4)	.000000038	Trace of pink.

No difference in color could be made out between the control and No. (4). Hence so far as the oxidation of alkaline phenolphthalin can be accomplished by blood alone without hydrogen peroxide, 0.00000038 gram of blood at a dilution of 3 c. c., or approximately 1 part of blood in 8,000,000, is about the limit of the reaction.

THE QUANTITY OF PHENOLPHTHALIN OXIDIZED UNDER THE INFLUENCE OF BLOOD IS PROPORTIONAL TO THE QUANTITY OF BLOOD PRESENT.

It was shown by Kastlė and Amoss (80) that the oxidizing power of the blood of different individuals is proportional to the hemoglobin content. It seemed of interest, therefore, to determine in this connection the oxidizing power of the blood of the same individual at different concentrations. Accordingly 3.5 milligrams of blood from one individual was dissolved in 100 c. c. of water. Five c. c. of this solution were made up to 10 c. c. with distilled water, 10 c. c. were made up to 50 c. c., 10 c. c. were made up to 100 c. c. These solutions, therefore, had the relative concentrations of 1.0, 0.5, 0.2, and 0.1, respectively. The oxygen-carrying power of these solutions was then tested toward the alkaline phenolphthalin-hydrogen peroxide reagent, using 2 c. c. of the reagent and 1 c. c. of each of the blood solutions. The four tubes containing these mixtures were allowed to stand one hour at ordinary temperature, at the end of which time they were compared as to color in the Duboscq colorimeter with the following results:

Concentration	1.0	0.5	0.2	0.1
Scale reading	1.0	2.0	4.6	9.6

The oxidizing power of these solutions was also tested toward alkaline phenolphthalin, using 2 c. c. of the reagent with 1 c. c. of

each of the blood solutions. The tubes containing these substances were allowed to stand twenty-four hours at ordinary temperature, at the end of which time they were compared in the Duboscq colorimeter with a fresh standard of phenolphthalein in alkali containing 0.0318 gram of phenolphthalein in 21 c. c. of N/10 sodium hydroxide and sufficient water to bring the total volume of the solution up to 100 c. c. The following are the results of these comparisons:

Concentration	1.0	0.5	0.2	0.1
Scale reading	.5	1.1	2.3	5.2

The standard was set at 0.2 division on the scale of the colorimeter.

It is evident from these results that the oxygen-carrying power of blood towards an alkaline solution of phenolphthalin containing hydrogen peroxide and its oxidizing power toward alkaline phenolphthalin alone, is directly proportional to the concentration of the blood.

In my opinion, two things are necessary for the more exact determination of hemoglobin than is possible with the instruments now in use for this purpose; first, to weigh the blood used in the determination instead of attempting to measure such a viscous liquid in pipettes of such narrow caliber as are ordinarily employed in hematologic work, and, second, to use a colorimetric method depending on the oxidation of phenolphthalin by blood, whereby a coloring matter of great intensity of color is obtained. I hope, therefore, at some future time to work out the details of a method for the exact determination of hemoglobin based on these principles.

ON THE EFFECT OF VARIOUS ANIMAL TISSUES ON THE OXIDATION OF ALKALINE PHENOLPHTHALIN BY BLOOD ALONE AND IN THE PRESENCE OF HYDROGEN PEROXIDE.

It soon became apparent in the course of this investigation that the oxidation of phenolphthalin in alkaline solution by blood alone and in the presence of hydrogen peroxide was considerably retarded by extracts of various animal tissues. In order to determine to what extent this occurred, the following experiments were carried out: A guinea pig was anesthetized and killed by bleeding, and a solution of the pig's blood was prepared by dissolving 0.0225 gram of the blood in 250 c. c. of water. This was labeled solution No. (1). After the death of the animal the following tissues were removed and labeled as indicated: Liver, (2); bone marrow, (3); pancreas, (4); spleen, (5); muscle, (6); lung, (7); suprarenals, (8); brain, (9). One-tenth gram of each of these tissues was rubbed up in a mortar with 10 c. c. of blood solution No. (1) and the several solutions numbered to correspond with the numbers of the tissues used. These solutions were then compared in oxidizing power with the blood solution towards the alkaline phenolphthalin containing hydrogen

peroxide, using 2 c. c. of the reagent and 0.1 c. c. of the solution of blood or of blood and tissue. After a short interval all of these tissues showed a retarding influence on the oxidation. Arranged in order of the amount of phenolphthalein formed as shown by the color of the tubes, these solutions stood in the following order:

Blood.
Liver = Bone marrow = Lung = Suprarenals.
Spleen.
Pancreas.
Brain = Muscle.

The greatest retardation was shown by the muscle and brain. At the end of fifteen minutes the tube containing blood alone was deep purplish red, whereas tubes (6) and (9) were only faint pink. When compared in the colorimeter with standard phenolphthalein, these three tubes gave the following readings:

[Dec. 17, 1908.]

Tube.	Contents.	Colorimeter scale readings.	
		Test.	Standard.
Number (1).....	Blood.....	5	2.0
Number (6).....	Blood and muscle.....	5	.2
Number (9).....	Blood and brain.....	5	.4

On standing one hour solutions (3), (5), and (7) showed even a greater coloration than (1). It should be borne in mind, however, that these three tissues, bone marrow, spleen, and lung, are highly vascular, and hence solutions (3), (5), and (7) probably actually contained somewhat more blood than solution (1).

The conduct of these solutions was also tested towards alkaline phenolphthalin alone, using 2 c. c. of the reagent and 1 c. c. of each of the solutions. These tests were allowed to stand twenty-four hours at ordinary temperature, when the colors were compared with the phenolphthalein standard, with the following results:

Tube.	Contents.	Colorimeter scale readings.	
		Test.	Standard.
Number (1).....	Blood.....	5	3.4
Number (2).....	Blood and liver.....	5	1.4
Number (3).....	Blood and marrow.....	5	1.7
Number (4).....	Blood and pancreas.....	5	.7
Number (5).....	Blood and spleen.....	5	2.0
Number (6).....	Blood and muscle.....	5	.1
Number (7).....	Blood and lung.....	5	1.7
Number (8).....	Blood and suprarenals....	5	(?)4.2
Number (9).....	Blood and brain.....	5	.4

Here again we note a marked retarding effect on the oxidizing power of blood towards alkaline phenolphthalin on the part of all the tissues, with the possible exception of the suprarenals, and in this case the solution had become turbid and hence difficult to read in the colorimeter.

The solutions of blood and of blood containing the several tissues were kept on ice for twenty-four hours, at the end of which time they were again tested towards alkaline phenolphthalin solution, using 2 c. c. of the reagent and 1 c. c. of the several solutions. The tests were allowed to stand twenty-four hours at ordinary temperature, at the end of which time they were compared in the colorimeter with the phenolphthalein standard with the following results:

[Dec. 18, 1908.]

Tube.	Contents	Colorimeter scale readings.	
		Test.	Standard.
Number (1)	Blood	5	1.8
Number (2)	Blood and liver	5	1.3
Number (3)	Blood and marrow	5	3.0
Number (4)	Blood and pancreas	5	3.0
Number (5)	Blood and spleen	5	1.8
Number (6)	Blood and muscle	5	.1
Number (7)	Blood and lung	5	2.0
Number (8)	Blood and suprarenals	5	2.0
Number (9)	Blood and brain	5	.3
Control	Water	Not measurable.	

Comparing these results with those obtained with the same solution on December 17, 1908, we note a considerable falling off in oxidizing power in the blood alone (1). Solutions (2), (5), (6), (7), and (9) show about the same activity on the two dates, and solutions (3) and (4) show a considerable increase in activity. It is also interesting to note that whereas when first mixed with blood both bone marrow and pancreas retard the oxidizing power of blood towards alkaline phenolphthalin alone, they seem to accelerate its action after twenty-four hours, or possibly they prevent, in some way, its decomposition by water. The great retarding effect of muscle (6) and brain (9) tissues is still seen with solutions of blood and these tissues that have stood twenty-four hours. As to the precise cause of these remarkable effects, nothing definite can be said at present. It is known, however, that most of the fresh animal tissues possess considerable reducing power, and it is conceivable that these have a greater affinity for oxygen than phenolphthalin itself.

TESTING FOR BLOOD IN PATHOLOGICAL FLUIDS AND SECRETIONS.

The fact that animal tissues in many cases retard the oxidation of phenolphthalin under the influence of blood indicates that the testing for blood in various animal tissues and secretions under normal and pathological conditions is altogether a different matter from testing for blood in pure aqueous solutions, and that the delicacy of such tests is very likely to be interfered with. As a matter of fact, strictly chemical tests for blood such as the guaiacum test have been more extensively used for the recognition of blood stains than in the examination of body fluids for blood. (See Wittstein, 204, and also Schuster, 165.) Our attention was first directed to this phase of the subject through the study of the peroxidase reaction of certain normal and pathologic urines. In the presence of hydrogen peroxide and phenolphthalin certain urines containing blood gradually developed a deep blood-red color, after the addition of N/10 sodium hydroxide, due to the production of phenolphthalein, whereas other urines known to contain blood failed entirely to show this change of color. Evidently, therefore, certain urines contain substances which retard the oxidation of phenolphthalin by blood and hydrogen peroxide. Similar difficulties have been encountered by other observers, and in no instance has a method of blood testing been devised which will recognize in urine, feces, gastric contents, etc., as small an amount of blood as can be detected in water.

THE ADSORPTION OF BLOOD PIGMENTS BY VARIOUS COLLOIDAL AND FINELY DIVIDED SUBSTANCES.

The attempt has been made to overcome the difficulty in testing for blood in pathologic fluids and secretions referred to above by treating the urine or other fluid with a substance which would absorb the blood pigment and leave the interfering substances in solution.

The earliest observations in this field were made by Rose (134), who found that such substances as aluminium hydroxide, ferric hydroxide, etc., have the power of adsorbing blood coloring matters from their aqueous solutions. These observations have been confirmed by Fleming (53). Later Struve (177) made use of tannin for this purpose, and other observers, among them Dragendorf (48), Schwartz (167), Berg (17), have employed zinc acetate for this purpose, and still others, among them Bird (20), and Klein (87), have used a mixture of tannin and zinc acetate. Heller has also proposed a test for blood in urine which depends upon the fact that the earthy phosphates of urine precipitated on warming with alkali have the power of absorbing any blood-coloring matter which the urine may contain, in consequence of which the precipitate takes on a pink or

rose red color depending on the amount of blood present. Arnold (6) has adapted Heller's method to the examination of blood spots by dissolving the spot in an aqueous solution of caustic potash, adding urine, and heating the mixture. Similarly Parisot (cited by Le Canu, 99) adopted the plan of heating the urine, when if blood is present it is found in the coagulum.

BLOOD IN URINE.

Obviously Heller's test in its original form is more or less crude unless further controlled by some such test as the phenolphthalin reaction, for the reason that as compared with the quantities of blood which can be recognized by phenolphthalin the quantities of blood required to impart a visible coloration to such a precipitate are relatively large.

In connection with the phenolphthalin test we have employed the adsorption and concentration of the blood pigments from urines containing blood with good results, as may be seen from the following: 0.0026 gram of blood was dissolved in 100 c. c. of the urine of the same individual from whom the blood was obtained and labeled (1). Some of the original urine was reserved for comparison and labeled (2). One c. c. of urines (1) and (2) were mixed with 2 c. c. of redistilled water and two drops of thick alumina cream, and by way of further comparison a third tube was prepared containing 3 c. c. of redistilled water and two drops of alumina cream; this was labeled (3). The tubes containing these mixtures were then shaken and filtered through small filters and the residues washed with small amounts of distilled water. Very small amounts of the residues thus obtained were added to 2 c. c. of the phenolphthalin-hydrogen peroxide reagent and allowed to stand five minutes at ordinary temperature, at the end of which time the solutions showed the following colors:

(1) Reddish purple.
(2) Faint pink.
(3) Faint pink.

It is evident, therefore, that by this procedure we were able to recognize 0.000026 gram of blood at a total dilution of 3 c. c., or approximately 8 parts of blood per 1,000,000. As a matter of fact we really recognized a considerably smaller quantity than this for the reason that only a small amount of the aluminium hydroxide residue containing the blood was employed in making the test. Or looked at in another way, we have been able to recognize definitely the oxidizing effect of 26 parts of blood in 1,000,000 parts of urine. This, I think, is a much smaller amount than could be recognized with certainty by spectroscopic or microscopic tests, or indeed by

any method, unless it be by the precipitin or other biologic tests. These experiments were repeated on other samples of urine containing blood with like results.

BLOOD IN SALIVA.

Similar results have been obtained with saliva, as may be seen from the following: 0.0036 gram of human blood was dissolved in 100 c. c. of fresh saliva, and the solution labeled (1); some of the same saliva was kept for comparison and labeled (2). One c. c. of solutions (1) and (2) were mixed with 2 c. c. of water and three drops of alumina cream. The mixtures were then thoroughly shaken and filtered, and the residues washed with redistilled water. Small amounts of these residues were added to 2 c. c. of the phenolphthalin-hydrogen peroxide reagent. After standing a few minutes at ordinary temperature, solution (1) became deep purplish red, whereas (2) showed only a faint pink coloration. Small amounts of the two residues were then added to alkaline phenolphthalin containing no hydrogen peroxide. At the end of five hours solution (1) had become pink, whereas (2) remained colorless. At the end of twenty-four hours (1) was dark purplish red, while (2) was faint pink. The unfiltered and filtered salivas were also tested for blood directly by means of alkaline phenolphthalin and hydrogen peroxide, and also by means of alkaline phenolphthalin alone, without previous treatment with aluminium hydroxide. With the unfiltered salivas, 1 c. c. of (1) with 2 c. c. of the alkaline phenolphthalin-hydrogen peroxide reagent developed a deep pink color on standing twenty minutes, whereas (2) showed only a faint trace of pink under the same conditions, and the filtered solutions gave similar results. The two salivas, filtered and unfiltered, were also tested with alkaline phenolphthalin alone, using 1 c. c. of the saliva and 2 c. c. of the alkaline solution of phenolphthalin. Solution (1) showed a light pink color after twenty-four hours, while (2) showed only a trace of pink.

It is possible, therefore, to recognize 36 parts of blood in 1,000,000 parts of saliva by direct test with the phenolphthalin reagent, but much more satisfactory results are obtained by previous treatment with aluminium hydroxide.

ADSORPTIVE POWER OF SOIL FOR BLOOD.

In view of the highly adsorptive power of aluminium and ferric hydroxides for blood, it occurred to me that possibly soil would also exhibit adsorptive power towards the blood pigments. In order to test the matter, 0.0026 gram of human blood was dissolved in 25 c. c. of water and 1 gram of soil added. This mixture was well shaken and a portion of it filtered. Both the filtrate and the residue showed the presence of blood when tested with the alkaline phenolphthalin-

hydrogen peroxide reagent. The residue gave much the stronger test, however, indicating some adsorption of blood. Some of the mixture was then boiled and filtered. One c. c. of the filtrate with 2 c. c. of the alkaline phenolphthalin-hydrogen peroxide reagent gave only a faint pink color after standing, whereas a very small amount of the residue gave with 2 c. c. of the reagent a deep purplish red color after standing a few minutes. Hence the adsorptive power of soil for blood is greatly augmented by boiling. A control test was carried out using 25 c. c. of water and 1 gram of the same sample of soil, but no blood. Neither the filtrate nor the residue, before or after boiling, gave a color with the alkaline phenolphthalin-hydrogen peroxide reagent.

Hence by means of the alkaline phenolphthalin-hydrogen peroxide reagent we were able to detect 1 part of blood in 9,600 parts of water containing 1 gram of soil, and from such a solution the blood is completely adsorbed by the soil on boiling. In testing soil for blood, therefore, it is advantageous to boil the soil with twenty to thirty times its volume of water, filter, and test the conduct of the residue toward the alkaline phenolphthalin-hydrogen peroxide reagent. Should such a test turn out to be negative, it is reasonably certain that no blood is present in the soil, or if any, not more than traces. Should a positive test be obtained under these conditions, it is reasonably certain that blood is present, and this should be confirmed by other tests.

BLOOD IN MILK.

In certain diseased conditions it becomes a matter of some importance to be able to recognize small amounts of blood in milk. (See "The Significance of Leucocytes and Streptococci in Milk," by William Whitfield Miller, Article No. 13, Bulletin No. 41, Hygienic Laboratory; "Milk and Its Relation to the Public Health".)

Some preliminary observations were made on cow's milk containing moderately large amounts of mouse's blood. Such milk, raw and after boiling, gave excellent tests for blood with Adler's benzidin test (2); indeed, this reagent seems to be admirably suited to the recognition of blood in milk. On the other hand, with phenolphthalin the color developed much more slowly than with aqueous solutions of blood, indicating the presence of substances which, like animal tissues generally, greatly retard the oxidation.

With milk the effect of acid coagulation was studied with the view of determining the distribution of the blood between the curd and the whey and the possibility of the adsorption of the blood pigment by casein. In order to determine the effect of acid coagulation, 2 c. c. of the fresh milk containing mouse's blood was coagulated by the addition of two or three drops of dilute acetic acid. The curdled

mass was then filtered and the curd washed with distilled water. The whey thus obtained had a golden-yellow color and gave, with benzidin and also with alkaline phenolphthalin containing hydrogen peroxide, excellent tests for blood. On the other hand, the curd was practically white and showed but little blood by the benzidin and phenolphthalin tests. With benzidin the curd showed only a faint blue color; with alkaline phenolphthalin containing hydrogen peroxide a little of the curd gave a deep pink color after standing for some time.

A specimen of fresh cow's milk, labeled (1), and another specimen of the same containing mouse's blood, labeled (2), were kept at ordinary temperature for six days. Both specimens had curdled and both were filtered. The serum of (1) was found to be clear and almost colorless; the serum of (2) was clear and salmon pink in color. One drop of each filtrate was then added to 2 c. c. of the alkaline phenolphthalin-hydrogen peroxide reagent; (1) remained colorless, whereas (2) became pink immediately and ultimately deep purplish red.

The residues left after filtering these two specimens of milk were washed with small amounts of distilled water and tested with the alkaline phenolphthalin-hydrogen peroxide reagent: milk (1) remained colorless, and (2) gave a pink color after long standing.

It is evident from these results that on acid coagulation the blood contained in cow's milk passes almost entirely into the whey, and that the casein possesses but little if any adsorptive power for the blood pigments, the small amounts of blood present in the casein probably not being adsorbed but merely held in mechanical suspension by the casein.

Further tests for blood in milk have been carried out on human milk containing known amounts of human blood. Solutions of human blood in human milk were prepared as follows:

(1) 0.0024 gram of blood was dissolved in 1 c. c. of water; to this solution were added 5 c. c. of human milk *B*.
(2) 0.0051 gram of blood was dissolved in 1 c. c. of water; to this solution were added 5 c. c. of human milk *B*.
(3) 1 c. c. of solution (1) was made up to 10 c. c. with human milk *B*.
(4) Consisted of human milk *B* alone.
(5) Consisted of a second specimen of human milk, *D*, alone.

These solutions were then tested towards the alkaline phenolphthalin-hydrogen peroxide reagent, using 2 drops of the milk and 2 c. c. of the reagent. After standing three minutes the solutions showed the following colors:

(1) Red.
(2) Deep purplish red.
(3) Pink.
(4) Trace of pink.
(5) Trace of pink.

Similar tests were made on these specimens of milk after boiling, using the same quantities of milk and of the reagent as were employed in testing the raw specimens. At the end of three minutes the solutions showed the following colors:

(1) Red.
(2) Deep red.
(3) Pink.
(4) Trace of pink.
(5) Trace of pink.

The conduct of these milks, raw and boiled, was also tested towards alkaline phenolphthalin alone, using 1 drop of each specimen of milk and 2 c. c. of the reagent. After standing all night at the ordinary temperature the solutions showed the following colors:

	Raw.	Boiled.
(1)	Purplish red.	Purplish red.
(2)	Deep purplish red.	Deep purplish red.
(3)	Pink.	Pink.
(4)	Trace of pink.	Trace of pink.
(5)	Trace of pink.	Trace of pink.

Milk (3) was distinctly darker in color than (4) and (5). The colors of (1) and (2) were compared in the Duboscq colorimeter with the following results:

	Readings on colorimeter scale.	
	Raw.	Boiled.
(1)	4.0	4.0
(2)	2.0	1.8

These results signify, of course, that approximately twice as much phenolphthalein had been produced in (2) as in (1), and in this connection it is interesting to note that (2) contained approximately twice as much blood as (1) in the original solution, viz:

(1) 0.0024 gram.
(2) 0.0051 gram.

Hence by means of phenolphthalin we are not only able to detect very small amounts of blood in milk, but also to determine the relative amounts thereof in several samples by colorimetric comparison. These 5 specimens of human milk, raw and boiled, were also tested for blood by the benzidin test, using 1 drop of milk, 0.5 c. c. of distilled water, 3 drops of acetic acid, 0.5 c. c. of benzidin solution, and 0.5 c. c. of a 3 per cent solution of hydrogen peroxide, with the following results:

	Raw.	Boiled.
(1)	Light bluish green.	Decided blue.
(2)	Deep indigo blue.	Deep indigo blue.
(3)	Colorless.	Colorless.
(4)	Colorless.	Colorless.
(5)	Colorless.	Colorless.

On milk (1) a somewhat better test for blood was obtained with the benzidin test on the boiled specimen. As a test for blood in milk, phenolphthalin has been found to be a somewhat more delicate reagent than benzidin. It has the further advantage that the color produced is reasonably stable in alkaline solution, whereas the color produced with the benzidin reagent fades rapidly and slight changes of color are therefore apt to be overlooked.

It is also evident from our results that we can detect 1 part of blood in 25,000 parts of milk when only 1 drop of the milk is used in making the test, and by making use of aluminum hydroxide with the whey of milk that has been curdled by acid it is more than probable that still smaller quantities of blood could be recognized in milk. As it is, however, either the phenolphthalin or the benzidin test is sufficiently delicate to enable us to detect much smaller quantities of blood than would ever be likely to occur in the milk in diseased conditions of the mammary glands, and by means of phenolphthalin the amounts of blood in the milk in such diseases could be followed quantitatively from day to day.

These five samples of human milk were kept in ordinary cork-stoppered bottles from January 30 to April 5, 1909, when they were again tested with the alkaline phenolphthalin-hydrogen peroxide reagent, using 2 drops of the milk and 2 c. c. of the reagent. After standing three minutes the solutions showed the following colors:

(1) Red.
(2) Deep purplish red.
(3) Pink.
(4) Trace of pink.
(5) Trace of pink.

It is evident, therefore, that the blood in these specimens had persisted unchanged for over two months, despite the fact that no efforts had been made to preserve the milk from the ordinary bacterial changes which occur in this fluid.

BLOOD IN GASTRIC CONTENTS.

0.0057 gram of human blood was dissolved in 10 c. c. of fresh gastric contents, and the solution labeled (1); a portion of the original contents was kept for comparison and labeled (2). Both specimens were allowed to stand until the solid contents had settled. Two drops of each of the specimens were then added to 2 c. c. of the alkaline phenolphthalin-hydrogen peroxide reagent. Solution (1) gave a deep purplish red color after standing a few minutes, whereas (2) showed only a faint trace of pink. Even better tests with phenolphthalin were obtained after boiling the two specimens of gastric contents. Solution (1) gave the benzidin test both raw and after boiling, whereas (2) gave no reaction. Hence in 0.1 c. c. portions

of stomach contents we were able to detect 1 part of blood in 1,750 parts of the stomach contents. In ordinary clinical work it is not likely that any attention would be paid to quantities of blood smaller than this, and hence no attempts have been made to recognize smaller quantities. I have no doubt, however, that by the use of aluminium hydroxide and the phenolphthalin reagent, much smaller quantities of blood could be detected in gastric contents.

Reference has been made to a recent communication by Pozzi-Escot (128) on the use of phenolphthalin as a reagent for blood, in which he severely criticises the work of Deléarde and A. Benoit (16) on this subject, and seems inclined to doubt the usefulness of this substance both as a test for blood and as a reagent for the oxidases. As a matter of fact, however, since this compound was first proposed by Kastle and Shedd (83) as a reagent for the oxidases it has given excellent results, both in the hands of Kastle and other chemists, and has proven especially valuable in recent investigations on the peroxidases. In my opinion, therefore, these adverse criticisms of this reagent on the part of Pozzi-Escot, for the work for which it has thus far been employed, are absolutely unjustifiable in the light of the facts, and it is a subject which can not be dismissed on the basis of a few faulty observations. As a matter of fact, phenolphthalin is not only a beautiful reagent for the oxidases and peroxidases, but it lends itself admirably to quantitative investigations in this field, since the product of the oxidation—phenolphthalein—is of perfectly definite composition and can be determined colorimetrically with great accuracy. This can certainly not be said of a great many substances which have been used in work of this kind. Further, that it is a valuable reagent for blood can scarcely be doubted. According to Pozzi-Escot (128) an alkaline solution of phenolphthalin containing hydrogen peroxide is valueless as a reagent for blood for the reason that it yields phenolphthalein with the following substances besides blood, viz, extract of malt, saliva, ash of blood, pus, the greater number of organic secretions, plant extracts, certain urines absolutely free from blood, and a great number of metalic salts, such as those of cobalt, manganese, iron, lead, etc. These observations do not agree with our own experience for at least the greater number of these substances. In order to determine to precisely what extent his criticisms are justified, however, I have recently tested the conduct of saliva, extract of malt, potato peel, horseradish, human blood, and copper sulfate towards the following phenolphthalin reagents:

1. The oxidase reagent, containing 0.032 gram of phenolphthalin, with 1 c. c. N/10 sodium hydroxide, made up with distilled water to 100 c. c.
2. Alkaline phenolphthalin, containing 0.032 gram of phenolphthalin, with 21 c. c. of N/10 sodium hydroxide, made up with distilled water to 100 c. c.

3. The peroxidase reagent, containing 0.032 gram of phenolphthalin, with 1 c. c. of N/10 sodium hydroxide and 0.1 c. c. of 3 per cent hydrogen peroxide.
4. The alkaline phenolphthalin—hydrogen peroxide reagent, containing 0.032 gram of phenolphthalin, 21 c. c. of N/10 sodium hydroxide, and 0.1 c. c. of 3 per cent hydrogen peroxide, made up with distilled water to 100 c. c.

Tubes were prepared containing 2 c. c. of each of the above reagents and 1 c. c. of the solution to be tested or of distilled water. These were allowed to stand for one hour at ordinary temperature, at the end of which time 0.2 c. c. of N/10 sodium hydroxide was added to each of the tubes in which reagents 1 and 3 had been employed, and the colors noted. The results of these observations are given in Table I.

TABLE I.

Substance.	Color produced with reagent—			
	(1)	(2)	(3)	(4)
Water	Colorless	Colorless	Very light pink	Very light pink.
Malt (active)	Colorless	Colorless	Deep purplish red	Very light pink.[a]
Malt (boiled)	Colorless	Colorless	Light pink	Very light pink.[a]
Saliva (active)	Colorless	Colorless	Light pink	Very light pink.[a]
Saliva (boiled)	Colorless	Colorless	Colorless	Very light pink.[a]
Potato (active)	Pink	Yellowish	Deep purplish red	Light pinkish salmon.
Potato (boiled)	Colorless	Yellowish	Yellowish	Light pinkish salmon.
Blood (active)	Colorless	Pink	Colorless, rapidly becoming pink and then red.	Deep purplish red.
Blood (boiled)	Colorless	Pink	Colorless, rapidly becoming pink and then red.	Deep purplish red.
Horse-radish (active)	Colorless	Trace of pink.	Deep purplish red instantly.	Faint pink.
Horse-radish (boiled)	Colorless	Colorless	Purplish pink, much lighter than unboiled specimen.	Trace of pink.
M/1000 copper sulfate	Colorless	Colorless	Deep purplish red practically instantly.	Deep purplish red.

[a] Lighter than water control.

In order to observe the effect of allowing these substances to stand in contact with the alkaline phenolphthalin reagent (2), certain of these tubes were allowed to stand, and examined at the end of three hours and twenty-four hours, with the following results:

Substance.	Color produced with reagent (2) after standing—	
	3 hours.	24 hours.
Water	Colorless	Trace of pink.
Blood (active)	Purplish pink	Deep purplish red.
Horse-radish (active)	Trace of pink	Light purplish red.
Horse-radish (boiled)	Colorless	Faint pink.
M/1000 copper sulfate	Trace of pink	Faint pink.

It is evident from these results that saliva, extract of malt, potato peel, horse-radish, blood, and copper sulfate conduct themselves very differently toward the four phenolphthalin reagents. Properly interpreted they go to show, first, that the potato peel contains both an oxidase and a peroxidase; second, that saliva, extract of malt, and horse-radish contain a peroxidase; third, that blood contains neither an oxidase nor a peroxidase, but that both raw and boiled it is capable of oxidizing phenolphthalin in alkaline solution either alone or through the agency of hydrogen peroxide; fourth, that copper sulfate at great dilutions contains nothing which acts like an oxidase or peroxidase, but that like blood it is capable of strongly inducing the oxidation of phenolphthalin in alkaline solution by hydrogen peroxide, but that it differs from blood in not being able to oxidize an alkaline solution of phenolphthalin alone, or if it does so, it is only with extreme slowness as compared with blood; and we can readily distinguish between an aqueous extract of horse-radish and blood, the former containing probably the most powerful peroxidase known, by the fact that it (horse-radish) readily oxidizes a neutral solution of phenolphthalin containing hydrogen peroxide, whereas blood does not, and further by the fact that the peroxidase of horse-radish does not oxidize either an alkaline solution of phenolphthalin alone (2) or one containing hydrogen peroxide (3), or at least only with extreme slowness as compared with blood, and thirdly by the fact that the slight oxidizing power which the peroxidase of horse-radish exhibits toward reagents (2) and (4) is entirely lost on boiling, whereas boiled blood solutions are very active toward both of these reagents.

Concerning the action of pus, it may be said that my own experience has been that pus differs from blood in that it contains a true peroxidase the activity of which is lost on boiling. Thus I have found unboiled extracts of pus to oxidize reagent (3), whereas with the boiled extracts no oxidation occurred. This has been the experience of other chemists. Thus Brandenburg (24) has observed that pus contains a nucleo-proteid having the properties of a peroxidase and that its peroxidase activity is lost on boiling. Hence, by means of the phenolphthalin reagents we could readily distinguish between blood and pus.

Similarly with regard to urine, Doctor Roberts and myself have found that only urine containing pus exhibits true peroxidase reactions. Such urines never oxidize an alkaline solution of phenolphthalin containing hydrogen peroxide unless they contain blood, and as already pointed out certain urines may even contain blood and still not oxidize phenolphthalin in the presence of alkali and hydrogen peroxide, on account of the presence of restraining (reducing) substances, and I myself have never encountered a normal urine that would show either true peroxidase reactions or that would induce the oxidation of an alkaline solution of phenolphthalin by hydrogen peroxide.

The following observations have been made on the effect of evaporation and incineration on the power of blood and copper sulfate to induce the oxidation of an alkaline solution of phenolphthalin by hydrogen peroxide:

A solution of blood was prepared containing 4.4 milligrams of human blood in 100 c. c., and also a M/1000 solution of copper sulfate.

1. 1 c. c. portions of each of these solutions were evaporated to dryness on a steam bath in platinum dishes; the residues were then dissolved in 1 c. c. of water and 2 c. c. of reagent (4) added.
2. 1 c. c. portions of each of these solutions were evaporated to dryness on the steam bath in platinum dishes and incinerated at a low red heat; the residues were then treated with 1 c. c. of water and 2 c. c. of reagent (4) added.

By way of comparison, tubes were prepared containing—

3. 1 c. c. of distilled water and 2 c. c. of reagent (4).
4. 1 c. c. of the blood solution and 2 c. c. of reagent (4).
5. 1 c. c. of the M/1000 copper sulfate solution and 2 c. c. of reagent (4).

At the end of five minutes these tubes showed the following colors:

Tube.	Color.		
	Water.	Blood.	Copper sulfate.
Number 1		Deep red (slow)	Deep purplish red.
Number 2		Faint pink	Deep purplish red.
Number 3	Faint pink		
Number 4		Deep purplish red	
Number 5			Deep purplish red.

We see, therefore, that as the result of evaporation blood loses some of its oxidizing power, whereas the activity of copper sulfate, as might be expected, remains undiminished. On incineration, blood loses, completely or nearly so, its power to induce the oxidation of an alkaline solution of phenolphthalin by hydrogen peroxide; whereas the activity of copper sulfate suffers practically no change as the result of incineration.

The effect of evaporation and incineration on the oxidizing power of blood towards alkaline phenolphthalin alone, reagent (2) was also tried. 1. 1 c. c. of the blood solution containing 0.044 milligram of blood was mixed with 2 c. c. of reagent (2). 2. The residue left from the evaporation of 1 c. c. of this same blood solution was dissolved in 1 c. c. of water and 2 c. c. of reagent (2) added. 3. The ash left from the incineration of 1 c. c. of the same blood solution was dissolved in 1 c. c. of distilled water and 2 c. c. of reagent (2) added. After twenty-four hours these tubes showed the following colors:

1. Deep red.
2. Light red.
3. Faint trace of pink.

Hence we see again that the effect of evaporation is to lessen the oxidizing power of blood and the effect of incineration is to destroy it or at least to lessen it very greatly.

Further, in order to determine to what extent the salts of the heavy metals might be confused with blood, the conduct of a number of these substances toward an alkaline solution of phenolphthalin, with and without hydrogen peroxide, has been studied. Solutions of the several salts named in the following tables were prepared by dissolving 10 milligrams of each of these salts in 100 c. c. of distilled water. These were then compared as to oxygen-carrying power toward reagents (2) and (4) with a solution of human blood containing 10 milligrams of blood in 100 c. c. Tubes were prepared containing 2 c. c. of the reagent and 1 c. c. of water, blood, or the solution of the salt.

The results obtained with reagent (2), alkaline phenolphthalin, are given in Table II:

TABLE II.

Number of tube.	Substance.	Color.		
		Immediate.	After 1 hour.	After 24 hours.
1	Water	Colorless	Colorless	Trace of pink.
2	Blood	Colorless	Deep pink	Deep purplish red.
3	Chromic alum	Colorless	Colorless	Colorless.
4	Potassium chromate	Yellow	Faint yellowish	Faint pink.
5	Potassium dichromate	Light yellow	Faint yellow	Faint yellow.
6	Ferrous sulfate	Faint yellow	Faint yellow	Yellowish.
7	Ferrous ammonium sulfate	Faint yellow	Faint yellow	Yellowish.
8	Ferric alum	Faint yellow	Faint yellow	Faint yellowish.
9	Potassium ferrocyanide	Light pink	Faint pink	Light pink.
10	Potassium ferricyanide	Deep purplish red.	Deep purplish red.	Deep purplish red.
11	Sodium nitroprusside	Faint trace of pink.	Faint yellowish	Reddish purple.
12	Nickel nitrate	Colorless	Colorless	Faint pink.
13	Cobalt chloride	Colorless	Colorless	Trace of pink.
14	Cupric chloride	Faint pink	Trace of pink	Light pink; brown precipitate.
15	Cupric sulfate	Trace of pink	Trace of pink	Faint pink; brown precipitate.
16	Cupric sulfate, M/1000	Trace of pink	Trace of pink	Light pink; brown precipitate.
17	Manganous sulfate	Yellow	Yellowish	Trace of pink; brown precipitate.
18	Manganous chloride	Yellowish	Yellowish	Trace of pink; brown precipitate.
19	Silver nitrate	Faint pink	Reddish	Reddish brown.
20	Mercuric chloride	Colorless	Colorless	Faint pink.
21	Lead chloride	Colorless	Colorless	Faint pink.
22	Lead acetate	Colorless	Colorless	Faint pink.
23	Potassium chlorplatinate	Colorless	Faint pink	Decided pink.
24	Sodium chloride	Colorless	Colorless	Trace of pink.
25	Potassium nitrate	Colorless	Colorless	Trace of pink.

In order to form a better idea of the extent of the oxidation in certain of these solutions, the following, Nos. 1, 9, 10, 11, and 23, were compared in the Duboscq colorimeter with solution No. 2, containing blood, with the following results:

Solution.	Substance.	Readings on colorimeter scale.	
		Substance.	Blood (No. 2).
Number 1	Water	10	0.1
Number 9	Potassium ferrocyanide	10	.4
Number 10	Potassium ferricyanide	10	2.0
Number 11	Sodium nitroprusside	10	2.0
Number 23	Potassium chlorplatinate	10	.7

Or, in terms of the color of the solution of blood arbitrarily made equal to 100, the colors produced with the other substances have the following values:

Substance.	Color.
Blood	100
Water	1
Potassium ferrocyanide	4
Potassium chlorplatinate	7
Potassium ferricyanide	20
Sodium nitroprusside	20

It is evident from these results that at the concentration of 100 milligrams per liter none of the 23 substances investigated have an oxygen-carrying power towards alkaline phenolphthalin at all comparable with that of blood, only 3 out of the 23 showing decided oxidizing power, viz, silver nitrate, No. 19, potassium ferricyanide No. 10, and sodium nitroprusside, No. 11, and even with these only one-fifth as much phenolphthalein was produced as with blood.

The results obtained with reagent (4), alkaline phenolphthalin containing hydrogen peroxide, are given in Table III:

TABLE III.

No. of Tube.	Substance.	Color, after fifteen minutes.
1	Water	Faint trace of pink.
2	Blood	Deep purplish red.
3	Chromic alum	Deep pink.
4	Potassium chromate	Faint trace of pink.
5	Potassium dichromate	Faint pink.
6	Ferrous sulfate	Faint trace of pink.
7	Ferrous ammonium sulfate	Faint trace of pink.
8	Ferric alum	Faint trace of pink.
9	Potassium ferrocyanide	Faint trace of pink.
10	Potassium ferricyanide	Decided pink.
11	Sodium nitroprusside	Trace of pink.
12	Nickel nitrate	Faint trace of pink.
13	Cobalt chloride	Faint yellowish.
14	Cupric chloride	Deep purplish red.
15	Cupric sulfate	Deep red.
16	Cupric sulfate, M/1000	Deep purplish red.
17	Manganous sulfate	Yellowish.
18	Manganous chloride	Yellowish.
19	Silver nitrate	Brownish, turbid.
20	Mercuric chloride	Decided pink.
21	Lead chloride	Decided pink.
22	Lead acetate	Trace of pink.
23	Potassium chlorplatinate	Trace of pink.
24	Sodium chloride	Trace of pink.
25	Potassium nitrate	Faint trace of pink.

The only substances, therefore, showing decided oxygen-carrying power towards reagent (4) are blood, copper salts, and chromic alum, in order of activity. Mercuric chloride, No. 20, lead chloride, No. 21, and potassium ferricyanide, No. 10, also show some oxygen-carrying power; this is slight, however, as compared with blood.

A comparison of the conduct of such of these substances as show certain resemblances to blood, towards the two phenolphthalin reagents, reveals certain interesting differences between such substances and blood. Thus we see that while silver nitrate oxidizes phenolphthalin in alkaline solution it fails to oxidize it when hydrogen peroxide is present. As already pointed out, copper salts rapidly oxidize an alkaline solution of phenolphthalin containing hydrogen peroxide, but fail to oxidize it in the absence of hydrogen peroxide, or oxidize it very slowly, much more slowly than blood. Chromic alum also oxidizes an alkaline solution of phenolphthalin containing hydrogen peroxide, but failed to oxidize it in the absence of the peroxide. Potassium chlorplatinate oxidizes an alkaline solution

of phenolphthalin, but fails to oxidize it when hydrogen peroxide is present. Lead and mercuric chlorides oxidize it slowly when hydrogen peroxide is present, but fail to oxidize an alkaline solution of phenolphthalin alone. Potassium ferricyanide rather rapidly oxidizes an alkaline solution of phenolphthalin in the absence of hydrogen peroxide, but oxidizes it more slowly when hydrogen peroxide is present. In other words, despite the fact that a few of the salts of the heavy metals exhibit certain resemblances to blood in their conduct toward one or the other phenolphthalin reagents, it would seem that when tested with both reagents they all display certain well-marked differences from blood, whereby we could readily tell the difference between blood and any one of these substances. It might be possible to prepare a solution or a stain which could not be told from a solution of blood or a blood stain by means of the phenolphthalin reagents, but up to this time such a solution or stain has not been encountered.

To attempt, therefore, to convey the impression that phenolphthalin is without utility as a reagent for blood and the oxidases, as has been done by Pozzi-Escot, appears to me to be entirely unwarranted and unjustifiable in the light of the facts. As a matter of fact, all really delicate tests have to be controlled and used with considerable circumspection and discrimination in order to arrive at the proper conclusion from the findings, and it not infrequently happens that the less one knows of analytical methods the more certain he is of the results of the analysis.

THE EXAMINATION OF A NUMBER OF UNKNOWN STAINS BY MEANS OF THE PHENOLPHTHALIN REAGENTS.

In order to form a better idea of the practical utility of the alkaline phenolphthalin reagents (2) and (4), in the actual examination of blood spots, 25 spots or stains upon filter paper, not exceeding 4 square millimeters in area, were prepared by Mr. Elvove, one of the workers in the Chemical Division of the Hygienic Laboratory, and submitted to the writer for examination. At the time of the examination the nature and composition of the stains were known only to Mr. Elvove. Within two hours after these unknown stains had been submitted to the writer three of them, viz, Nos. 3, 12 and 17, had been found to contain blood, and Nos. 1, 2, 5, 11, 22, and 25 had been found to contain artificial coloring matters, whereas the remaining specimens were found to contain neither blood nor a soluble dye, the solubility in water, color of the aqueous solution, and conduct towards alkaline phenolphthalin, with and without hydrogen peroxide, being the only means employed in arriving at these conclusions. In

the following table is given the composition of the stains submitted by Mr. Elvove, together with the results of my examination:

No. of stain.	Composition of stain, furnished by Elvove.	Found by Kastle.
1	Aniline red and jeweler's rouge[a]	Artificial coloring matter.
2	Benzopurpurin	Do.
3	Rabbit blood	Blood.
4	Burnt sienna	
5	Cochineal and jeweler's rouge	Artificial coloring matter.
6	Cupric ferrocyanide	
7	Cupric oxide	
8	Cuprous oxide	
9	Mercuric iodide and jeweler's rouge	
10	Mercuric oxide and jeweler's rouge	
11	Brick red ink (Higgins)	Artificial coloring matter.
12	Human blood	Blood.
13	Indian red	
14	Jeweler's rouge	
15	Manganous manganite	
16	Manganese dioxide	
17	Mouse blood	Blood.
18	Red paint	
19	Litharge and jeweler's rouge	
20	Lead peroxide and jeweler's rouge	
21	Potassium permanganate	
22	Red ink	Artificial coloring matter.
23	Silver chromate	
24	Silver oxide and jeweler's rouge	
25	Vermillion	Artificial coloring matter.

[a] Wherever jeweler's rouge was used with another substance, approximately equal volumes of the two substances were mixed together and a sample of the mixture taken.

The results of these tests are sufficient to give an idea of the practical utility of the phenolphthalin tests for blood. By no other method, unless it may possibly be the spectroscopic or precipitin tests, could these stains have been examined in the time actually consumed in making these tests with phenolphthalin. A further point of interest in connection with the general utility of phenolphthalin as a reagent for blood is that a number of vigorous oxidizing agents and powerful oxygen-carriers entered into the composition of the stains prepared by Mr. Elvove, and yet no difficulty was experienced in determining which of these contained blood, despite the fact that in most cases the original colors of the various stains matched so closely that only the most expert examination could detect any differences in their color and general appearance.

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LIST OF HYGIENIC LABORATORY BULLETINS OF THE PUBLIC HEALTH AND MARINE-HOSPITAL 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.

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*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.

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*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.)

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TREASURY DEPARTMENT
Public Health and Marine-Hospital Service of the United States

HYGIENIC LABORATORY.—BULLETIN NO. 52
OCTOBER, 1909

REPORT No. 3

ON

THE ORIGIN AND PREVALENCE OF TYPHOID FEVER IN THE DISTRICT OF COLUMBIA

(1908)

By

M. J. ROSENAU, L. L. LUMSDEN
and JOSEPH H. KASTLE

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1909

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CHARTS AND MAPS.

INTRODUCTION.

By request of the Commissioners of the District of Columbia the undersigned were appointed a board by the Surgeon-General of the Public Health and Marine-Hospital Service on July 2, 1906, for the purpose of making an investigation of the origin and prevalence of typhoid fever in the District of Columbia Since that date this investigation has been continuous, and this bulletin gives the results of our studies on typhoid fever in the District of Columbia during the season of 1908. Our first report on the subject, for the year 1906, is contained in Hygienic Laboratory Bulletin No. 35, and our second report, that for 1907, in Hygienic Laboratory Bulletin No. 44.

This year our studies included a continuation of the epidemiological investigations of the disease during the typhoid-fever season. This makes the third consecutive year of these studies. The data for the epidemiological part of the work were collected by one of us (Lumsden). Special mention is made of this fact for the reason that we believe that differences between the results for any two of the three years may be attributed to differences in conditions, as the factors of personal equation have not varied.

In addition to the epidemiological data collected from personal visits to every case reported during the typhoid season (May 1 to November 1) we made during that season bacteriological examinations of the raw and filtered Potomac River water, also numerous examinations of the tap water, the applied water, etc.

At the suggestion of Professor Sedgwick, made at the meeting of the advisory board of the Hygienic Laboratory, we made an intensive study of the problem in a selected district comprising 32 city blocks and containing 5,300 persons. This study was made by Passed Assistant Surgeon Norman Roberts. We found no cases of clinical typhoid fever among these people which had not been diagnosed as such and reported to the health officer. A special search was also made for bacillus carriers in this area. Specimens of feces were collected from about 1,000 healthy persons and examined for the presence of typhoid bacilli. Most of these examinations were made by Assistant Surgeon W. W. Miller, assisted by Dr. Walter D. Cannon. Three of the specimens—one of urine and two of feces—were found

to contain typhoid bacilli. The facts and conclusions of that work are fully discussed in the text.

Upon our request, Dr. L. O. Howard, Chief of the Bureau of Entomology, Department of Agriculture, made a study of fly abundance in relation to the prevalence of typhoid fever in the District of Columbia. But little connection between the germ of typhoid fever and the fly as its disseminator could be made out.

The prevalence of typhoid fever in the District of Columbia showed but slight differences between the two years 1907 and 1908. Our studies showed that about 50 per cent of the cases during the typhoid seasons of these two years were definitely attributable to importation, contact with previous cases in the febrile stage of the disease, and to infected milk.

The evidence strongly suggests that more cases were due to personal contact and to infected milk than were traceable to these factors.

The city water supply during the typhoid seasons of these two years was, according to bacteriological standards, of good sanitary quality, and it does not seem probable that such water could have been directly responsible for more than an insignificant part of the infection. We believe, therefore, that in order to cause a further and a marked reduction in the prevalence of typhoid fever in Washington it will be necessary to carry out measures to prevent the conveyance of infection by milk and by personal contact. Our studies indicate that if the market milk supplied the city were pasteurized under official supervision, and an efficient campaign made to diminish contact infection, there would result a very great diminution in the amount of typhoid fever in the District of Columbia.

It is beginning to be realized generally that the solution of the typhoid-fever problem and the final suppression of the disease depend upon accurate knowledge gained by special studies of the disease in endemic areas rather than by the necessarily hurried studies of acute outbreaks. For a full appreciation of the typhoid-fever problem in Washington it is necessary to have comparable data from other cities. It is therefore gratifying to note that careful and valuable studies of this particular problem are being made in at least three other large American cities—Richmond, Va., Pittsburg, Pa., and Baltimore, Md.

Levy and Freeman[a] have contributed a particularly valuable report on typhoid fever at Richmond, Va.

In Pittsburg the typhoid situation is being studied by a typhoid-fever commission composed of Drs. J. F. Edwards, W. T. Sedgwick,

[a] Levy, Ernest C., and Freeman, Allen W.: Certain conclusions concerning typhoid fever in the South, as deduced from a study of typhoid fever in Richmond, Va. Old Dominion Journ. Med. and Surg., vol. 7, Nov., 1908.

Samuel G. Dixon, John W. Boyce, and Milton J. Rosenau, assisted by Dr. E. G. Matson and Messrs. Frank E. Wing and Morris Knowles, under the patronage of the Russell Sage Foundation. The report on the Pittsburg situation has not yet been published, the work is still in progress.

A very interesting and instructive review of the typhoid-fever situation in Baltimore, Md., for the four years 1904, 1905, 1906, and 1907 is presented by Dr. C. Hampson Jones in the annual report of the health department of the city of Baltimore, Md., for 1907.

We again wish to acknowledge our indebtedness to Dr. William C. Woodward, health officer of the District of Columbia, for assistance and many courtesies; also, to the authorities of the various hospitals.

We have again profited by consultation with the advisory board of the Hygienic Laboratory. At a recent meeting of the members of that board our findings and general conclusions for this report were reviewed.

M. J. Rosenau,
Director Hygienic Laboratory, Chairman.
L. L. Lumsden,
Passed Assistant Surgeon.
Joseph H. Kastle,
Chief of Division of Chemistry, Recorder,
United States Public Health and Marine-Hospital Service.

Washington, D. C., *April 16, 1909.*

Report No. 3 on the Origin and Prevalence of Typhoid Fever in the District of Columbia (1908).[a]

In 1908, as in 1907,[b] our study of the prevalence of typhoid fever in the District of Columbia comprised an epidemiological investigation of all cases of typhoid fever reported to the District health office during the period extending from May 1 to November 1. In both years we have made an especial study of the cases reported in this period, because typhoid fever in the District of Columbia during these six months is much more prevalent than in the other months of the year. Usually about 70 per cent of the cases for the whole year are reported in this period.

From May 1 to November 1, 1908, there were reported 679 cases as gainst 675 cases for the corresponding period of 1907. Of the cases in the 1908 period 8 were abandoned because no accurate information in regard to them could be obtained and 6 were dropped from our records because the diagnosis was determined by autopsy or by further clinical observation to be incorrect. Deducting these 14 cases from the 679, there are left 665 cases for consideration in this report.

DIAGNOSIS.

This year, as in 1907, it was not practicable for us to study the cases in clinical detail; but, judging by the histories obtained in frequent instances, the results of blood cultures, Widal tests, etc., we are of the opinion that again some of the cases were incorrectly diagnosed and should not have been placed on the records as cases of typhoid fever.

Reasoning along very general lines, after our study of the situation in 1907, we presented the view (Hyg. Lab. Bull. 44, Report No. 2, p. 12) that the number of cases of typhoid fever in the District of Columbia which are unrecognized and not reported exceeds considerably the number reported as typhoid fever under mistaken diagnoses.

In the summer of 1908 a house-to-house canvass, covering 32 blocks, located in different sections of the city, and having a population of 5,364, was made and not a case of clinical typhoid fever which had not been reported as such was discovered. On the other hand,

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[a] Manuscript submitted for publication April 16, 1909.

[b] Rosenau, M. J.; Lumsden, L. L.; and Kastle, Joseph H.: Report No. 2 on the origin and prevalence of typhoid fever in the District of Columbia, 1907. Hyg. Lab. Bull. No. 44, May, 1908.

some of the reported cases in these blocks had been diagnosed on what appeared to be scant clinical evidence and without any attempt at confirmation by laboratory tests.

A better acquaintance with the situation inclines us to believe that practically every case of clinical typhoid fever occurring in the District of Columbia is reported to the health officer. On account of the frequent association of the medical profession and the lay people with the disease, many cases of continued fever of obscure nature, but not typhoid, are, however, diagnosed as typhoid. In other words, few cases of clinical typhoid fever are ever overlooked, but the official records contain more typhoid fever than actually exists in a clinical sense. How much more could only be determined by persistent and prolonged laboratory confirmation of the clinical diagnoses. This we expect to do at a future investigation. We are inclined to believe, from our observations of the situation and from experimental data, that perhaps from 10 to 20 per cent of the cases reported as typhoid fever are really some other disease. This may in part account for Washington's relatively high typhoid rate when compared with that of other American cities.

In a community where typhoid fever has been markedly prevalent for a number of years, as it has been in Washington, it is but natural that physicians should form somewhat of a habit of reporting doubtful cases as typhoid fever.

The correct diagnosis of cases is, of course, of first importance both in the obtainment of correct data for epidemiological studies and in the adoption of proper measures to prevent the spread of the disease.

In most American cities physicians report to the health office, for official confirmation of diagnosis, suspected cases of certain communicable infectious diseases, such as tuberculosis, diphtheria, smallpox, etc. The communicability of the infection of typhoid fever from the sick to the well is now thoroughly recognized, and, considering the widespread prevalence of typhoid fever in America, we believe that it is of prime importance from a public-health standpoint for official diagnosis of cases of this disease to be required. To the competent and careful physician, the assistance of official expert opinion and of laboratory facilities would be welcome, while to the incompetent or careless physician such assistance would be important.

Specimens for examination at the laboratory can be obtained with so little inconvenience to the patient, that there seems to be no excuse for physicians to fail to have laboratory tests made to aid in the diagnosis of all suspected cases.

Specimens of the stools and urine, of course, may be obtained without any inconvenience to the patient, and bacteriological examination of these excreta by comparatively recent methods will demonstrate the typhoid bacillus in a large percentage of cases.

The pricking of the ear lobe or finger tip to obtain a drop of blood for the Widal test causes so little pain that, in frequent instances, a sleeping child will not be awakened by the procedure. The Widal tests will give positive results at some time in the course of the illness for about 95 per cent of cases. Positive results are rarely obtained before the tenth or twelfth day of fever, and this lateness of the development of the agglutinating property of the blood prevents the Widal test from being of much aid in early diagnosis.

The puncture of a vein at the elbow joint, in order to obtain a few cubic centimeters of blood for bacteriological examination by culture, causes a slight amount of pain, it is true, but when the operation is done properly it is entirely free from danger and causes no more distress to the patient than does the application of a swab to the throat in order to obtain cultures in cases of suspected diphtheria. The great advantage of the blood-culture method is that it gives in the earliest stages of the illness positive results and maximum information.

In about 90 per cent of cases in the first week of fever the blood culture test will demonstrate the typhoid bacillus, usually in pure culture. The later in the attack the blood is taken the less are the chances of demonstrating the organism. Thus Heinrich Kayser,[a] working in Germany, obtained positive results in 96 per cent of cases examined in the first week, in 65 per cent examined in the second week, in 42 per cent in the third week, and in 35 per cent in the fourth week. Kayser took only 2.5 c. c. of blood for each examination.

Since beginning our investigations of typhoid fever here in 1906 we have offered to the physicians of the District of Columbia the facilities of the Hygienic Laboratory, U. S. Public Health and Marine-Hospital Service, to assist them in the diagnosis of suspected cases. So far, comparatively few requests for blood cultures have been received. It appears that the value and ease of application of this test are not generally appreciated.

METHOD OF INVESTIGATION.

In the study of the cases this year the following form was used and all facts called for by this form were carefully investigated for each case. The form is the same as the one used in 1907:

TYPHOID FEVER CASE CARD.

Date of investigation, ———. Case No. ——.
Name, ———.
Age, ——. Color, ———. Sex, ———. Nationality, ———.
Probable date of onset, ———. Date definite symptoms, ———.
Name and address of physician, ———.

[a] Munch. med. Woch., 1906, p. 823.

RESIDENCE.

How long resident in District of Columbia, ———.
Residence when taken sick, ———; from ——— to ———.
Previous residences, ———; from ——— to ———.
Subsequent residences, ———; from ——— to ———.
Temporary absences from District of Columbia within thirty days prior, ———.
Number of occupants, ——; ages, ——.
Number of occupants who have had typhoid, ——; when, ———.
Newcomers in house within three months prior, ——.
Newcomers in house had typhoid? ——.
Servants:
White: Resident, ——. Typhoid? ——.
Nonresident, ——. Typhoid? ——.
Colored: Resident, ——. Typhoid? ——.
Nonresident, ——. Typhoid? ——.
Typhoid at homes of servants? ——. When? ——.
Disposal of sewage, ——. Water-closet in house? ——.
Water-closet in yard? ——. Privy? ——. Location? ——.
General sanitary condition of residence, ——.

OCCUPATION.

Place, ———; from ——— to ———.
Other cases, ———.

WATER.

Within thirty days prior, ———. Solely, ———. Principally, ———. Occasionally, ———.

FOOD.

Within thirty days prior, ———.
Where taken, ———.
Milk (how used), ———; from ———. Boiled? ——. Pasteurized? ——.
Ice cream? ——. Where? ——.
Uncooked fruits and vegetables, ——.
Shellfish, ——.

CONTACT.

Association thirty days prior with patients in febrile stage, ——.
Association with suspected cases, ——.
Association with persons who have had typhoid within six months, ——; one year, ——; two years, ——; three years, ——; four years, ——; five years, ——.
Association thirty days prior with persons in contact with patients in febrile stage,——.
Treatment of stools and urine of patients, ——.
Other precautions, ——.
Remarks: ———.
Summary: ———.

Signature: ——— ———.

Our method of conducting the investigation of the cases has been the same for each of the three years in which we have studied typhoid fever in the District of Columbia. All residences within the District from which cases were reported were visited and inspected. For the data the patients themselves were questioned when their condition permitted it. If their condition did not permit it the statements of other members of the family or household were taken.

Frequently several visits to a home were necessary before all the information desired could be obtained for a given case. By exercising care and patience it was endeavored to eliminate as much error as possible from the figures. Having followed the same plan of study for each of the three years we believe that differences in our findings for any two of the years are due to change of conditions and scarcely, if in any wise, to change of methods of study.

In each of the three years the epidemiological investigation of the individual cases has been conducted by one of us (Lumsden), so that differences in the results from personal equation have been eliminated.

INTENSIVE STUDY OF SPECIAL DISTRICTS.

In addition to the above epidemiological investigations there was made in 1908 an intensive study of the typhoid fever situation in 32 blocks of the city. This part of the work was done under our direction by Passed Assistant Surgeon Norman Roberts. The study consisted in a house to house canvass of the blocks. Effort was made to obtain full histories of all cases of illness in these sections and to determine if any of the cases were typhoid fever.

In the course of this work over 1,000 specimens of feces were obtained from 993 persons and examined at the Hygienic Laboratory for the presence of the typhoid bacillus. The specimens were obtained from suspected cases of illness and from healthy persons, particularly women and children, with a view of determining what proportion of the population harbors typhoid bacilli without having symptoms of the disease.

In the course of this special investigation Doctor Roberts also collected data in regard to sanitary conditions, water and milk supplies, etc., of the households in those sections.

The results of this special study are presented in the following table:

TABLE No. 1.—*Results of intensive investigation of special districts, 32 city blocks.*

(Miscellaneous data covering all families visited.)

Locality.	No. of Houses.	Number of families (1,101).		Population.	Servants.	Visitors.	Drinking water.			
		White.	Colored.				Raw.	Boiled.	Bottled.	Melted ice.
Block No. 1	52	51	4	233	11	3	49	7	.....	.....
Block No. 2	38	34	6	230	10	5	33	9	1	1
Block No. 3	48	38	14	250	8	1	44	8	1	1
Block No. 4	52	30	23	244	5	4	46	8	.....	.....
Block No. 5	46	48	7	242	10	4	48	7	.....	.....
Block No. 6	35	28	9	275	3	6	34	4	.....	2
Block No. 7	44	42	6	221	12	10	37	11	.....	.....
Block No. 8	58	48	11	226	6	3	43	14	1	.....
Block No. 9	33	20	13	212	0	7	33	0	.....	.....
Block No. 10	45	12	34	208	0	7	32	3	.....	.....
Block No. 11	42	23	20	180	3	4	39	3	.....	.....
Block No. 12	27	29	2	153	6	3	24	5	1	.....
Block No. 13	26	8	19	111	0	0	24	3	.....	.....
Block No. 14	42	28	13	195	0	2	37	4	.....	.....
Block No. 15	52	28	24	294	0	1	49	2	.....	.....
Block No. 16	26	15	11	125	1	0	26	0	.....	.....
Block No. 17	23	16	7	91	1	0	21	11	.....	.....
Block No. 18	8	8	0	40	0	0	8	0	.....	.....
Block No. 19	47	21	26	245	2	7	39	8	.....	.....
Block No. 20	19	19	0	62	3	6	14	3	1	.....
Block No. 21	39	11	25	161	1	10	26	7	1	2
Block No. 22	45	16	28	254	0	5	43	3	.....	.....
Block No. 23	20	0	20	92	0	1	18	2	.....	.....
Block No. 24	10	0	10	57	0	0	9	0	.....	.....
Block No. 25	10	0	10	41	0	0	10	0	.....	.....
Block No. 26	14	1	13	69	1	0	13	1	.....	.....
Block No. 27	18	0	18	104	0	0	17	1	.....	.....
Georgetown	18	18	0	96	2	3	17	1	.....	.....
Northwest	39	39	0	162	25	7	27	13	.....	.....
Northeast	36	33	3	184	6	19	34	2	.....	.....
Southeast	29	12	20	174	5	17	28	4	.....	.....
Southwest	28	29	0	133	2	11	26	1	.....	2
Total	1,069	705	396	[a] 5,364	123	146	858 85%	145 15%	6	8

[a] White, 3,392; colored, 1,972.

TABLE No. 1.—*Results of intensive investigation of special districts, 32 city blocks*—Continued.

Locality.	Milk used.							Screening of houses.			Condition of house as to repair.		Condition of house as to cleanliness.	
	Raw.	Home heated.	Commercially pasteurized.	Condensed.	Rarely used.	None.	Of unknown origin.	Throughout.	Partial.	None.	Bad.	Good.	Bad.	Good.
Block No. 1	47	4	1	5	.....	2	.....	40	5	9	1	55	9	47
Block No. 2	32	3	0	3	.....	3	.....	30	5	5	5	35	5	34
Block No. 3	28	6	11	3	5	1	4	27	7	18	5	47	4	46
Block No. 4	28	6	4	7	4	5	1	25	4	24	12	41	7	45
Block No. 5	37	2	4	7	2	3	2	33	12	10	5	49	10	51
Block No. 6	25	4	0	6	1	3	.....	23	9	6	3	36	.....	37
Block No. 7	22	11	4	9	.....	3	.....	34	6	9	4	45	4	44
Block No. 8	37	9	2	6	.....	4	3	45	.....	12	7	50	6	52
Block No. 9	17	6	11	5	.....	2	1	21	1	10	1	31	4	27
Block No. 10	22	8	1	7	4	3	1	20	1	25	7	39	1	44
Block No. 11	28	6	0	3	1	6	.....	30	2	10	6	35	1	38
Block No. 12	13	12	5	2	.....	0	2	26	.....	3	1	28	1	27
Block No. 13	8	2	0	3	.....	14	.....	19	.....	8	8	18	3	19
Block No. 14	22	4	3	4	.....	5	3	39	.....	9	5	36	1	39
Block No 15	48	5	7	3	.....	7	1	42	.....	10	5	47	1	49
Block No. 16	17	0	1	1	.....	6	1	16	.....	10	2	23	2	24
Block No. 17	15	3	0	1	.....	4	.....	18	.....	5	2	21	1	22
Block No. 18	7	0	0	0	.....	1	.....	6	.....	1	2	5	.....	6
Block No. 19	30	4	3	2	3	4	1	30	9	7	10	36	7	36
Block No. 20	12	3	4	1	.....	0	.....	17	.....	1	0	18	.....	18
Block No. 21	22	1	1	7	3	2	.....	20	6	10	7	29	4	30
Block No. 22	14	6	2	4	.....	7	15	29	.....	17	4	42	3	43
Block No. 23	4	1	0	2	.....	6	7	15	.....	4	5	14	3	15
Block No. 24	2	1	1	2	.....	3	3	1	.....	9	2	8	4	6
Block No. 25	0	1	1	7	.....	0	1	6	.....	4	1	9	1	8
Block No. 26	3	1	0	1	.....	7	2	7	.....	7	3	11	.....	14
Block No. 27	3	0	0	9	.....	0	6	2	.....	16	5	13	8	10
Georgetown	12	2	2	3	.....	3	.....	16	.....	2	2	16	.....	16
Northwest	21	13	4	0	.....	0	4	39	.....	0	1	38	.....	39
Northeast	21	3	0	7	1	7	1	24	4	7	5	31	.....	33
Southeast	21	1	1	3	.....	3	2	20	1	11	1	31	.....	30
Southwest	16	1	2	6	.....	6	3	21	1	6	2	26	3	25
Total	614	129	75	129	24	110	64	741	73	285	129	963	93	974
	54%	11%	7%	11%	2%	10%	6%	68%	6%	26%	12%	88%	11%	89%

TABLE NO. 2.—*Giving number of cases of diseases other than typhoid fever (present or recent) reported during house-to-house canvass of the special districts.*

Abscess	3	Paralysis	2
Appendicitis	1	Pleurisy	1
Boils	1	Pneumonia	4
Bowel trouble (chronic or recurrent)	2	Ptomaine poisoning	1
Bronchitis	3	Rheumatism	10
Chickenpox	1	Scarlet fever	1
Diabetes	2	Senility	1
Dyspepsia (chronic or recurrent)	35	Summer complaints	73
Fistula in ano	1	Tonsilitis	7
Gynecologic	3	"Threatened typhoid"	3
Heart trouble	2	Tuberculosis	9
Jaundice	1	Whooping cough	4
Kidney trouble	2	Worms	2
Malaria	11	Undetermined, but fairly severe	8
Measles	4		
Nervous trouble	12	Total	212

Observed incidence of "summer complaint" as to age and race.

	White.	Colored.
Under 2 years	11	6
2 to 15 years	14	3
Over 15 years	29	8
Total all ages	54	19
Grand total	73	

TABLE NO. 3.—*Giving number of persons in the 32 blocks who had had typhoid fever in previous years and the results of the bacteriologic examination of their feces for B. typhosus.*

Number of years since attacks of typhoid fever occurred.	Specimens obtained.	Result of examination. +	Result of examination. −	Specimens refused.	Total.
10 years and over (no specimens asked for)	113				
Between 9 and 10 years	1	0	1	7	8
8 years	2	0	2	2	4
7 years	4	0	4	13	17
6 years	8	0	8	7	15
5 years	4	0	4	8	12
4 years	11	0	11	7	18
3 years	8	0	8	4	12
2 years	17	0	17	6	23
1 year	6	0	6	5	11
Within the past year	10	0	10	2	12
Sick or convalescent at time of visit	9	7	2	3	12
Total	80	7	72	64	144
Total previous typhoid reported					257

This table shows that in a population of 5,364 canvassed 257 or 4.8 per cent gave a history of having had typhoid fever.

Map No. 1.—MAP OF WASHINGTON, SHOWING LOCATION OF CITY BLOCKS IN WHICH AN INTENSIVE STUDY OF TYPHOID FEVER WAS MADE FROM JULY 15 TO OCTOBER 24, 1908.

Blocks shaded red.

TABLE No. 4.—*Cases of diseases other than typhoid fever from which specimens of feces were obtained.*

Diseases.	Number of cases.	Result of examination for *B. typhosus*.	
		+	−
Summer complaints	37	0	37
Dyspepsia	7	0	7
Malaria	4	0	4
Tonsilitis	3	0	3
Liver trouble	3	1	2
Chronic intestinal catarrh	2	0	2
Appendicitis	1	0	1
Pneumonia	1	0	1
Gynecologic	1	0	1
Tubercular peritonitis	1	0	1
Undetermined, but severe	1	0	1
Total	61	1	60

The first house-to-house canvass was begun on July 15, that being about the time when typhoid fever in Washington is at the beginning of the six weeks of highest prevalence, and completed on September 30.

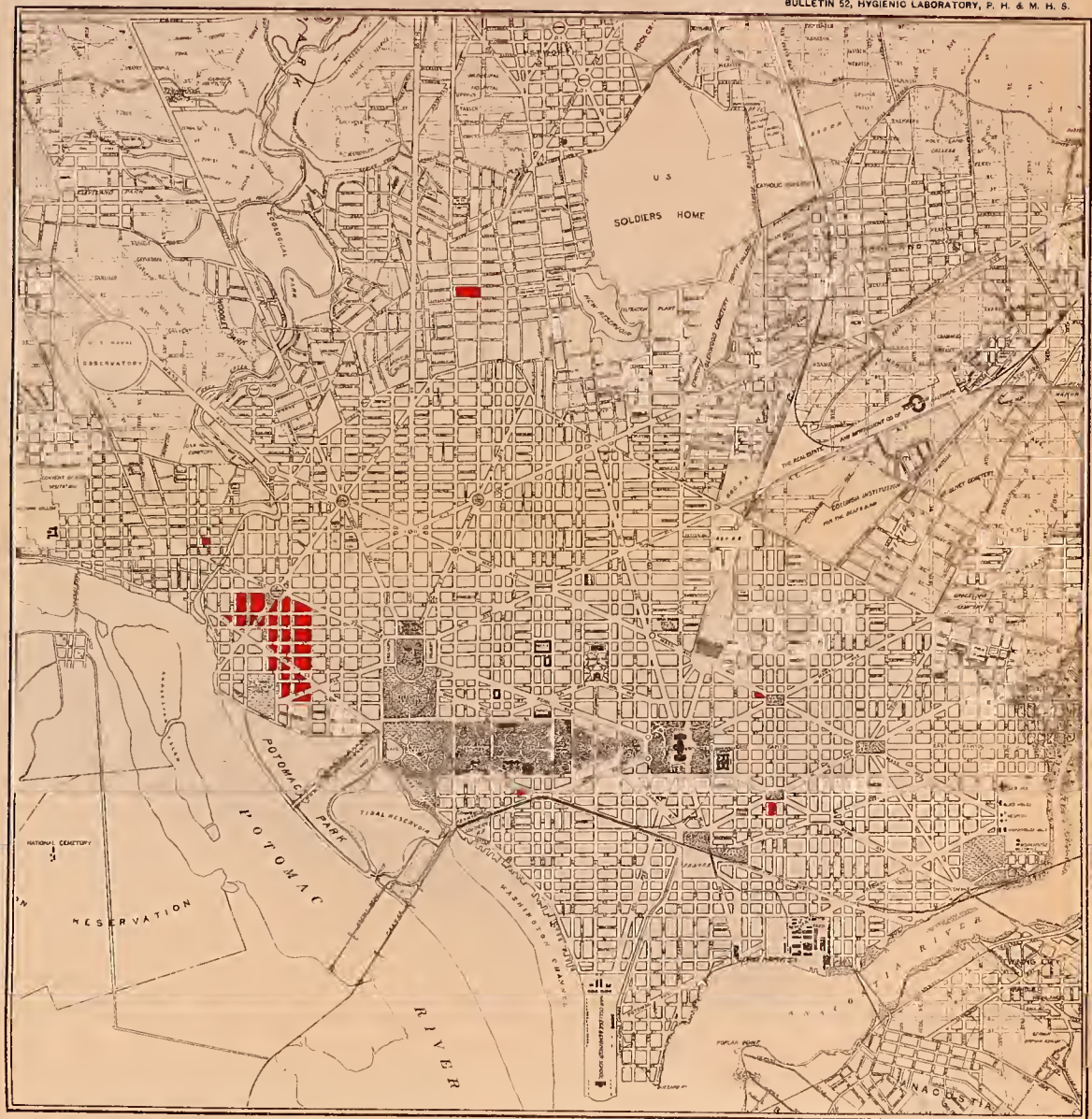
On this first canvass the specimens for laboratory examination and the general data as to population, condition of the houses, cases of illness, water and milk used, etc., were obtained.

A second canvass of the same 32 blocks was begun on October 1 and completed on October 24. On this second canvass data were obtained as to cases of illness which had developed since the time of the first visit. So the cases of illness recorded in the table include all that could be learned of in these 32 blocks during the period extending from July 15 to about October 24.

To collect the data, every household in the blocks was visited and some member or members of the household interviewed. Where there were cases of illness attended by physicians the physicians were communicated with in regard to the nature of the illness. Throughout this study all physicians practicing, and all persons residing, in the sections were requested by circular letter to inform Doctor Roberts when any cases suspected to be typhoid fever developed.

Map No. 1 shows the location of the blocks selected for this intensive study. These blocks represent fairly the average sanitary and social conditions of the city of Washington.

Upon our request, Dr. L. O. Howard, Chief of the Bureau of Entomology, U. S. Department of Agriculture, and consulting entomologist to the United States Public Health and Marine-Hospital



Map No. 1.—MAP OF WASHINGTON, SHOWING LOCATION OF CITY BLOCKS IN WHICH AN INTENSIVE STUDY OF TYPHOID FEVER WAS MADE FROM JULY 15 TO OCTOBER 24, 1908.

Blocks shaded red.

Service, also made a special study of the fly abundance in these selected blocks. For the results of this intensive study of 32 city blocks in relation to bacillus carriers, see page 114.

IMPORTED CASES.

In determining the number of cases in which the infection was contracted out of the District of Columbia the same method was followed as in our study of the cases in 1907 and as published in Report No. 2, Hygienic Laboratory Bulletin No. 44.

The cases fall under the following heads:

	Number of cases.
(*a*) Infection undoubtedly contracted out of the District of Columbia	103
(*b*) Infection probably contracted out of the District of Columbia	20
(*c*) Infection contracted in or out of the District of Columbia, chances about equal	33
(*d*) Infection undoubtedly contracted in the District of Columbia	438
(*e*) Infection probably contracted in the District of Columbia	71
Total	665

Considering the cases on the percentages of chances of the infection having been contracted out of the District of Columbia, they were as follows:

	Cases.
(*a*) 103 cases, chances 100 per cent	103
(*b*) 20 cases, chances 75 per cent	15
(*c*) 33 cases, chances 50 per cent	17
(*d*) 438 cases, chances 0 per cent	0
(*e*) 71 cases, chances 12.5 per cent	9
665 cases. Total	144

On this estimate, about 22 per cent of the 665 cases investigated by us this year contracted the infection out of the District of Columbia as against about 26 per cent of the 670 cases in 1907, and about 15 per cent of the 866 cases in 1906.

No detailed epidemiological investigation was made of the cases which were considered as certainly or almost certainly having contracted the infection out of the District. Therefore, most of the data in this report refer to the 542 cases comprised under the heads c, d, and e of the above classification.

RACE.

Three hundred and sixty-eight, or 67.9 per cent, of the cases were among whites, and 174, or 32.1 per cent, among negroes.

According to the police census for the spring of 1908 the population of the District of Columbia consists of 241,920 whites and 97,483 negroes, a total of 339,403. Thus negroes compose 28.73 per cent of the population, while the cases of typhoid fever among them composed 32.1 of the total number reported, showing this year

a somewhat higher morbidity rate among negroes than among whites. In the corresponding period (May 1 to November 1) of 1907 the conditions were reversed. The negroes then comprised 29.18 per cent of the population and furnished only 26 per cent of the typhoid-fever cases.

In the 1906 period (June 1 to November 1) negroes composed 29.1 per cent of the population and furnished 32.1 per cent of the typhoid fever cases.

For a full discussion of the racial distribution of cases for the calendar year, see page 26, under "Prevalence."

NATIONALITY.

The following table gives the nationality of the cases, along with the number of foreign-born, in the District of Columbia for 1900, according to the United States census report. For some of the nationalities the population has no doubt changed considerably since 1900, so that the figures in the table are not strictly comparable:

Nationality.	Number of cases.		Number of native and foreign-born in District of Columbia, United States Census, 1900.
	1908.	1907.	
American	522	495	258,599
Austrian	2	0	187
Danish	0	1	88
English	2	3	2,299
French	1	0	389
German	3	5	5,857
Hungarian	0	1	48
Irish	1	5	6,220
Italian	5	11	930
Russian	3	1	807
Scotch	1	0	574
Swedish	2	1	234
Total	542	523	.....

SEX.

Of the 542 cases, 314 occurred in males and 228 in females. Males compose about 48 per cent and females about 52 per cent of the population of the District of Columbia; 59.77 per cent of the cases were among males, showing, as was the case in the groups studied by us in 1906 and 1907, a somewhat higher typhoid-fever morbidity rate among the males.

The higher rate among males is probably due largely to men being more exposed to infection by occupation habits and the more frequent practice of taking meals at lunch counters, eating houses, restaurants, etc., away from the place of residence. This view is supported by the fact, as shown in the table of ages of the cases given below, that the

greater prevalence of the disease among males falls especially upon those between the ages of 15 and 34 years.

Another condition probably contributing to the higher rate among males is that in the summer months relatively more women than men are away from Washington. Many of the men, after their families are gone, take their meals at various eating places instead of at home and so are exposed to more possible sources of infection in food.

AGES.

The following table gives the cases classified by age and sex, along with the population of the District of Columbia in 1900 (United States Census):

Ages, years.	Number of cases.			Population, 1900.			Ratio to total population.	
	Male.	Female.	Total.	Male.	Female.	Total.	1908.	1907.
0–4	18	7	25	11,683	11,467	23,150	1–926	1–1,052
5–9	39	42	81	11,708	12,023	23,731	1–293	1–293
10–14	47	42	89	10,953	11,781	22,734	1–255	1–234
15–19	59	27	86	71,362	13,452	24,814	1–288	1–335
20–24	52	38	90	13,774	17,736	31,510	1–350	1–398
25–29	35	26	61	13,345	16,410	29,755	1–487	1–419
30–34	33	17	50	11,911	12,857	24,768	1–495	1–619
35–39	11	13	24	10,409	11,472	21,881	1–912	1–810
40–44	10	11	21	8,679	9,255	17,934	1–854	1–2,562
45–49	4	0	4	6,876	7,750	14,626	1–3,656	1–914
50–54	3	2	5	6,371	6,928	13,299	1–2,659	1–6,649
55–59	0	0	0	5,285	5,001	10,286	.....	1–3,428
60–64	2	2	4	4,123	4,078	8,201	1–2,050	1–4,100
65–69	1	1	2	2,431	2,604	5,035	1–2,517	.....
70–74	0	0	0	1,611	1,829	3,440	.....	1–1,720
75–79	0	0	0	796	1,049	1,845	.....	.....
80–84	0	0	0	351	567	918	.....	.....
85–89	0	0	0	132	206	338	.....	.....
90–94	0	0	0	26	73	99	.....	.....
95–99	0	0	0	10	29	39	.....	.....
Over 99	0	0	0	6	14	20	.....	.....
Age not known	0	0	0	162	133	295	.....	.....
Total	314	228	542	132,004	146,714	278,718	.....	.....

The youngest person affected was 19 months and the oldest 69 years.

The figures given of the population in age periods are those of the United States Census report and are the latest returns available. As the census was taken in 1900 and the cases occurred in 1908, the figures from which the ratios are derived are not strictly comparable, but give a fairly accurate estimate.

The largest number of cases proportionate to population again this year, as in 1907, occurred among persons from 10 to 14 years of age. The next highest ratio, in 1907, was in periods from 5 to 9; but this

year it was in persons from 15 to 19. It will be noted that this year, as in 1907, the rate among children under 15 years of age was disproportionately high.

By decades, 106 of the cases were under 10 years of age, 175 between 10 and 20, 151 between 20 and 30, 74 between 30 and 40, 25 between 40 and 50, and 11 over 50.

The special prevalence of the disease among children observed during all three of the years in which we have studied the situation suggests that milk and contact are two important factors in the transmission of the infection in Washington. (See pages 15–17, Report No. 2, Hyg. Lab. Bull. No. 44.)

LENGTH OF RESIDENCE IN THE DISTRICT OF COLUMBIA.

The following table gives the length of residence in the District of Columbia of the persons affected. The cases for 1907 of corresponding duration of residence in the District of Columbia are placed in a parallel column.

Length of residence.	Number of cases.	
	1908.	1907.
Under 6 months	19	20
6 months to 1 year	10	15
1 to 2 years	22	28
2 to 3 years	21	16
3 to 4 years	13	19
4 to 5 years	13	17
5 to 10 years	55	62
10 to 15 years	28	26
15 to 20 years	26	16
20 to 30 years	19	18
30 to 40 years	6	7
40 to 50 years	2	4
50 to 60 years	1	1
All of life	294	239
Not determined	13	35
Total	542	523

The ages of the persons affected in the 1908 period and who had resided in the District all of their lives were as follows:

Ages.	Cases.	Ages.	Cases.
0 to 4 years	22	35 to 39 years	8
5 to 9 years	63	40 to 44 years	0
10 to 14 years	65	45 to 49 years	2
15 to 19 years	59	50 to 54 years	2
20 to 24 years	36	65 to 69 years	1
25 to 29 years	21		
30 to 34 years	15	Total	294

Over 50 per cent of the cases occurred among persons who had resided in the District of Columbia all their lives. So this year, as in 1907, the disease does not seem to have prevailed to any very unusual extent among newcomers.

However, it should be noted that in both years the number of cases among persons who had resided in the District less than 5 years is much larger than (nearly twice) the number among persons who had resided in the District from 5 to 10 years, and over three times as large as the number among those who had resided in the District from 10 to 15 years.

In considering these figures it should be remembered that the transient element of Washington's population is rather large, and that among persons having resided here less than 10 years there is of course a large proportion of children.

PREVALENCE.

The following table shows the cases according to dates of definite onset:

Onsets by days.

Day.	Apr.	May.	June.	July.	Aug.	Sept.	Oct.
1	0	1	0	0	2	3	11
2	0	2	0	6	8	5	4
3	0	0	2	5	5	4	5
4	0	1	2	5	6	1	2
5	0	1	2	1	3	6	5
6	0	3	0	2	9	3	10
7	0	3	0	5	1	6	6
8	1	1	0	6	2	1	3
9	0	0	1	4	3	3	10
10	0	3	3	4	3	5	6
11	1	0	1	3	2	6	2
12	0	3	3	6	4	1	3
13	0	1	2	3	4	2	5
14	0	0	3	1	9	3	5
15	0	2	0	3	3	4	3
16	0	1	5	3	4	3	3
17	0	2	3	2	3	0	4
18	3	1	5	9	3	4	3
19	0	0	2	2	5	1	1
20	2	1	6	7	7	2	2
21	0	0	3	2	4	2	0
22	0	0	0	2	3	1	4
23	1	1	3	3	3	3	0
24	0	3	1	4	3	4	0
25	1	0	3	4	2	2	0
26	2	5	2	3	4	4	0
27	0	0	4	5	1	1	0
28	0	1	2	2	3	1	1
29	1	1	0	6	6	2	0
30	2	3	6	4	6	7	0
31	.....	0	.....	3	0	.....	0
Total cases (542)	14	40	64	115	121	90	98

As in our previous reports the cases are tabulated and charted according to date of onset of definite symptoms, it having been found that in the majority of instances accurate statements as to date of onset of the first slight symptoms were not obtainable.

In view of the generally accepted period of incubation for typhoid fever, a period of about 15 to 21 days previous to date of definite onset as given probably will represent for the majority of the cases the time at which infection occurred.

If the period of incubation of typhoid fever were constant, epidemiological studies of the disease would be simplified to a great extent; but the period seems to be quite variable when compared with that of certain other diseases, such as vaccinia, smallpox, measles, etc. Furthermore, it is difficult to definitely define the incubation period of typhoid fever, as some persons apparently begin to have symptoms almost immediately upon receiving the infection, while others go at least three weeks or perhaps longer before having symptoms. Then there is the period of prodromal symptoms, which varies greatly in duration in different cases. So that knowing the time that a patient has developed definite symptoms or has taken to bed enables us to estimate but roughly in individual cases just when the infection was contracted.

It has been observed that when large amounts of concentrated infection are ingested, as in milk outbreaks, the period of incubation is apparently shortened and in many cases prodromal symptoms are absent. It is possible, therefore, that if very small quantities of infection are ingested the period of incubation and the period of prodromes in some cases may be very much prolonged.

Judging by observations made in pronounced outbreaks attributed to infection in water, the evidence is strongly against the majority of the cases having very much prolonged incubation periods. The outbreak at Scranton, Pa., reported by Wainwright[a] offers a striking example. There the active period of the epidemic (by report of cases) ceased abruptly about 25 days after the infected water supply was shut off. Considering the volume of water implicated at Scranton it does not seem reasonable to suppose that the individuals affected there could have received very large doses of infection; that is, assuming that the typhoid bacillus does not multiply in water under certain conditions.

The supposition that typhoid bacilli at times may undergo tremendous multiplication in water under natural conditions is not supported by experimental evidence. Of course it is possible that only the highly resistant bacilli survive in water, causing pronounced outbreaks, and consequently a very small number—perhaps only one—of such organisms may in some instances undergo rapid multiplication after getting into the alimentary canal of man and cause infection.

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[a] N. Y. Med. Rec., June 1, 1907.

Varying degrees of virulence (and infectiveness) of different strains of typhoid bacilli may be the explanation of some of the peculiar features of typhoid fever observed in epidemiological studies.

It may be that in communities where typhoid fever prevails endemically the period of incubation varies within much wider limits than is the case in epidemics when a large number of persons get the infection from a common source, and all presumably from the same strain of organism.

The striking seasonal prevalence of typhoid fever observed in Washington and in many other endemic foci may possibly find its explanation as follows: The infectious organisms from various sources disseminated through the community by water, milk, raw shellfish, personal contact, flies, salads, etc., during the winter and spring months are, when ingested by persons, incapable of causing the disease for the majority of the cases at that time; but they become stored in the bodies of these persons and with the advent of warm weather other factors become operative and disease results.

It is known that persons may carry the typhoid bacillus for years without clinical evidence of typhoid fever. It is conceivable that such persons may finally contract the fever. In fact, Davies and Hall[a] report a carrier (Mrs. H.) who apparently caused three outbreaks of enteric fever and who finally had herself an irregular febrile diarrheal affection which the authors considered to be typhoid fever. If this diagnosis was correct it means that Mrs. H. had a "period of incubation" of at least three years; that is, the typhoid bacilli were contained in her intestinal tract for a period of three years before she herself contracted the disease.

Such occurrences may explain the development of the case at the Government Hospital for the Insane (see p. 35).

The following table shows the progress of the disease as indicated by dates of onset of the cases in periods of a half month.

The number of cases for corresponding periods in 1907 and in 1906 are placed in parallel columns.

Period.	1908.	1907.	1906.
April 1–15, incomplete	2	.....	.....
April 16–30, incomplete	12	.....	.....
May 1–15, complete	21	15	.....
May 16–31, complete	19	12	.....
June 1–15, complete	19	15	32
June 16–30, complete	45	27	32
July 1–15, complete	54	39	101
July 16–31, complete	61	63	131
August 1–15, complete	64	99	139
August 16–31, complete	57	67	70
September 1–15, complete	53	54	59
September 16–30, complete	37	54	41
October 1–15, incomplete	80	52	98
October 16–31, incomplete	18	17	22

[a] Lancet, November 28, 1908, p. 1585.

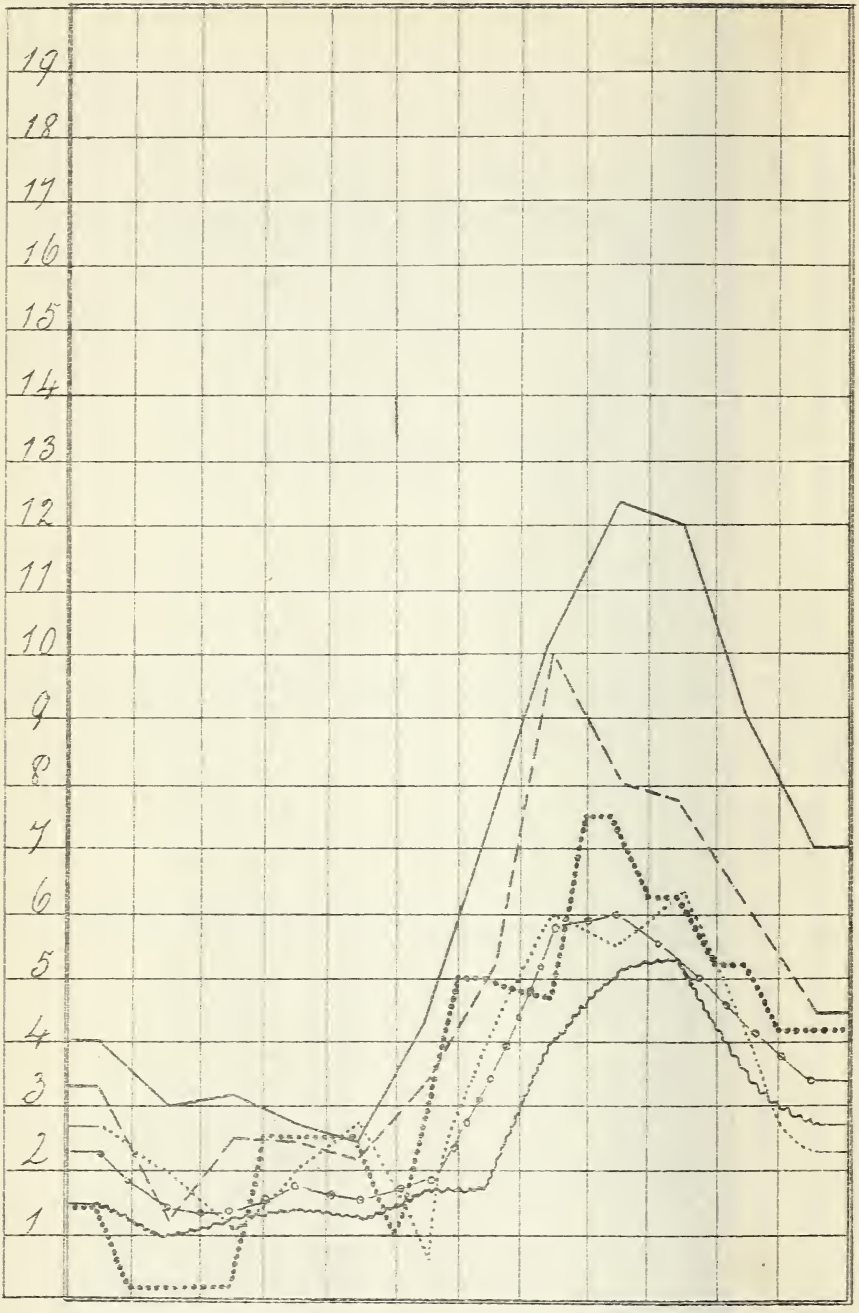

CHART NO. 3, SHOWING MONTHLY DEATH RATE PER 100,000 OF POPULATION FROM TYPHOID.

The average population computed on the Twelfth Census is approximately:

Washington (1885–1897)	240,000
Washington (1898–1907)	300,000
Washington (1907)	300,000
Baltimore (1888–1897)	470,000
Boston (1888–1897)	500,000

During the three months July, August, and September there were in 1906, 541 cases; in 1907, 376 cases; in 1908, 326 cases. In the preceding month of June, 1906, 64 cases; 1907, 42 cases; 1908, 64 cases.

The rate increased quite sharply in the latter half of June, and reached its height early in July, continued high until the early part of September, when there was a marked decrease in the rate.

The sharp rise in the curve in October was due largely to the occurrence of a pronounced milk outbreak, which was responsible for about 45 of these cases.

Chart No. 1 shows by curves the rate of prevalence of the disease for corresponding periods of the three years 1906, 1907, and 1908.

Chart No. 2 shows the cases by dates of onset and by attributed causation.

It is interesting to note that the pronounced summer rise in the curve has become decidedly less each year. The curves for 1907 and 1908 differ in that the rise in 1908 occurred earlier and the high rate continued more uniformly and later—until the early part of September.

The marked rise from about July 20 to August 20 which occurred in 1907 and 1906 was absent in 1908. (See Chart No. 1.)

In the summer months—June, July, and August—of 1908 there occurred 300 cases, as against 310 in the same peroid of 1907 and 505 in the same period in 1906.

For what may be termed the typhoid months here—July, August, and September—there developed in 1908 326 case; in 1907, 376 cases; and in 1906, 541 cases. It is evident that for the summer and early fall the disease was decidedly less prevalent in 1908 and in 1907 than in 1906. In 1908 the prevalence was slightly less than in 1907.

It will be noted (Chart No. 1) that, though the typhoid-fever curve has changed considerably in height for the different years, it has shown in each of the three years a marked summer rise. In other words, the difference has been more in degree than in character.

Chart No. 3 shows by curves the monthly death rate from typhoid fever since 1888 in Washington, Baltimore, and Boston.

While rather marked differences occurred in the prevalence of the disease in Washington for the warm season of the three years, the rates for the winter, spring, and late fall seasons were remarkably parallel. (See table, p. 38.) Thus the total cases reported during January, February, March, April, May, October, November, and December of the three years were as follows: In 1908, 449 cases; 1907, 451 cases; 1906, 444 cases.

It is evident, therefore, that the difference in rate for the three years has been due to the difference in extent of operation of some

factors, or perhaps of some one factor, which exert their effect particularly in warm weather.

The cases definitely attributed to infection in milk were not distributed uniformly in regard to these seasons for the three years. They were as follows:

Year.	Onset of cases.	Number of cases.	
1908	September 24 to October 24	52	
1907	June 8 to August 17	28	
1906	July 16 to August 20	35	79
	July 30 to August 13	12	
	October 2 to October 21	32	

It is interesting to note that there was a marked difference in the prevalence of the disease among the white and the colored races for the warm and cool seasons of 1908 and 1907.

In 1908 there were reported in the cool season (January, February, March, April, May, October, November, and December) 449 cases, of which 95 were colored (cases to population, 0.97 per 1,000) and 354 white (cases to population, 1.46 per 1,000), ratio of colored to white being 1 to 3.7.

In the warm season (June, July, August, and September) of 1908 there were reported 487 cases, of which 167 were colored (cases to population, 1.71 per 1,000) and 320 white (cases to population, 1.32 per 1,000), the ratio of colored to white being 1 to 1.9.

In 1907 there were reported in the cool season 451 cases, of which 98 were colored and 353 white, ratio of colored to white being 1 to 3.6.

In the warm season of 1907 there were reported 494 cases, of which 112 were colored and 382 white, ratio of colored to white being 1 to 3.4.

It appears from these figures that there was little difference in the ratios of colored to white cases for the two seasons of 1907; but if that year is divided into periods—one including the eight months January, February, March, April, May, June, November, and December and the other the four months July, August, September, and October—there is a marked difference in the ratios for these two periods.

Thus, in the former (cooler) of these periods there were reported 58 colored cases (cases to population, 0.6 per 1,000) and 281 white cases (cases to population, 1.2 per 1,000), the ratio of colored to white being 1 to 4.8.

In the latter (warm) of these periods there were reported 155 colored cases (cases to population, 1.61 per 1,000) and 454 white cases

(cases to population, 1.94 per 1,000), the ratio of colored to white being 1 to 2.9.

The effect of changing the position of the months of June and October in the divisions of the year to bring out the strikingly comparable ratios for the two years is of especial interest when considered in connection with the fact that in 1907 the beginning of the summer weather was delayed, June being quite a cool month for this section of the country. (See Chart No. 4.)

The mean temperatures of June for the past five years were as follows:

June, 1904	76° F.
June, 1905	71. 8° F.
June, 1906	75. 2° F.
June, 1907	65. 9° F.
June, 1908	71. 8° F.

Therefore, by having June and October fall in one division of the year for 1908 and in another for 1907 we have the two years divided into periods of much more nearly parallel temperature curves.

It was evident that during the warm weather of 1908 and 1907 the proportion of cases among the colored population was much higher than it was in the cool weather of those years.

In 1906 also the proportion among the colored in warm weather was higher, but not as strikingly so. Thus in January, February, March, April, May, June, November, and December there were reported 239 white cases and 92 colored cases, the ratio of colored to white being 1 to 2.4; while in July, August, September, and October there were reported 537 white and 238 colored cases, the ratio of colored to white being 1 to 2.2.

On dividing the year into periods to correspond in months with those given above for 1908, there were reported in 1906 during the cool season (January, February, March, April, May, October, November, and December) 445 cases, of which 109 were colored (cases to population, 1.14 per 1,000) and 336 white (cases to population, 1.45 per 1,000), the ratio of colored to white being 1 to 3.

While in the warm season (June, July, August, and September) there were reported 681 cases, of which 221 were colored (cases to population, 2.32 per 1,000) and 460 white (cases to population, 1.98 per 1,000), the ratio of colored to white being 1 to 2.

In 1905 the ratio of colored to white cases for the first of the above periods was 1 to 2.8 and for the second period (June, July, August, and September) 1 to 2.5.

In 1904 the ratio of colored to white cases for the cool season was 1 to 3.3, and for the warm season (June, July, August, and September) 1 to 2.3.

Summarized, the figures are as follows:

TABLE No. 4.—*Showing racial prevalence in relation to season.*

	Case rate per 1,000 of population.	Ratio of colored to white cases.	Ratio of colored to white population.
1908.			
Cool months—January, February, March, April, May, October, November, December:			
White	1.46	1 to 3.7	
Colored	.97		
Warm months—June, July, August, September:			1 to 2.4
White	1.32	1 to 1.9	
Colored	1.71		
1907.			
Cool months—January, February, March, April, May, June, November, December:			
White	1.20	1 to 4.8	
Colored	.60		
Warm months—July, August, September, October:			1 to 2.4
White	1.94	1 to 2.9	
Colored	1.61		
1906.			
Cool months—January, February, March, April, May, October, November, December:			
White	1.45	1 to 3	
Colored	1.14		
Warm months—June, July, August, September:			1 to 2.4
White	1.98	1 to 2	
Colored	2.32		
1905.			
Cool months—January, February, March, April, May, October, November, December:			
White	1.29	1 to 2.8	
Colored	1.07		
Warm months—June, July, August, September:			1 to 2.3
White	2.20	1 to 2.5	
Colored	2.05		
1904.			
Cool months—January, February, March, April, May, October, November, December:			
White	1.47	1 to 3.3	
Colored	1.02		
Warm months—June, July, August, September:			1 to 2.3
White	1.82	1 to 2.3	
Colored	1.84		

These figures show that during the cool season typhoid fever in the District of Columbia is relatively more prevalent among the whites and that this relatively higher prevalence among the whites has been more marked during the past two years (1907 and 1908).

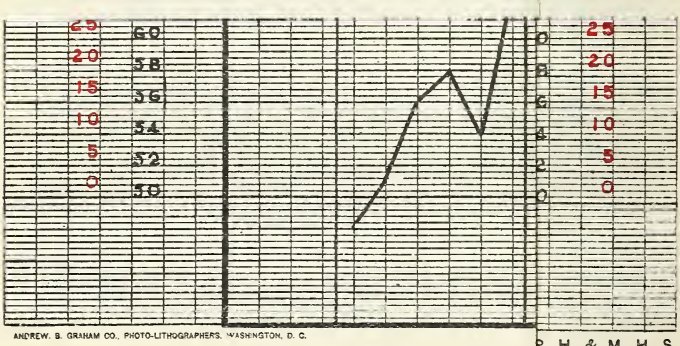

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Chart No. 4.—SHOWING RELATION O COLUMBIA.

RELATION OF INCREASED TEMPERATURE TO INCREASED PREVALENCE OF TYPHOID FEVER.

Chart No. 4 shows in curves the relation of temperature to the incidence of typhoid fever in the District of Columbia for the periods (May 1 to November 1) of the three years 1906, 1907, and 1908.

The temperature curve is compiled from figures furnished us by the United States Weather Bureau and based on the mean daily temperature in five-day periods.

It should be noted that in 1906 the typhoid curve rose to reach its maximum about a month subsequent to, and roughly paralleled, the rise in the temperature curve. As the typhoid curve would have to be set back probably about three weeks in order for it to represent the cases by time of probable infection the relation of the rise in this curve to that of the temperature is striking. The typhoid curve, however, begins to decline quite sharply in the early part of August and the decline continues through the remainder of August and through September, while the temperature curve reaches its maximum early in July and from then continues at about the same height until the latter part of September, which was about six weeks after the marked decline in the typhoid fever rate had begun.

From this it appears that the increasing causation of typhoid fever occurred coincidently with the increasing temperature; but there was a marked decrease in the causation of typhoid fever about ten weeks prior to the decline in temperature, and therefore independent of the continued high temperature.

It should be borne in mind, however, that the fall in the typhoid fever rate before that in the summer temperature may have been due to the majority of the susceptible persons in the community already having had the disease.

In 1907 the marked increase in the incidence of cases occurred about a month subsequent to the marked rise in temperature. It is interesting to note that in this year the rise in both the temperature and the typhoid curve occurred about a month later than that of 1906 and 1908. If the typhoid curve for the 1907 period were set back about a month, the rises and falls in the typhoid and temperature curves would present a rather striking parallelism.

If the same thing is done for the 1908 period, the parallelism in the rises and falls of the typhoid and temperature curves is for that year also quite striking.

The seasonal (summer) prevalence of typhoid fever observed here may be due to the summer temperature being followed by one or both of the following conditions:

1. Increased distribution of the infection in the community (seed).
2. Increased susceptibility of persons to the disease (soil).

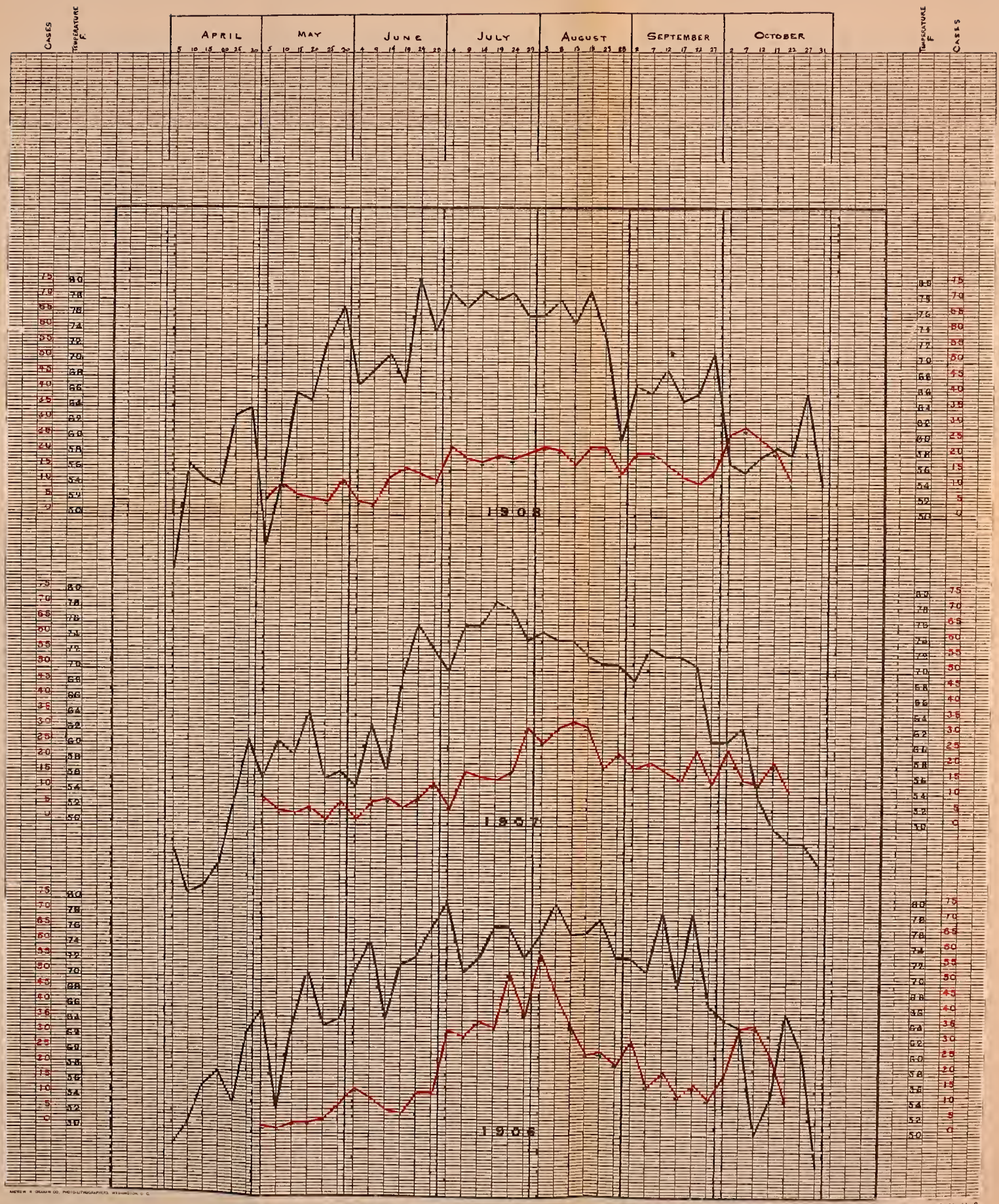

Chart No. 4.—SHOWING RELATION OF SUMMER TEMPERATURE TO THE PREVALENCE OF TYPHOID FEVER IN THE DISTRICT OF COLUMBIA.

——— = Temperature curve in five-day means.

——— = Cases of typhoid fever, by date of definite onset, in five-day periods.

In view of the present incomplete knowledge of the subjects of both seed and soil in the etiology of typhoid fever it is not possible to state positively which is the more concerned in the increased summer incidence of the disease.

FLIES.

The part played by flies in the transmission of typhoid-fever infection has been a subject of much discussion in recent years, particularly since the publication of the report of the army commission.[a]

Among the many suggested explanations of the typhoid fever in Washington, flies have been presented as being perhaps one of the major factors in the spread of the disease. Believing that, if this were so, the seasonal abundance of flies should show a relation to the seasonal prevalence of typhoid fever, we, at the beginning of our work in 1908, requested Prof. L. O. Howard, chief of the Bureau of Entomology, Department of Agriculture, to have made a study of the flies so that their abundance could be correlated in curves with the prevalence of typhoid fever.

The following excerpt of a letter from Professor Howard to us outlines the method followed in making the study:

> Our collections were started on June 19 and closed on October 19, 1908. Members of the Bureau of Entomology were supplied with sheets of fly paper, with instructions to expose a double sheet every other day, except Sunday, for a period of 48 hours. The sheets were then returned to the bureau and the flies carefully counted and recorded, together with such data as could be secured concerning conditions of near-by stables and manure dumps. Certain parts of the city among the slums and along the water front were not reached by members of the bureau just mentioned. Therefore, two assistants were especially employed and made regular rounds on bicycles, furnished for the purpose, collecting fly-laden sheets and distributing fresh fly paper three times a week at a large number of stations selected so as to supplement the stations already established by regular employees of the bureau, and thus to cover practically the whole city within the city limits. Absence of the Sunday records was allowed for where exposure was made in residences by recording only two-thirds of the Monday morning catch. In the case of fly papers exposed in meat shops and restaurants it was found that flies were usually so plentiful that the maximum catching capacity of the paper was reached in 48 hours. In all, sixty-two stations were located throughout the city during the progress of the experiment. But, on summing up the records, it was found that only twenty-three stations were sufficiently continuous to be of value in tabulating the results. Of these, there were eight in the northwest, five in the southwest, six in the southeast, and four in the northeast.

Charts Nos. 5 and 6 were prepared and furnished us by Professor Howard. Chart No. 5 shows the abundance of flies ascertained for the different sections of Washington. Chart No. 6 shows the relation seasonally of the abundance of flies to the prevalence of typhoid fever.

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[a] Reed, Walter, Vaughan, Victor C., and Shakespeare, E. O.: Report on the origin and spread of typhoid fever in the U. S. military camps during the Spanish war of 1898. Washington, Govt. Printing Office, 1904. Vol. 1, 721 p.; Vol. 2, charts.

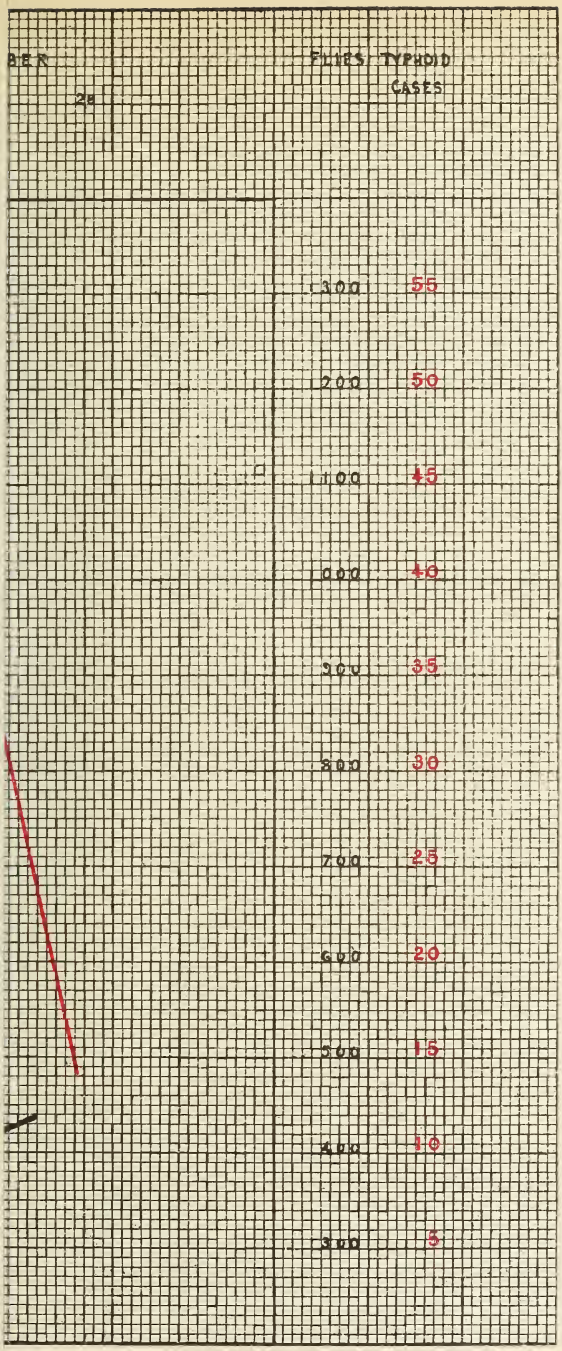


Chart N THE PREVALENCE OF TYPHOID FEVER.

accounted-for cases being charted.

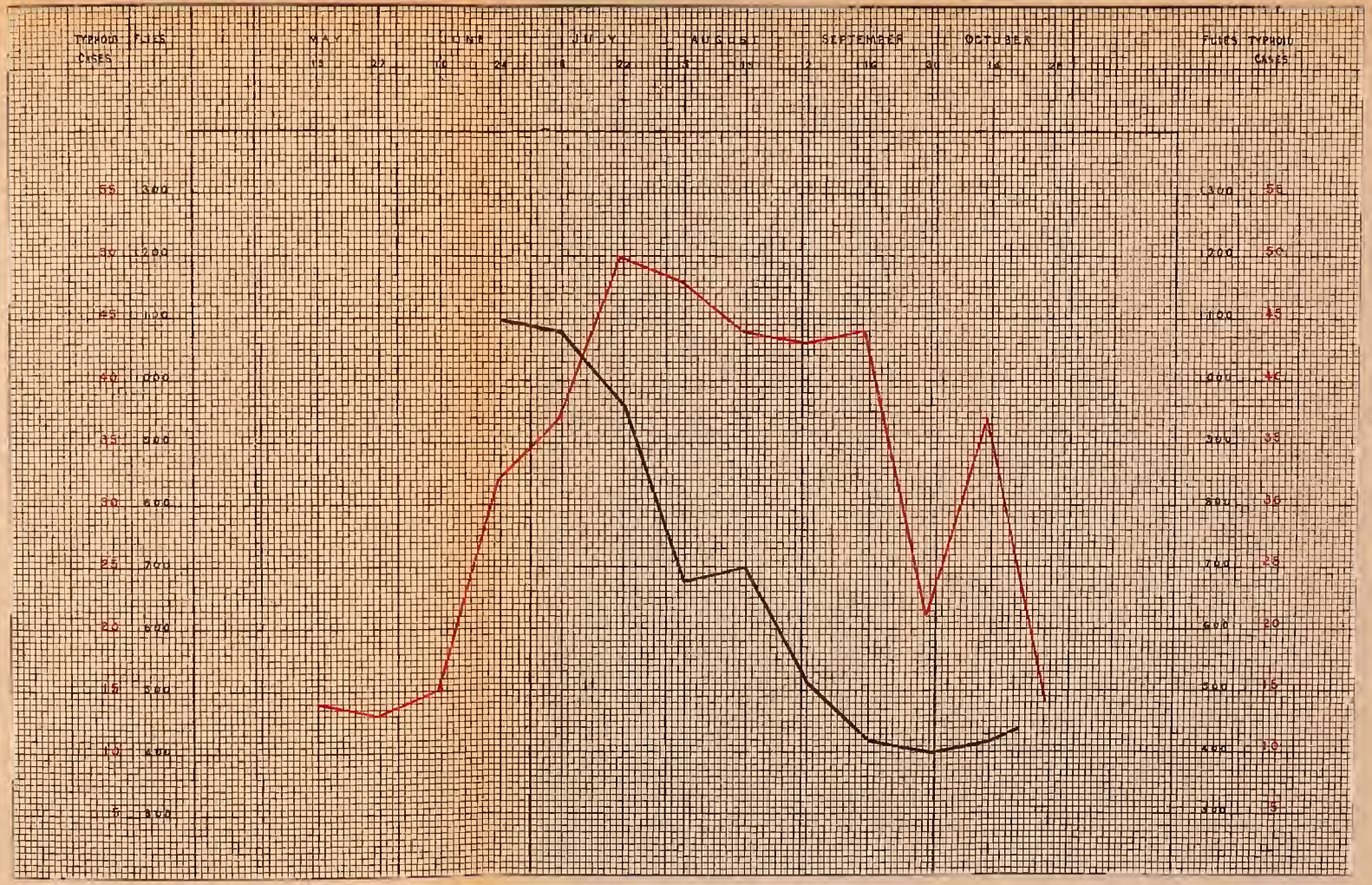


Chart No. 7.—SHOWING, BY CURVES, ABUNDANCE OF FLIES (*MUSCA DOMESTICA*) IN RELATION TO THE PREVALENCE OF TYPHOID FEVER.

HORNE & GRAHAM CO., PHOTO-LITHOGRAPHERS, WASHINGTON, D. C.

Cases due to direct contact with previous cases and those due to milk infection are not included, only the unaccounted-for cases being charted.

——— Seasonal abundance of flies, fourteen-day averages (Howard's data).

——— Seasonal prevalence ot typhoid fever, fourteen-day averages (our data).

It will be observed that the flies were at their maximum abundance about July 1. The number from that time diminished fairly uniformly from week to week until August 5, when the average number caught was only about one-half as much as the average on July 1. The number rose for the week ended August 12, and then continued to fall through the remainder of August and the greater part of September. A rise in the curve is noted for the two weeks ended October 21.

The typhoid fever curve (see Chart No. 1) reached its maximum about July 1 and continued along at about the same height until about the 10th of September, when a decline began which continued fairly uniform until the latter part of September. The rise in the curve in the latter part of September and the early part of October was due largely to a milk outbreak (see p. 102).

Chart No. 7, prepared by us, shows the fly abundance and the number of cases of typhoid fever in periods of two weeks. In this chart the cases of typhoid fever attributed to milk infection and to direct contact with previous cases are eliminated, only the cases due to causes undetermined being counted. By lengthening the periods many of the sharp turns in the curves as presented on charts Nos. 5 and 6 are removed and the relation of the curves can be followed with better understanding.

As the cases are charted by date of definite onset, probably about three weeks subsequent to the time of infection for the majority of the cases, the curve for the abundance of flies, if moved forward three weeks, should parallel the curve of prevalence of typhoid fever, provided a relation of cause and effect existed. If this is done, the parallelism certainly is not sufficiently striking to warrant the conclusion that flies play much of a rôle in the transmission of typhoid fever in Washington.

The chances of dissemination of infection by flies in a well-sewered city of course are much less than in a camp when proper care is not taken in the disposal of excreta. It is difficult to estimate what part flies play in the spread of the disease in Washington, but the evidence is quite strong that it is relatively a small part. That flies are a minor factor for the city as a whole does not preclude the possibility that in certain limited localities they from time to time may spread the infection. Therefore, in carrying out preventive measures against typhoid fever, the number of flies should be lessened by a proper control of their breeding places, and their access to infectious matter and food stuffs prevented.

GEOGRAPHICAL DISTRIBUTION.

Maps 2, 3, 4, 5, 6, 7, and 8 show the distribution of the cases by place of residence when the infection was considered to have been contracted.

Again this year, as in 1907 and 1906, the distribution was quite general; but there was in 1908 a noticeable concentration of cases in certain sections.

The marked grouping of cases in the Georgetown section of the city, which occurred in September and October, was due to a milk outbreak which was confined largely to that section.

Smaller groups of cases, attributed largely to contact infection, may be noted on the maps in south Washington, in Twining City, and in Ivy City. These smaller groups are in sections where the general sanitary conditions are poorer than they are in the greater part of the city.

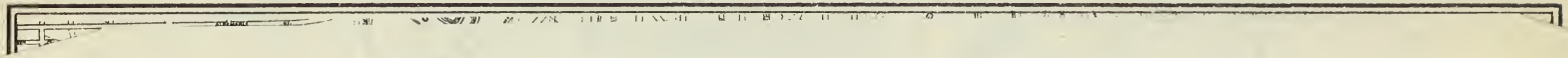
Apart from the local grouping of cases, there appeared to be in the southern sections of Washington as a whole a higher prevalence of typhoid fever due to causes undetermined than in the northern sections. In fact, a general survey of the distribution of the cases gives the impression that typhoid fever in Washington is caused by factors some of which operate generally and scatter infection broadcast over the city, while others operate locally and cause the small groups of cases observed to occur in certain sections.

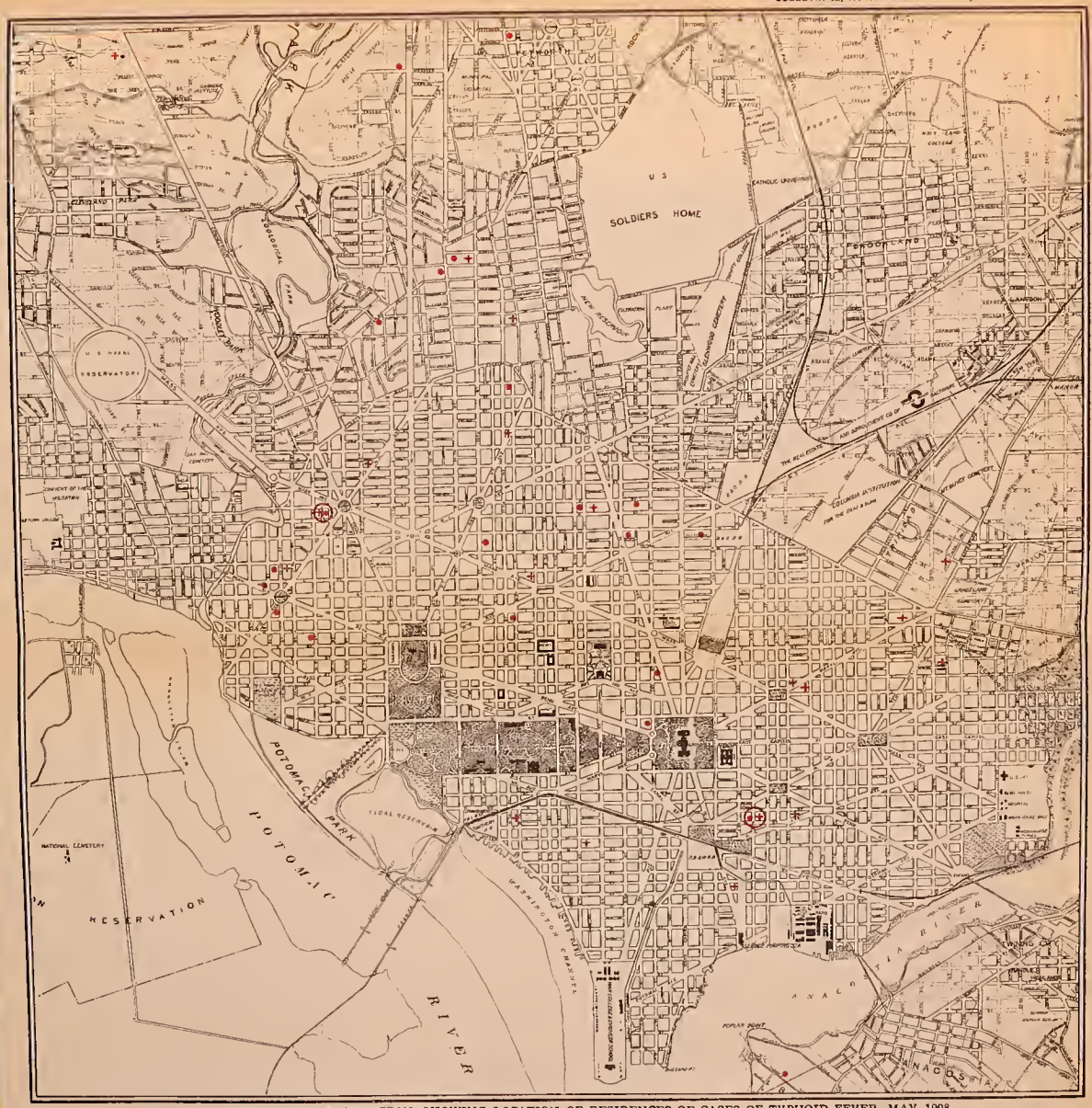
Map No. 8 shows the distribution of cases by place of residence in the 53 vital statistical districts of the District of Columbia.

The following table gives the population and the number of cases of typhoid fever which developed from May 1 to November 1, 1908, in each of the vital statistical districts:

TABLE No. 5.—*Showing population and incidence of typhoid fever in each vital statistical district in the District of Columbia.*

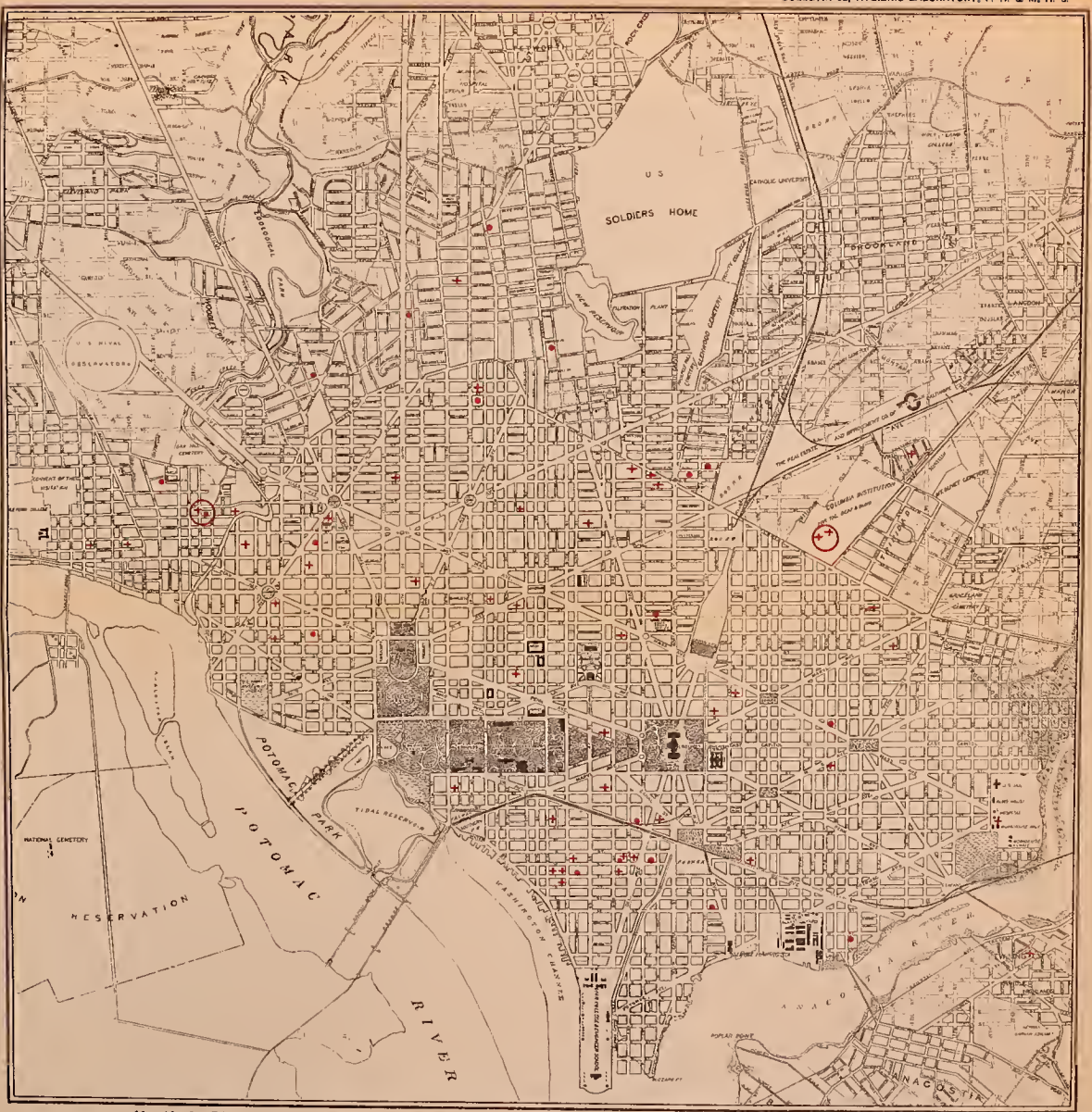
Vital statistical districts.	Population.			Number of cases of typhoid fever developing from May 1 to November 1, 1908.				Case rate per 10,000 of population.
	White.	Colored.	Total.	Unaccounted for.	Attributed to direct contact.	Attributed to milk infection.	Total.	
1	11,710	6,105	17,815	13	0	1	14	7.8
2	6,392	4,994	11,386	9	0	2	11	9.6
3	7,638	3,416	11,054	21	2	1	24	21.6
4	663	2,108	2,771	1	0	1	2	7.2
5	10,276	8,697	18,973	15	4	0	19	10.1
6	15,487	3,285	18,772	10	0	0	10	5.3
7	9,522	978	10,500	12	2	0	14	13.3
8	14,884	2,626	17,510	32	4	0	36	20.5
9	13,192	11,872	25,064	29	2	0	31	12.3
10	13,397	4,034	17,431	17	3	0	20	11.4





Map No. 2.—THE CITY OF WASHINGTON, SHOWING LOCATION OF RESIDENCES OF CASES OF TYPHOID FEVER, MAY, 1908.

● Cases having definite onset May 1-15.
✚ Cases having definite onset May 16-31.
Two or more dots or crosses in a single circle indicate two or more cases in the same house.
Imported cases not charted.

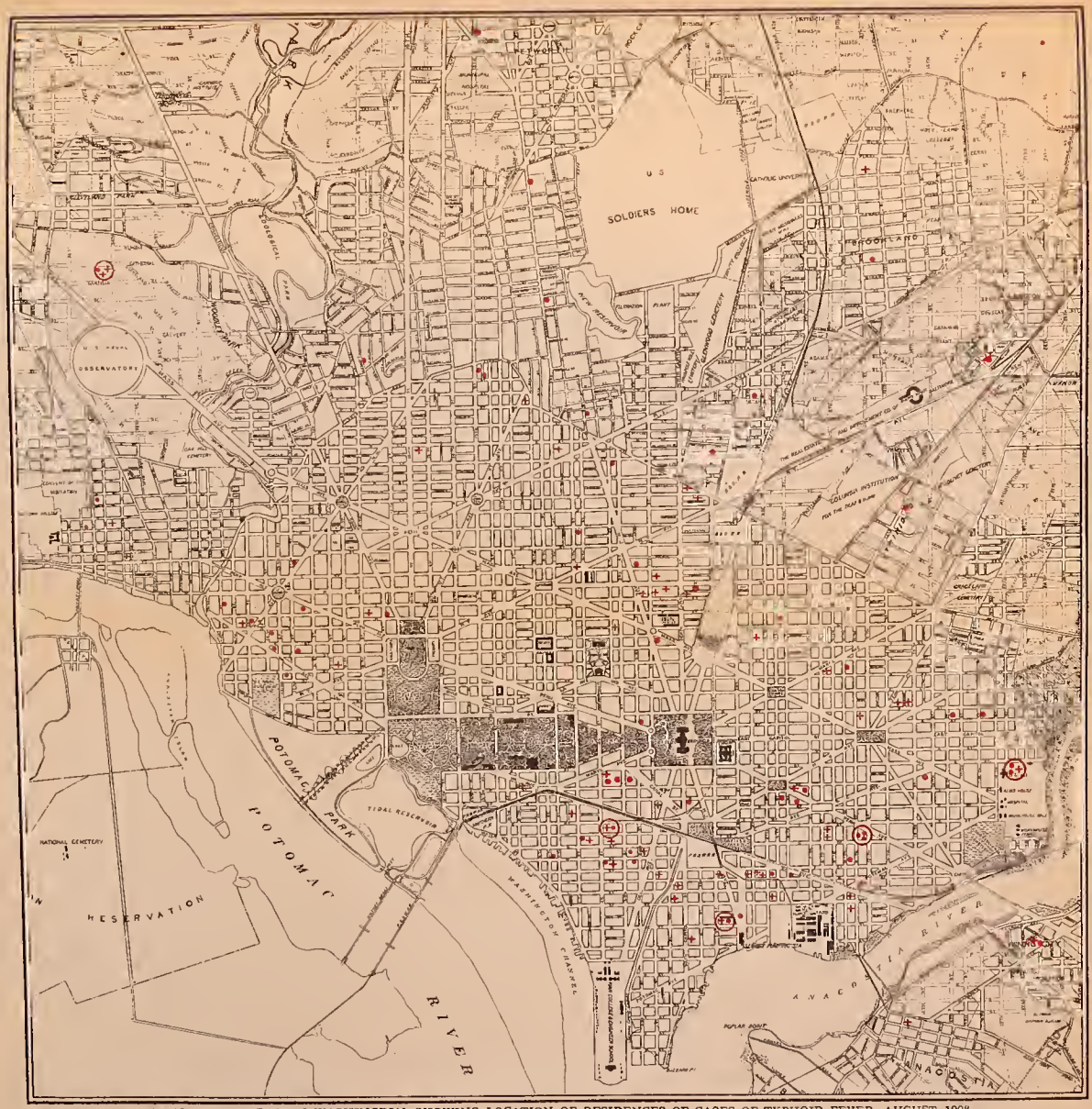


Map No. 3.—THE CITY OF WASHINGTON, SHOWING LOCATION OF RESIDENCES OF CASES OF TYPHOID FEVER, JUNE, 1908.

● Cases having definite onset June 1-15.
+ Cases having definite onset June 16-30.
Two or more dots or crosses in a single circle indicate two or more cases in the same house.
Imported cases not charted.

Map No. 4.—THE CITY OF WASHINGTON, SHOWING LOCATION OF RESIDENCES OF CASES OF TYPHOID FEVER, JULY, 1908.

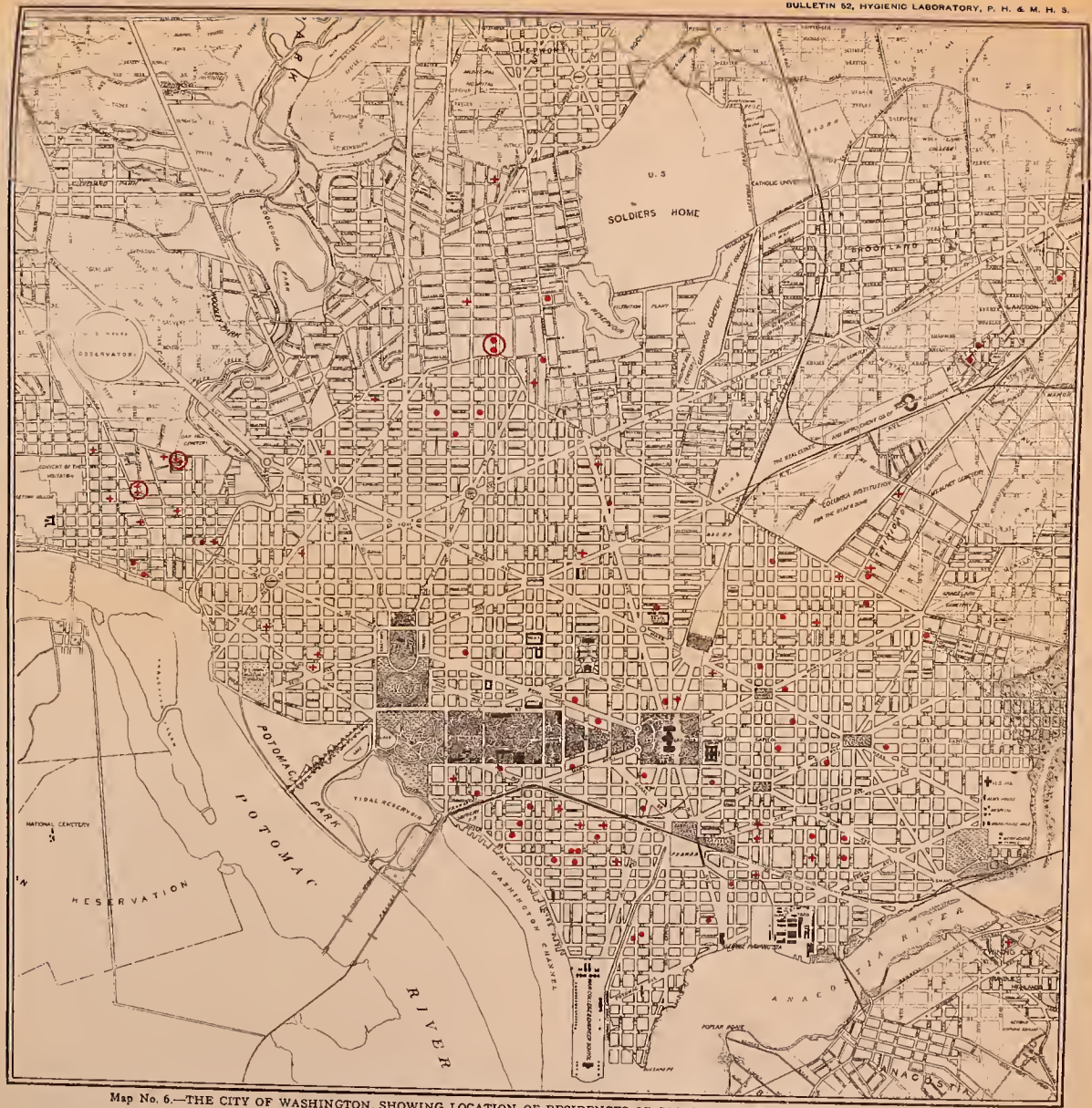
● Cases having definite onset July 1-15.
✚ Cases having definite onset July 16-31.
Two or more dots or crosses in a single circle indicate two or more cases in the same house.
Imported cases not charted.



Map No. 5.—THE CITY OF WASHINGTON, SHOWING LOCATION OF RESIDENCES OF CASES OF TYPHOID FEVER, AUGUST, 1908.

- Cases having definite onset August 1–15.
- Cases having definite onset August 16–31.

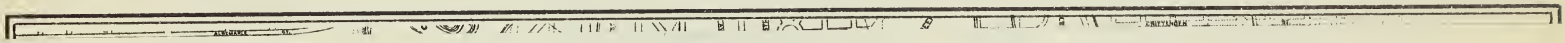
Two or more dots or crosses in a single circle indicate two or more cases in the same house.
Imported cases not charted.

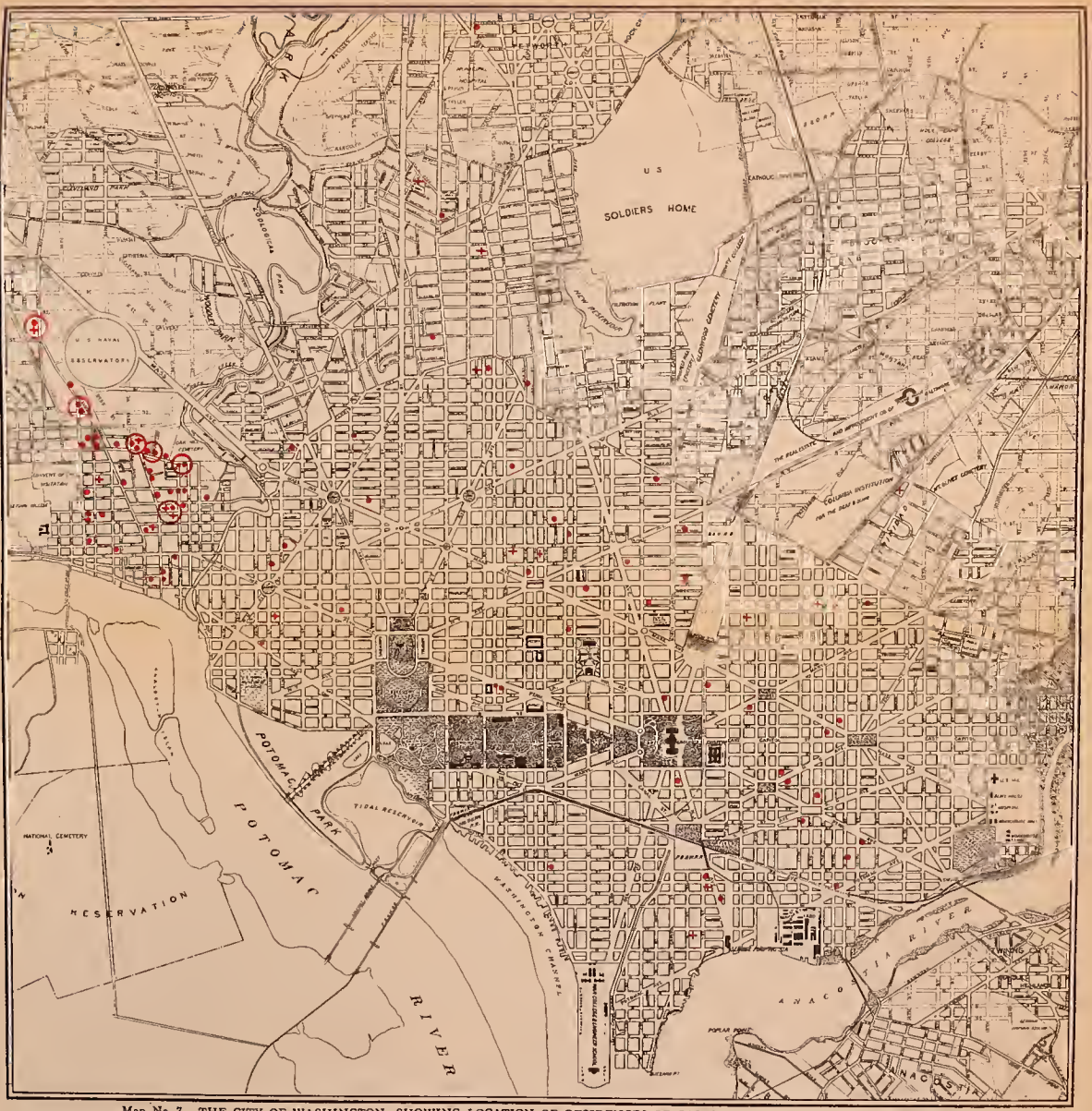


Map No. 6.—THE CITY OF WASHINGTON, SHOWING LOCATION OF RESIDENCES OF CASES OF TYPHOID FEVER, SEPTEMBER, 1908.

- Cases having definite onset September 1-15.
- Cases having definite onset September 16-30.

Two or more dots or crosses in a single circle indicate two or more cases in the same house.
Imported cases not charted.





Map No. 7.—THE CITY OF WASHINGTON, SHOWING LOCATION OF RESIDENCES OF CASES OF TYPHOID FEVER, OCTOBER, 1908.

● Cases having definite onset October 1-15.
+ Cases having definite onset October 16-31.
Two or more dots or crosses in a single circle indicate two or more cases in the same house.
Imported cases not charted.

TABLE NO. 5.—*Showing population and incidence of typhoid fever in each vital statistical district in the District of Columbia*—Continued.

Vital statistical districts.	Population.			Number of cases of typhoid fever developing from May 1 to November 1, 1908.				Case rate per 10,000 of population.
	White.	Colored.	Total.	Unaccounted for.	Attributed to direct contact.	Attributed to milk infection.	Total.	
11	2,489	7,381	9,870	26	5	0	31	31.5
12	3,124	3,674	6,798	7	0	0	7	10.2
13	4,348	1,132	5,480	10	0	0	10	18.2
14	15,327	2,263	17,590	22	1	0	23	13.0
15	10,475	2,780	13,255	16	3	0	19	14.3
16	6,364	2,167	8,531	22	5	0	27	31.6
17	12,688	2,404	15,092	20	0	0	20	13.2
18	9,817	1,533	11,350	20	5	0	25	22.0
19	2,818	890	3,708	6	2	0	8	21.5
20	2,522	1,851	4,373	4	1	0	5	11.4
21	887	506	1,393	1	1	0	2	14.3
22	124	915	1,039	0	0	0	0	0.0
23	410	87	497	1	0	0	1	20.0
24	719	55	774	2	3	0	5	67.1
25	3,594	1,000	4,594	4	2	0	6	13.0
26	2,648	594	3,242	1	0	0	1	3.0
27	1,755	2,567	4,322	1	0	0	1	2.3
28	503	216	719	0	0	0	0	0.0
29	870	12	882	2	0	0	2	22.6
30	2,105	1,156	3,261	13	7	0	20	61.3
31	2,421	161	2,582	4	1	0	5	19.3
32	2,801	223	3,024	3	0	0	3	9.9
33	6,340	1,250	7,590	1	0	0	1	1.3
34	695	2,981	3,676	3	0	0	3	8.1
35	8,150	3,288	11,438	9	3	0	12	10.4
36	5,281	399	5,680	7	1	0	8	14.0
37	1,641	240	1,881	1	0	0	1	5.3
38	1,148	24	1,172	0	0	0	0	0.0
39	812	46	858	2	0	0	2	23.3
40	1,144	294	1,438	3	0	0	3	20.8
41	625	67	692	2	0	0	2	28.9
42	269	275	544	1	0	0	1	18.3
43	1,491	127	1,618	1	0	0	1	6.1
44	4,244	469	4,713	1	0	0	1	2.1
45	732	1,048	1,780	4	0	0	4	22.4
46	2,288	547	2,835	3	1	0	4	14.1
47	833	194	1,027	1	3	1	5	48.6
48	5,148	2,359	7,507	6	3	28	37	49.2
49	1,452	690	2,142	6	0	2	8	37.3
50	6,225	1,144	7,369	11	2	13	26	35.2
51	1,048	156	1,204	3	0	1	4	33.2
52	80	92	172	0	0	2	2	116.2
53	304	111	415	1	0	0	1	24.0
	241,920	97,483	339,403	410	66	52	528	15.5

The districts in which there was a high prevalence of the disease—20 or more cases per 10,000 of population—and certain facts regarding these districts are presented in the following table:

Data regarding vital statistical districts having a high prevalence of typhoid fever—20 or more cases per 10,000 population.

Vital statistical districts.	Relatively large proportion colored race.	Relatively rural section.	Relatively poor sanitary conditions.	In older low-lying section of city.	Supplied with city water.			Supplied with city sewerage system.			Relatively large number of cases due to contact infection.	Relatively large number of cases due to milk infection.	Remarks.
					Wholly.	Partly.	No.	Wholly.	Partly.	No.			
3	+	−	±	+	+	....	....	+	....	....	−	−	
8	−	−	+	+	+	....	....	+	....	....	±	−	
11	++	−	+	+	+	....	....	+	....	....	+	−	
16	−	−	+	+	+	....	....	+	....	....	+	−	
18	−−	−	+	+	+	....	....	+	....	....	+	−	
19	−	−	+	±	....	+	....	....	+	....	+	−	
23	−	+	+	−	....	....	+	....	....	+	−	−	Small population.
24	−−	+	+	−	....	+	....	....	+	....	++	−	5 cases (3 due to contact).
29	−−	+	±	−	....	+	....	....	....	+	−	−	
30	±	±	+	−	....	+	....	....	+	....	++	−	20 cases (7 due to contact).
39	−−	±	−	−	....	+	....	....	+	....	−	−	
40	−	+	±	−	....	+	....	....	+	....	−	−	
41	−−	+	±	−	....	+	....	....	+	....	−	−	Small population.
45	++	−	−	−	+	....	....	+	....	....	−	−	
47	−	±	−	−	....	+	....	....	+	....	++	±	5 cases (3 contact, 1 milk).
48	±	−	−	±	....	+	....	....	+	....	+	+++	37 cases (3 contact, 28 milk).
49	±	−	+	+	+	....	....	+	....	....	−	+	8 cases (2 milk).
50	−	−	±	+	....	+	....	....	+	....	±	++	26 cases (2 contact, 13 milk).
51	−−	+	+	−	....	+	....	....	....	+	−	+	
52	++	+	±	−	....	+	....	....	+	....	−	+++	All due to milk infection.
53	±	+	±	−	....	+	....	....	+	....	−	−	Small population.
	.....	....	....	....	7	13	1	7	11	3	.....	.....	

The following table shows the districts in which the prevalence of the disease was low—less than 10 cases per 10,000:

Data regarding vital statistical districts having a low prevalence of typhoid fever—10 or less cases per 10,000 population.

Vital statistical districts.	Relatively large proportion colored race.	Relatively rural section.	Relatively poor sanitary conditions.	In older low-lying section of city.	Supplied with city water.			Supplied with city sewerage system.			Relatively large number of cases due to contact infection.	Relatively large number of cases due to milk infection.	Remarks.
					Wholly.	Partly.	No.	Wholly.	Partly.	No.			
1	+	−	−	−	+	....	....	+	....	....	−	−	
2	+	−	−	±	+	....	....	+	....	....	−	±	
4	++	−	+	+	+	....	....	+	....	....	−	+	
6	−	−	−	−	+	....	....	+	....	....	−	−	
22	++	+	±	−	....	....	+	....	....	+	−	−	No cases in population of 1,039.
26	−	+	−	−	....	....	+	....	....	+	−	−	Government Hospital for Insane.
27	++	+	±	−	....	+	....	....	....	+	−	−	
28	−	+	±	−	....	....	+	....	....	+	−	−	No cases in population of 719.
32	−	+	±	−	....	+	....	....	+	....	−	−	
33	−	−	−	−	....	+	....	+	....	....	−	−	
34	++	−	±	−	+	....	....	....	+	....	−	−	
37	−	+	−	−	....	+	....	....	....	+	−	−	Includes Soldiers' Home.
38	−	±	−	−	....	+	....	....	+	....	−	−	No cases in population of 1,172.
43	−	−	−	−	+	....	....	....	+	....	−	−	
44	−	−	−	−	+	....	....	....	+	....	−	−	
	.....	.....	.....	.....	7	5	3	5	5	5	.....	.....	

Judging only by the data in these tables it appears that the factors which seemed to contribute to the high prevalence of typhoid fever were poor sanitary conditions, location in old low-lying sections of the city, direct contact infection, and milk infection; while the effect of large proportions of negroes in the population, location in relatively rural or urban sections, and of being supplied with the city water and sewerage systems (see maps Nos. 9 and 10) seemed to be slight.

These statements must not be taken as conclusions, but simply the evidence presented by the limited data in the tables.

The Government Hospital for the Insane, with a population of about 3,000, but with artesian-well water, pasteurized milk, and generally good sanitary conditions, again this year was notably free from infection, only one case having occurred there.

This case was the first to occur among the inmates of the institution since the fiscal year ending June 30, 1903. The onset of the case was on July 10, and the diagnosis was confirmed by blood culture at the Hygienic Laboratory. The man had been in close

confinement in the institution for two years. He was a markedly demented case and was confined in a ward with about thirty others. His isolation was the more complete in that he had had no visitors from the outside, nor had he, so far as known, eaten any food except that supplied by the institution.

He used the same water, milk, and general food supplies that were used by the other inmates and the attendants at the institution, yet his was the only case which occurred.

It is difficult to explain how infection could have reached this one man without also reaching others living in the somewhat isolated institution. Several possible ways, however, suggest themselves. Flies had rather free access to the ward, and they may have deposited infection on some piece of food which this individual ate. There were in the three or four weeks prior to the onset of illness of this man two cases of typhoid fever in a house about one-half mile from the institution. The excreta from these cases were handled very carelessly, and flies were abundant.

The man was allowed to walk about the grounds of the institution, but always under guard. He had a habit of seizing upon various things, such as cigar butts, banana peels, etc., and putting them into his mouth. In this way he might have picked up something infected which had been thrown over the fence into the institution grounds from grounds adjoining, and which were frequented by picnicking parties. The possibility of there being a bacillus carrier in the ward to account for the infection occurred to us, but examinations of specimens of stools and urine of the eleven inmates who had been admitted to the ward within the year previous were all negative for the typhoid bacillus.

No history could be obtained of any cases or suspected cases of typhoid fever in the households of the ward attendants who went to their own homes at night. Still it is possible that the infection was brought in on the hands or clothing of the hospital attendants.

The mystery of this one case stands out remarkably. He was the only one of about 3,000 persons, living under practically the same conditions, to develop typhoid fever.

This case was the only one to occur among the inmates of the institution during a period of five years. No other cases developed in the institution within the eight months following the onset of this case.

In view of the facts, it seems that water and milk as possible factors can be excluded. Such vegetables as are eaten uncooked were supplied the institution from the market, and the man had eaten uncooked tomatoes and blackberries during the month previous to his illness. The chances of infection having been conveyed to this

man on vegetables seems remote, in view of the fact that no other cases occurred.

The man had eaten no shellfish. So these, as a possible factor, can be excluded.

That no other cases developed in the ward, where there were about thirty other demented cases—most of whom were very careless with their excreta—and the fact that the examination of the stools and urine of all the newcomers in the ward were negative for the typhoid bacillus, argues strongly against the view that the infection may have been caused by a bacillus carrier.

It is evident that all the known methods of introduction of recent infection to this man seem remotely applicable. A possibility of a prolonged incubation period in this case has to be considered.

Davies and Hall[a] report an instance of a bacillus carrier who, in the course of three years, apparently caused three outbreaks of typhoid fever, and then herself had an attack of fever which the authors considered clinical typhoid.

If the man contracted the infection before he came to the institution he had to do so two years before his onset of definite symptoms. If he were a bacillus carrier during this time some of the other inmates in the ward very probably, but of course not necessarily, would have been infected.

The solution of the mystery surrounding the causation of this case probably would throw much light on the problem of the causation of typhoid fever generally.

CASE AND DEATH RATES FOR CALENDAR YEARS 1908, 1907, AND 1906.

The total number of cases of typhoid fever in the District of Columbia reported to the health officer during the calendar year 1908 was 936, as against 945 in 1907 and 1,126 in 1906. Thus the typhoid-fever morbidity rate in the District of Columbia for the calendar year 1908 was 2.75 per 1,000 of population, or 1 person in every 362, as against 2.87 per 1,000 in 1907 and 3.45 per 1,000 in 1906.

Although the morbidity rate was slightly lower in 1908 than in 1907, the death rate was slightly higher.

The number of deaths from typhoid fever in the District of Columbia in 1908 was 124, being a death rate of 36.53 per 100,000 of population, as against 34.5 for 1907 and 49.3 for 1906.

Of the 124 deaths in 1908, 88 were among whites and 36 among negroes, giving a death rate among whites of 36.37 per 100,000 and among colored of 36.92 per 100,000.

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[a] Lancet, Nov. 28, 1908, p. 1585.

The following table shows the typhoid fever death rate per 100,000 of population in the District of Columbia for the calendar years 1906, 1907, and 1908:

Year.	White.	Colored.	Total.
1906	35.4	83.1	49.3
1907	32.99	38.46	34.5
1908	36.37	36.92	36.53

The percentage of deaths to cases (fatality rate) for the three years was as follows:

Year.	White.	Colored.	Total.
1906	10.3	24.2	14.4
1907	10.4	17.6	12.0
1908	13.0	13.7	13.2

In 1908 the fatality rate among the whites was higher and among the negroes lower than it has been in any other year of which there is record, that is, since 1903.

In the following table is given the number of cases and deaths from typhoid fever in the District of Columbia reported by months to the health office during the calendar years 1906, 1907, and 1908:

Month.	Cases.								
	White.			Colored.			Total.		
	1906.	1907.	1908.	1906.	1907.	1908.	1906.	1907.	1908.
January	24	46	25	3	7	12	27	53	37
February	15	28	13	6	4	1	21	32	14
March	13	23	21	5	2	4	18	25	25
April	29	20	35	6	8	8	35	28	43
May	31	31	32	13	6	7	44	37	39
June	32	30	41	26	4	21	58	34	62
July	123	61	63	58	21	45	181	82	108
August	200	145	110	93	46	52	293	191	162
September	105	146	106	44	41	49	150	187	155
October	129	102	127	43	47	26	171	149	153
November	57	68	50	25	19	21	82	87	71
December	38	35	51	8	5	16	46	40	67
Total	796	735	674	330	210	262	1,126	945	936

Month.	Deaths.								
	White.			Colored.			Total.		
	1906.	1907.	1908.	1906.	1907.	1908.	1906.	1907.	1908.
January	5	6	2	1	1	2	6	7	4
February	2	3	1	2	3	0	4	6	1
March	2	2	1	3	2	0	5	4	1
April	2	3	5	2	3	3	4	6	8
May	6	5	5	4	2	3	10	7	8
June	4	1	3	5	1	0	9	2	3
July	7	6	7	14	4	8	21	10	15
August	15	13	9	17	5	4	32	18	13
September	10	13	16	10	4	7	20	17	23
October	16	12	16	12	7	3	28	19	19
November	11	8	13	8	3	3	19	11	16
December	2	5	10	2	2	3	4	7	13
Total	82	77	88	80	37	36	162	114	124

As this table gives the cases by date of report to the health office, which date is usually two or three weeks (and sometimes more) subsequent to the date of onset of illness, and as it includes imported cases, it shows but roughly the actual chronological progress of the disease in the District of Columbia.

THE TYPHOID FEVER DEATH RATE OF WASHINGTON AS COMPARED WITH THAT OF OTHER CITIES:

TABLE NO. 6.—*Giving typhoid fever death rate per 100,000 of population by years for eight American cities.*

City.	1900.	1901.	1902.	1903.	1904.	1905.	1906.	1907.	1908.	Average.
Washington, D. C.	74.1	56.4	74.0	45.0	43.8	43.9	49.3	34.5	36.5	50.8
Richmond, Va.	103.7	56.8	73.4	74.3	54.3	46.1	47.0	44.2	32.8	61.4
Baltimore, Md.	34.9	27.2	41.9	35.4	36.8	35.8	32.8	40.7	31.4	35.2
Philadelphia, Pa.	34.7	33.6	43.6	69.4	52.8	47.6	72.4	59.3	34.8	49.8
New York, N. Y.	20.8	20.6	21.0	17.5	17.2	16.7	15.4	17.3	12.1	17.6
Boston, Mass.	25.5	25.0	24.2	20.5	22.9	19.6	20.2	10.5	25.8	21.6
Savannah, Ga.	29.6	28.9	44.1	54.1	78.8	40.1	40.8	.....	.....	45.2
New Orleans, La.	39.7	50.1	44.2	40.9	36.7	32.6	29.6	50.9	29.8	39.4

The death rates, as given in this table, were furnished by the health departments of the different cities.

Comparing the typhoid death rates of these cities it is evident that the rate for Washington has been, since 1900, relatively high, being exceeded only by that of Richmond, Va.

In comparing the typhoid death rates of different cities a number of conditions should be considered, and we have selected these cities because they offer conditions subject to comparison with those of Washington.

The following table presents these conditions, as nearly as we have been able to ascertain them, comparatively with corresponding conditions in Washington:

City.	Population.	Ratio of colored to white in population.	General sanitary condition.	Sewerage system.
Richmond	About one-third as much	Larger	Generally poorer	Worse.
Baltimore	Nearly twice as much	Smaller	do	Do.
Philadelphia	About five times as much	Much smaller	do	No better.
New York	About twelve times as much	do	do	No better.
Boston	Nearly twice as much	do	do	No better.
Savannah	About one-fifth as much	Larger	do	Worse.
New Orleans	About the same	About the same	do	Worse.

City.	Duration of warm-weather season.	Death rates.		
		1900–1905.	1906.	1907–8.
Washington		56.2	49.3	35.5
Richmond	About the same	68.1	47.0	48.5
Baltimore	do	35.3	32.8	36.0
Philadelphia	Somewhat shorter	46.9	72.4	47.0
New York	Shorter	18.9	15.4	14.7
Boston	Much shorter	22.9	20.2	18.1
Savannah	Longer	45.9	40.8	.....
New Orleans	Much longer	40.7	29.6	40.3

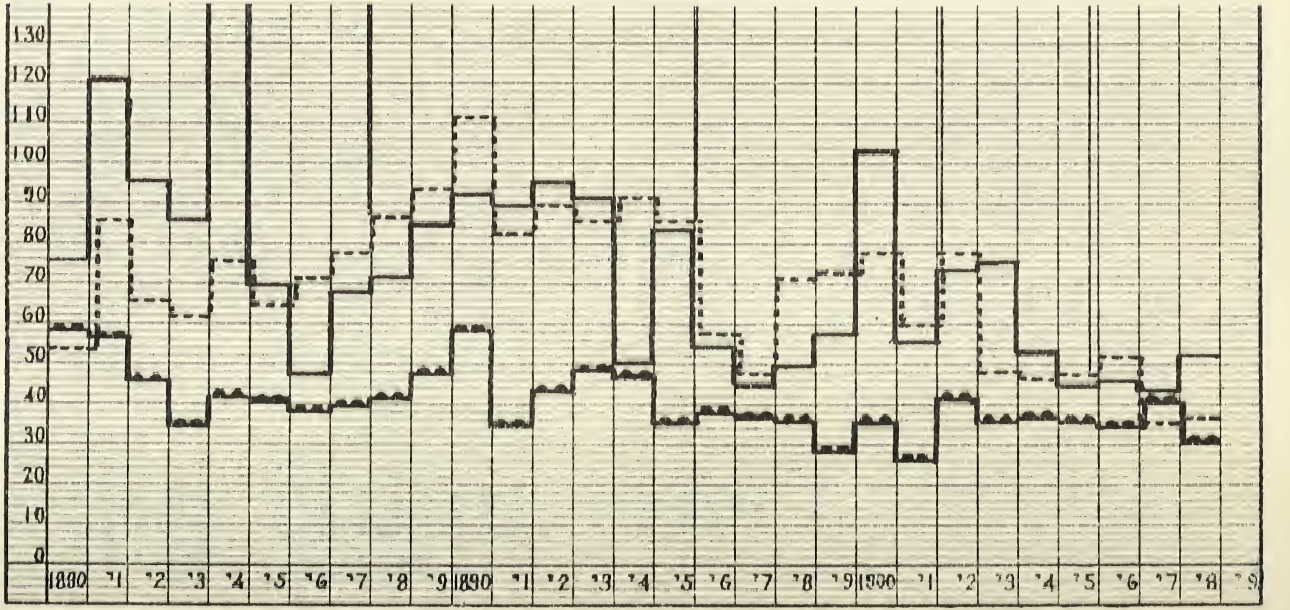
In the six years 1900 to 1906 the typhoid death rate in Washington was higher than in any other of these cities except Richmond, Va.

In 1906, the first year after the filtration of the water supply, the rate was higher than that of any other of the cities except Philadelphia. In 1907 and 1908 the rate in Washington was lower than those of Richmond, Baltimore, Philadelphia, and New Orleans.

It requires but a brief study of this table to force one inevitably to the conclusion that Washington has a much higher typhoid death rate than it should if only those conditions believed to affect the "prosodemic" typhoid are considered.

With the exception of Boston and New York, the conditions under which the milk supply is collected and handled are fully as well, if not better, guarded in Washington than in the other cities. Probably home heating of milk is done to a relatively much greater extent in New Orleans than in Washington, but this does not apply to Baltimore.

This broad, but superficial, comparison suggests that either water or some unknown factor has played a major rôle in the causation of Washington's typhoid fever.



BULLETIN 93, HYGIENIC LABORATORY, P. H. & M. H. S.

Chart No. 8.—TYPHOID FEVER DEATH RATE (INCLUDING TYPHO-MALARIA) FOR RICHMOND AND DISTRICT OF COLUMBIA 1880–1907, BY LEVY AND FREEMAN; DISTRICT OF COLUMBIA AND RICHMOND FOR 1908 AND BALTIMORE 1880–1908 ADDED THERETO.

——— Richmond. - - - - Washington. ●●● Baltimore.

THE PARALLELISM IN THE TYPHOID DEATH RATES OF RICHMOND, VA., AND WASHINGTON, D. C.

Levy and Freeman [a] recently have contributed a very interesting report on typhoid fever in Richmond, Va. They conclude that but a small part of Richmond's typhoid fever is due to the public water supply and that—

> The death rate from typhoid fever in Richmond, certainly for the past few years, has been almost entirely the "residual" or "prosodemic" rate of northern cities, made larger than in the North by reason of the greater length and intensity of the hot season, during which, both North and South, there is normally a marked increase in the typhoid death rate. A considerable part of this residual rate in Richmond is due to the large number of dry closets.

They present a chart showing a striking and really remarkable parallelism in the typhoid death rate of Richmond and Washington since 1880. We reproduce their chart with the typhoid death rate of Baltimore for corresponding years, and the rate of Richmond and Washington for 1908 added thereto. (See Chart No. 8.)

The authors argue that the fluctuations in the typhoid fever death rate of Washington were not due to changes in the water supply, because astoundingly parallel fluctuations occurred in the rate of Richmond, whose water supply during the entire period was practically unchanged, and they follow the argument with the statement that—

> We are reduced, so far as the writers can see, absolutely to the theory that there must be some little understood influences of a very widespread character, varying from year to year, which play a more important rôle in the epidemiology of typhoid fever than has as yet been discovered.

The curve for Baltimore does not fall in line in this remarkable parallelism, which greatly complicates the problem and the conclusions to be drawn from the curves.

Richmond and Washington, though obtaining their water supplies from different rivers still obtain water of the same general character—that is, surface river water exposed to very similar climatic conditions and to about corresponding diluted sewage pollution.

Baltimore obtains water of different character—lake water obtained from a fairly well protected watershed and then subjected to long storage. Baltimore's typhoid death rate from 1880 to 1907 was continuously lower than those of Richmond and Washington. In 1907 for the first time Baltimore's rate was higher than Washington's.

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[a] Levy, Ernest C., and Freeman, Allen W.: Certain conclusions concerning typhoid fever in the South, as deduced from a study of typhoid fever in Richmond, Va. Old Dom. journ. med. and surg., vol. 7, Nov., 1908.

DEATH RATES FROM TYPHOID AND CERTAIN OTHER DISEASES IN THE DISTRICT OF COLUMBIA.

The following table gives, by years, the death rate per 100,000 in the District of Columbia for certain infectious diseases:

Death rates per 100,000 in the District of Columbia for certain diseases.

Year.	Pneumonia.	Pulmonary tuberculosis.	Scarlet fever.	Diarrhea and enteric disease.			Typhoid fever.
				Under 2 years.	2 years and over.	Total, all ages.	
1900	137.7	278.9	4.4	131.9	26.2	158.2	74.1
1901	130.5	271.0	3.7	114.8	27.4	142.2	56.4
1902	156.5	224.6	2.0	107.7	30.5	138.2	74.0
1903	179.6	249.2	.6	90.6	30.2	120.8	45.0
1904	175.2	262.3	3.5	101.8	20.8	122.6	43.8
1905[a]	164.1	255.6	3.4	104.3	23.5	127.8	43.9
1906	154.1	239.2	2.5	97.4	23.6	121.0	49.3
1907	163.5	226.9	.6	98.6	23.7	122.7	34.5
1908	150.2	208.9	2.6	97.5	18.8	116.3	36.5

[a] Water filtered October 1905.

It will be observed that, with the exception of pulmonary tuberculosis, the death rate of which has gradually but progressively declined since 1904, there has been but little difference in the death rate from these diseases since the water supply of the city has been filtered (October, 1905). Some decrease in the death rate from diarrheal diseases is noted. The average rates for the three years 1903–1905 was 124, and for 1906–1908 was 120.

The death rate from all causes per 1,000 of population in the District of Columbia has been as follows:

Year.	Death rate per 1,000.
1900	20.61
1901	20.19
1902	18.95
1903	19.09
1904	19.61
1905	19.20
(Water supply filtered since October, 1905.)	
1906	19.35
1907	19.25
1908	18.03

For the three years 1900 to 1903 the average of the annual death rates from all causes was 19.92 per 1,000. For the three years 1903 to 1906 the average of the annual rates was 19.30; for the three years 1906 to 1909—that is, after the filtration of the public

water supply—the average of the annual rates was 18.88. Thus the reduction in the death rate from all causes since 1900 has been progressive.

There was a greater reduction in the rate between the 1900–1903 and 1903–1906 periods than between the 1903–1906 and 1906–1909 periods. So it is questionable how much of the reduction in the last three years has been due to the further improvement in the city's water supply by sand filtration. In this connection it should be remembered that an additional storage reservoir (Washington City) was put into operation in 1902.

These data are presented for what they are worth, in view of Hazen's theorem:

> For every death from typhoid fever avoided by the purification of public water supplies two or three deaths are avoided from other causes.[a]

SANITARY CONDITION OF RESIDENCES.

In the following table is given the general sanitary condition of the residences at which the persons affected had lived at the time when the infection was considered to have been contracted, the condition of residences of the cases studied in 1907 and 1906 being placed in parallel columns:

Condition of residence.[a]	Number of cases.		
	1908.	1907.	1906.
Good	82	98	253
Fairly good	206	228	243
Rather bad	184	140	158
Bad	70	57	92
Not determined	0	0	1
Total	542	523	747

[a] The definitions of the terms used in this table correspond to those used in our previous reports (Bulletins 35 and 44).

The percentages of cases at residences of these four classes for the three periods were as follows:

Condition of residence.	Percentage of cases.		
	1908.	1907.	1906.
Good	15.1	18.7	33.9
Fairly good	38	43.5	32.5
Rather bad	33.9	26.7	21.2
Bad	12.9	10.9	12.3

[a] Sedgwick and MacNutt. Science, August 14, 1908, p. 215.

The proportion of cases among persons at residences of which all the sanitary conditions were good has progressively diminished during the last three years.

In all three years the majority of the cases occurred among persons living at residences of good or fairly good sanitary conditions, but this majority has diminished from year to year. Thus, the percentages of cases at residences where the sanitary conditions were good or fairly good were: 1906, 66.3 per cent; 1907, 62.2 per cent; 1908, 53.1 per cent.

These figures suggest that since 1906 there has been here a diminution in the part played by agents such as disseminate the infection of typhoid fever generally in a community, and that the part played by agents such as affect especially persons living under the poorer sanitary conditions has been relatively increased.

In this connection it is interesting to note that the sanitary condition of the residences of the 52 cases this season definitely attributed to milk-borne infection was as follows: Good, 24; fairly good, 23; rather bad, 5; bad, 0.

DISPOSAL OF SEWAGE.

Of the 542 cases this season, 483 were among persons living at residences connected with the city sewerage system and at which there were water-closets, 56 at residences not connected with the city sewerage system and at which there were privies, 1 at an institution not connected with the city sewerage system but having water-closets which discharged into a local sewerage system, and 2 on a dredge the water-closets of which discharged into the Potomac River.

The following table shows the methods of disposal of sewage at the residences of the cases studied during the three years:

Sewage disposal.	Number of cases.		
	1908.	1907.	1906.
Residence connected with city sewerage system and having water-closets	483	462	671
Residence not connected with city sewerage system and at which there were privies	56	56	72
Residence having neither water-closets nor privies	0	1	2
Residence having water-closets which discharge into subsoil cesspool	0	1	1
Residence having water-closets which discharge into local sewerage system	3	3	0
Method not noted	0	0	1
Total	542	523	747

The locations of the water-closets in relation to the houses connected with the city sewerage system and in which the cases occurred were as follows:

Location of water-closets.	Number of cases.		
	1908.	1907.	1906.
In house only	198	212	240
In yard only	190	163	257
In both house and yard	95	87	174
Total	483	462	671

Of the 56 cases occurring at houses at which privies were in use, 16 had direct contact with cases in the febrile stage in the same house or house near by, 1 had direct contact with a suspected case, and 8 had indirect contact by persons or flies with patients in the febrile stage. This relatively high contact infection shows that when cases occur in houses at which privies are in use, the chances of infection being conveyed to other members of the household are much greater than when the houses are connected with the city sewerage system.

WATER.

Of the 542 cases, 515, or about 95 per cent, gave a history of having used unboiled Potomac River water supplied through the regular city system as the sole, principal, or occasional source of water for drinking purposes during the thirty days prior to the onset of illness.

Of the 523 cases investigated in 1906, 96.54 per cent gave a history of having used the unboiled Potomac water.

The following table gives the source of water used for drinking during the thirty days prior to onset of illness by the 542 cases investigated:

Water.	Number of cases.
Raw tap:	
Solely	346
Principally	112
Occasionally	57
Occasionally (?)	3
Boiled tap:	
Solely	1
Principally	30
Occasionally	2
Filtered tap:[a]	
Solely	1
Principally	3
Occasionally	0

[a] By "filtered" tap water is meant water filtered after it is drawn from the tap.

Water.	Number of cases.
Public wells and springs:	
Solely	1
Principally	1
Occasionally	12
Bottled:	
Solely	0
Principally	4
Occasionally	8
Private wells or springs in the District of Columbia:	
Solely	9
Principally	31
Occasionally	6
Various sources out of the District of Columbia:	
Principally	4
Occasionally	121

BACTERIOLOGICAL EXAMINATION OF THE POTOMAC WATER SUPPLY.

The examinations were made in the Division of Pathology and Bacteriology of the Hygienic Laboratory by Assistant Surgeon W. W. Miller, assisted by Assistant Surgeon Lasher Hart and Dr. Walter D. Cannon. The samples of water examined were as follows:

1. Raw Potomac River water taken from the inlet of Dalecarlia reservoir, or when Dalecarlia was by-passed, from the inlet at Georgetown reservoir (August 31 to November 26, 1908). This represents the Potomac River water as it is introduced into the first sedimentation reservoir.

2. Applied water taken from the Washington city reservoir from which the water is run on to the filter beds.

3. Filtered water taken from the outlet of the storage reservoir for the filtered water. This represents the mixed effluents from all the filter beds and the water just before it passes from the reservoir to the conduits for distribution to the city.

4. The tap water in different sections of the city. This represents the filtered water after it has traversed the water mains and as distributed in the three different pressure areas of the system, namely, (*a*) gravity, (*b*) first high, and (*c*) second high; the taps in Lafayette Square, Washington Circle, Franklin Square, and in southwest Washington being in the gravity pressure area, the tap at the Hygienic Laboratory being in the first high, and the tap at 3211 Thirteenth street NW., being in the second high pressure area.

In the following tables are given the results of the bacteriological examinations of the specimens of water:

THE RAW RIVER WATER.

Table showing the number of bacteria and presence of colon bacilli found in samples taken from the inlet of Dalecarlia or Georgetown reservoirs.

JANUARY.

Day of month.	Bacteria per c. c.	B. coli in—			Fermentation in lactose broth.		
		0.1 c. c.	1 c. c.	10 c. c.	0.1 c. c.	1 c. c.	10 c. c.
1							
2	860	−	−	+	−	−	+
3							
4							
5							
6							
7							
8							
9							
10							
11							
12							
13							
14							
15							
16							
17	29,000	−	+		−	+	+
18	19,000	−	+		−	+	+
19							
20	19,250	−	+		−	+	+
21	15,500	−	+		−	+	+
22	9,000	−	+		−	+	+
23	17,000	+			+	+	+
24	7,000	−	+		−	+	+
25	8,100	−	+		−	+	+
26							
27	6,600	−	+		−	+	+
28	4,750	−	−	+	+	−	+
29	3,900	−	−	+	−	−	+
30	2,300	−	−	+	−	−	+
31	6,000	+	+	+	+	+	+
Average	10,840						

RAW WATER.

FEBRUARY.

Day of month.	Bacteria per c. c.	B. coli in—			Fermentation in lactose broth.		
		0.1 c. c.	1 c. c.	10 c. c.	0.1 c. c.	1 c. c.	10 c. c.
1	2,500	−	−	+	−	−	+
2							
3	2,900	−	−	+	−	+	+
4	2,900	−	−	+	−	+	+
5	3,100	−	−	+	−	−	+
6	2,900	−	−	+	−	−	+
7	1,100	−	−	+	−	−	+
8	1,000	−	−	+	−	−	+
9							
10	3,200	−	−	+	−	+	+
11	3,900	−	+	+	−	+	+
12	3,650	−	−	−	−	−	−
13	850	−	−	+	−	−	+
14	1,400	−	−	+	−	−	+
15	2,550	−	−	+	−	−	+
16							
17	2,800	−	−	+	−	−	+
18	7,300	−	+	+	−	+	+
19	9,000	−	+	+	+	+	+
20	16,500	−	+	+	+	+	+
21	11,250	−	+	+	−	+	+
22							
23							
24	7,250	−	+	+	−	+	+
25	11,250	−	−	+	+	+	+
26	7,750	−	−	+	−	+	+
27	6,750	−	−	+	−	+	+
28	4,250	−	+	+	−	+	+
29	4,100	−	+	+	−	+	+
Average	5,006						

RAW WATER—Continued.

MARCH.

Day of month.	Bacteria per c. c.	B. coli in—			Fermentation in lactose broth.		
		0.1 c. c.	1 c. c.	10 c. c.	0.1 c. c.	1 c. c.	10 c. c.
1							
2	6,700	−	+	+	−	+	+
3	17,750	+	+	+	+	+	+
4	19,250	+	+	+	+	+	+
5	15,750	−	+	+	+	+	+
6	9,500	−	−	+	+	+	+
7	9,500	−	+	+	−	+	+
8							
9	9,000	−	+	+	−	+	+
10	6,500	−	+	+	−	+	+
11	5,850	−	+	+	−	+	+
12	5,550	−	+	+	−	+	+
13	9,300	+	+	+	+	+	+
14	4,900	−	+	+	−	+	+
15							
16	4,700	−	−	+	−	+	+
17	3,600	−	−	+	−	+	+
18	3,100	−	−	+	−	+	+
19	2,500	−	−	+	−	+	+
20	2,300			−	−	−	+
21	2,400	−	−	+	−	+	+
22							
23	2,600	−	+	+	−	+	+
24	1,600	−	+	+	−	+	+
25	2,900	−	+	+	−	+	+
26	5,000	−	+	+	−	+	+
27	3,900	−	+	+	−	+	+
28	1,500	−	−	−	−	+	+
29							
30	1,000	−	−	+	−	+	+
31	900	−	−	−	−	−	+
Average	6,052						

RAW WATER—Continued.

APRIL.

Day of month.	Bacteria per c. c.	B. coli in—			Fermentation in lactose broth.		
		0.1 c. c.	1 c. c.	10 c. c.	0.1 c. c.	1 c. c.	10 c. c.
1	800	−	−	+	−	−	+
2							
3	800	−	−	+	−	+	+
4	1,100	−	−	+	−	−	+
5	800	−	−	+	−	−	+
6	1,000	−	+	+	−	+	+
7	1,250	−	+	+	−	+	+
8	550	−	+	+	−	+	+
9	700	−	−	+	−	−	+
10	1,400	−	−	+	−	−	+
11	700	−	−	+	−	−	+
12							
13	1,750	−	−	+	−	−	+
14	550	−	−	+	+	−	+
15	350	−	−	+	−	−	+
16	550	−	−	+	−	−	+
17							
18	1,650	−	−	+	−	−	+
19	4,150	−	−	+	−	+	+
20	1,850	−	−	+	.....	−	+
21	1,350	.....	−	+	.....	+	+
22	1,850	−	−	+	−	−	+
23	2,050	−	−	+	−	−	+
24	750	−	−	+	−	−	+
25	600	−	−	+	−	−	+
26							
27	250	−	−	+	−	−	+
28	400	−	−	−	−	−	−
29	390	−	−	+	−	−	+
30	475	−	−	+	−	−	+
Average	1,087						

RAW WATER—Continued.

MAY.

Day of month.	Bacteria per c. c.	B. coli in—			Fermentation in lactose broth.		
		0.1 c. c.	1 c. c.	10 c. c.	0.1 c. c.	1 c. c.	10 c. c.
1	620	−	−	+	−	−	+
2							
3							
4							
5							
6							
7							
8	3,200	+	+	+	+	+	+
9							
10							
11	6,750	+	+	+	+	+	+
12	6,450	−	+	+	−	+	+
13	6,500	+	+	+	+	+	+
14	4,750	−	+	+	−	+	+
15	2,250	−	+	+	−	+	+
16	1,600	+	+	+	+	+	+
17							
18	2,500	−	+	+	−	+	+
19	1,800	−	+	+	−	+	+
20	5,500	+	+	+	+	+	+
21	8,500	+	+	+	+	+	+
22	8,500	+	+	+	+	+	+
23	11,750	+	+	+	+	+	+
24							
25	8,750	+	+	+	+	+	+
26	9,500	+	+	+	+	+	+
27	5,000	+	+	+	+	+	+
28	4,850	+	+	+	+	+	+
29	5,000	−	+	+	−	+	+
30							
31							
Average	5,461						

RAW WATER—Continued.

JUNE.

Day of month.	Bacteria per c. c.	B. coli in—			Fermentation in lactose broth.		
		0.1 c. c.	1 c. c.	10 c. c.	0.1 c. c.	1 c. c.	10 c. c.
1	4,750	−	+	+	+	+	+
2	3,400	−	+	+	−	+	+
3	2,900	+	+	+	+	+	+
4	2,400	−	+	+	−	+	+
5	2,750	−	+	+	−	+	+
6	1,850	−	−	+	−	−	+
7							
8	1,400	−	−	+	−	−	+
9	1,150	−	+	+	−	+	+
10	800	−	−	+	−	−	+
11	525	−	−	−	−	−	−
12	1,925	−	+	+	+	+	+
13	1,900	−	−	+	−	+	+
14							
15	4,200	−	+	+	−	+	+
16	18,800	−	+	+	+	+	+
17	9,650	+	+	+	+	+	+
18	4,900	−	+	+	−	+	+
19	2,950	+	+	+	+	+	+
20	2,300	−	+	+	−	+	+
21							
22							
23	1,200	−	−	+	−	−	+
24	675	−	−	+	−	−	+
25	1,025	−	−	+	−	−	+
26	250	−	−	+	−	−	+
27	200	−	−	+	−	+	+
28	400	−	−	+	−	−	+
29	255	−	−	+	−	+	+
30							
Average	2,902						

RAW WATER—Continued.

JULY.

Day of month.	Bacteria per c. c.	B. coli in—			Fermentation in lactose broth.		
		0.1 c. c.	1 c. c.	10 c. c.	0.1 c. c.	1 c. c.	10 c. c.
1	330	−	−	+	−	−	+
2	415				−	+	+
3	560	−	−	+	−	−	+
4							
5							
6	620	−	−	−	−	−	+
7		−	−	−	−	−	+
8	575	−	−	+	−	−	+
9		−	+	+	−	+	+
10	820	−	−	+	−	−	+
11	350	−	−	+	−	−	+
12							
13	775	−	−	+	−	−	+
14	375	−	+	+	−	+	+
15	525	−	−	+	−	−	+
16	400	−	−	+	+	−	+
17	800	−	−	−	−	+	+
18	1,500	−	−	+	−	−	+
19							
20	350	−	−	−	−	−	−
21				+	−	+	+
22	750	−	−	+	−	−	+
23	6,100	+			+	+	+
24	11,700		+		−	+	+
25	5,000	+	+		+	+	+
26							
27		+			+	+	+
28		−	−	+	+	+	+
29		+			+	+	+
30	27,000	+			+	+	+
31	10,000	+			+	+	+
Average	3,447						

RAW WATER—Continued.

AUGUST.

Day of month.	Bacteria per c. c.	B. coli in—			Fermentation in lactose broth.		
		0.1 c. c.	1 c. c.	10 c. c.	0.1 c. c.	1 c. c.	10 c. c.
1	26,000		+			+	+
2	7,250			+		+	+
3							
4	6,650	+			−	+	+
5	2,500				+	+	+
6					−	−	
7	1,900	+			−	+	+
8							
9							
10	3,600			+		+	+
11	5,650	−	+		+	+	+
12	650	−	+		+	+	+
13	800	−	+		−	+	+
14	375	−	+		−	+	+
15	380	−	+		−	+	+
16							
17	490	−	−	+	−	−	+
18	1,300	+			+	+	+
19	10,200	+			+	+	+
20	4,600	−	+		−	+	+
21	5,250	+			+	+	+
22	1,150	+			+	+	+
23							
24	550	−	+		−	+	+
25	850	−	+		+	+	+
26	4,450	+			+	+	+
27	31,000	−	+		+	+	+
28	5,750	−	−	+	+	+	+
29							
30							
31	5,500	−	−	+	+	+	+
Average	5,515						

RAW WATER—Continued.

OCTOBER.

Day of month.	Bacteria per c. c.	B. coli in—			Fermentation in lactose broth.		
		0.1 c. c.	1 c. c.	10 c. c.	0.1 c. c.	1 c. c.	10 c. c.
1							
2							
3							
4							
5							
6							
7							
8							
9							
10							
11							
12							
13							
14							
15							
16							
17							
18							
19	350			+	−	−	+
20	950			+	−	−	+
21				+	−	+	+
22	800		+		−	+	+
23	750		+		−	+	+
24	400			+	−	−	+
25							
26	250			+	−	+	+
27	800			+	−	−	+
28	700			+	−	+	+
29	900	+			+	+	+
30	850		+		−	+	+
31	1,300		+		−	+	+
Average	732						

RAW WATER—Continued.

NOVEMBER.

Day of month.	Bacteria per c. c.	B. coli in—			Fermentation in lactose broth.		
		0.1 c. c.	1 c. c.	10 c. c.	0.1 c. c.	1 c. c.	10 c. c.
1							
2	3,950	+	+	+	+		
3	650	−	+	+		+	
4	950	−	+	+		+	
5	2,200	−	+	+			+
6	2,600	−	+	+		+	
7	850	+	+	+		+	
8							
9	1,700	−	+	+		+	
10	500	+	+	+	+		
11	350	−	+	+			+
12	700	−	+	+		+	
13	900	−	−	−			−
14	550	−	−	+			+
15							
16	650	−	+	+		+	
17	500	−	−	+			+
18	650	−	+	+			+
19	750	−	−	−	−	−	−
20	550	−	−	+			+
21	700	+	+	+	+		
22							
23	800	−	+	+	−	+	
24							
25							
26							
27							
28							
29							
30							
Average	1,078						

APPLIED WATER.

JULY, 1908.

Day of month.	Bacteria per c. c.	Fermentation in lactose broth.		
		0.1 c. c.	1 c. c.	10 c. c.
1				
2				
3				
4				
5				
6				
7				
8				
9				
10				
11				
12				
13				
14				
15				
16				
17	310		+	+
18	465		−	+
19				
20	475		−	+
21				
22				
23	480		+	+
24				
25				
26				
27			+	+
28			+	+
29				
30	1,000		+	
31				
Average	546			

APPLIED WATER—Continued.

AUGUST, 1908.

Day of month.	Bacteria per c. c.	Fermentation in lactose broth.		
		0.1 c. c.	1 c. c.	10 c. c.
1				
2				
3			−	+
4				
5				
6				
7				
8				
9				
10			−	+
11				
12				
13			−	−
14				
15				
16				
17			+	+
18				
19				
20			+	+
21				
22			+	+
23				
24				+
25			+	+
26			+	+
27			+	+
28			+	+
29			+	+
30				
31			+	+

APPLIED WATER—Continued.

SEPTEMBER, 1908.

Day of month.	Bacteria per c. c.	Fermentation in lactose broth.		
		0.1 c. c.	1 c. c.	10 c. c.
1			+	+
2			+	+
3			−	+
4			+	+
5			−	+
6				
7				
8			+	+
9			+	+
10			−	+
11			+	+
12				
13				
14				
15			+	+
16			−	+
17			+	+
18				
19			−	+
20				
21			−	+
22				
23				
24			+	+
25			−	+
26			−	+
27				
28			−	+
29			+	+
30			+	+

APPLIED WATER—Continued.

OCTOBER, 1908.

Day of month.	Bacteria per c. c.	Fermentation in lactose broth.		
		0.1 c. c.	1 c. c.	10 c. c.
1			+	+
2			+	+
3			+	+
4				
5			+	+
6				
7				
8				
9				
10				
11				
12				
13				
14				
15				
16				
17				
18				
19				
20				
21				
22				
23				
24				
25				
26				
27				
28				
29				
30				
31				

There was little difference in the proportion of samples giving preliminary test for *B. coli* (fermentation in lactose broth) in "raw" water and in "applied" water.

FILTERED WATER.

DECEMBER, 1907.

Day of month.	Bacteria per c. c.	B. coli in—			Fermentation in lactose broth.		
		0.1 c. c.	1 c. c.	10 c. c.	0.1 c. c.	1 c. c.	10 c. c.
1							
2	150		−	+		−	+
3	145		−	+		−	+
4	200		−	+		−	+
5	170		−	+		−	+
6	130		−	−		−	−
7	140		−	+		−	+
8							
9	200		−	+		−	+
10	165		−	−		−	+
11	180		−	−		−	−
12	160		−	−		−	−
13	135		−	−		−	+
14	160		−	+		−	+
15							
16	330		−	+		−	+
17	210		−	+		−	+
18	250		+	+		+	+
19	515		+	+		+	+
20	950		+	+		+	+
21	900		+	+		+	+
22							
23	950		+	+		+	+
24	875		−	+		−	+
25							
26	525		−	+		−	+
27	775		−	+		−	+
28	875		−	−		−	+
29							
30	775		+	+		+	+
31	625		+	+		+	+
Average	419						

FILTERED WATER—Continued.

JANUARY, 1908.

Day of month.	Bacteria per c. c.	B. coli in—			Fermentation in lactose broth.		
		0.1 c. c.	1 c. c.	10 c. c.	0.1 c. c.	1 c. c.	10 c. c.
1							
2	875		−	+		−	+
3	675		−	−		−	+
4	425		−	+		−	+
5							
6	380		−	−		−	+
7	200		−	−		−	−
8	230		+	+		+	+
9	160		−	−		−	−
10	115		−	−		−	+
11	85		−	−		−	+
12							
13	130		−	−		−	+
14	235		+	+		+	+
15	625		−	−		−	−
16	690		−	+		−	+
17	535		−	+		−	+
18	410		−	+		−	+
19							
20	520		−	+		−	+
21	570		−	+		−	+
22	250		+	+		+	+
23	470		+	+		+	+
24	305		−	+		+	+
25	185		−	+		−	+
26							
27	150		−	−		−	+
28	110		−	−		−	+
29	235		−	−		−	+
30	100		−	−		−	−
31	230		−	−		−	−
Average	342						

FILTERED WATER—Continued.

FEBRUARY, 1908.

Day of month.	Bacteria per c. c.	B. coli in—			Fermentation in lactose broth.		
		0.1 c. c.	1 c. c.	10 c. c.	0.1 c. c.	1 c. c.	10 c. c.
1	120	.....	−	−	.....	−	−
2	.....	.....	.....	.....	.....	.....	.....
3	76	.....	−	−	.....	−	−
4	105	.....	−	−	.....	−	−
5	65	.....	−	−	.....	−	−
6	65	.....	−	−	.....	−	−
7	72	.....	−	−	.....	−	−
8	85	.....	−	+	.....	−	+
9	.....	.....	.....	.....	.....	.....	.....
10	240	.....	−	−	.....	−	+
11	85	.....	−	−	.....	−	−
12	140	.....	−	−	.....	−	+
13	80	.....	−	−	.....	−	−
14	85	.....	−	−	.....	−	+
15	.....	.....	.....	.....	.....	.....	.....
16	.....	.....	.....	.....	.....	.....	.....
17	55	.....	−	−	.....	−	−
18	.....	.....	.....	.....	.....	.....	.....
19	160	.....	−	−	.....	−	−
20	95	.....	−	−	.....	−	−
21	225	.....	−	+	.....	−	+
22	.....	.....	.....	.....	.....	.....	.....
23	.....	.....	.....	.....	.....	.....	.....
24	220	.....	−	+	.....	−	+
25	300	.....	−	−	.....	−	−
26	230	.....	−	−	.....	−	−
27	150	.....	−	−	.....	−	+
28	180	.....	−	+	.....	−	+
29	115	.....	−	−	.....	−	+
Average	134						

FILTERED WATER—Continued.

MARCH, 1908.

Day of month.	Bacteria per c. c.	B. coli in—			Fermentation in lactose broth.		
		0.1 c. c.	1 c. c.	10 c. c.	0.1 c. c.	1 c. c.	10 c. c.
1							
2	95		−	−		−	+
3	130		−	−		−	+
4	255		−	−		−	−
5	95		−	−		−	−
6	90		−	−		−	+
7	230		−	−		−	+
8							
9	70		−	−		−	−
10	80		−	−		−	−
11	80		−	−		−	−
12	60		−	−		−	+
13	155		−	−		+	+
14	76		−	−		−	−
15			−				
16	36					−	+
17	40		−	−		−	−
18							
19	34		−	−		−	−
20	36		−			+	−
21							
22							
23	29					+	+
24	48		−	−		−	+
25	68		−	−		−	−
26	60		−	−		−	−
27	60		−	−		−	+
28	30		−	−		−	−
29							
30	55		−	−		−	−
31	45		−	−		−	−
Average	81						

FILTERED WATER—Continued.

APRIL, 1908.

Day of month.	Bacteria per c. c.	B. coli in—			Fermentation in lactose broth.		
		0.1 c. c.	1 c. c.	10 c. c.	0.1 c. c.	1 c. c.	10 c. c.
1	60		−	−		−	−
2							
3	100		−	−		−	−
4	65		−	−		−	−
5	55		−	−		−	−
6	70		−	−		−	−
7	40		−	−		−	−
8	85		−	−		−	−
9	55		−	−		−	−
10	45		−			−	+
11	75		−	+		−	+
12							
13	115		−	−		−	−
14	45		−	−		−	−
15	30		−	−		−	−
16	80		−	−		−	−
17							
18	40		−	−		−	−
19	80		−	−		−	+
20	35		−	−		−	−
21	40		−	−		−	+
22	80		−	−		−	−
23	110		−	−		−	+
24	55		−	−		−	−
25	85		−	−		−	−
26							
27	25		−	−		−	−
28	45		−	−		−	−
29	28		−	−		−	−
30	24		−	−		+	−
31							
Average	58						

FILTERED WATER—Continued.

MAY, 1908.

Day of month.	Bacteria per c. c.	B. coli in—			Fermentation in lactose broth.		
		0.1 c. c.	1 c. c.	10 c. c.	0.1 c. c.	1 c. c.	10 c. c.
1	145		−	−		−	−
2							
3							
4							
5							
6							
7							
8	80		−	−		−	−
9	70		−	−		−	−
10							
11	20		−	−		−	−
12	50		−	−		−	−
13	70		−	−		−	−
14	55		−	−		+	−
15	45		−	−		−	−
16	35		−	−		−	−
17							
18	35		−	−		−	−
19	30		−	−		−	−
20	24		−	+		−	+
21	27		−	−		−	−
22	40		−	−		−	−
23	30		−	−		−	−
24							
25	60		−	−		−	−
26	165		−	−		−	+
27	35		−	−		−	−
28	49		−	−		−	−
29	50		−	−		−	−
30	40		−	−		−	−
31							
Average	56						

FILTERED WATER—Continued.

JUNE, 1908.

Day of month.	Bacteria per c. c.	B. coli in—			Fermentation in lactose broth.		
		0.1 c. c.	1 c. c.	10 c. c.	0.1 c. c.	1 c. c.	10 c. c.
1							
2	35		−	+		−	+
3	68		−	−		−	−
4	42		−	−		−	+
5	20		−	−		−	+
6	39		−	−		−	−
7							
8	36		−	−		−	−
9	33		−	−		−	−
10	15		−	−		−	−
11	32		−	−		−	−
12	104		−	−		−	−
13	59		−	−		−	−
14							
15	68		−	−		−	−
16	56		−	−		−	−
17	50		−	−		−	−
18	74		−	−		−	−
19	31		−	−		−	−
20	46		−	−		−	−
21							
22							
23	32		−	−		−	−
24	26		−	−		−	−
25	44		−	−		−	−
26	44					+	+
27	43		−	−		−	−
28							
29	53		−	−		−	−
30	30		−			−	+
Average	45						

FILTERED WATER—Continued.

JULY, 1908.

Day of month.	Bacteria per c. c.	B. coli in—			Fermentation in lactose broth.		
		0.1 c. c.	1 c. c.	10 c. c.	0.1 c. c.	1 c. c.	10 c. c.
1	59	.....	−	−	.....	−	−
2	26	.....	−	−	.....	−	−
3	34	.....	−	−	.....	−	−
4	.....	.....	.....	.....	.....	.....	.....
5	.....	.....	.....	.....	.....	.....	.....
6	44	.....	−	−	.....	−	+
7	.....	.....	.....	.....	.....	.....	.....
8	24	.....	−	−	.....	−	−
9	.....	.....	.....	.....	.....	.....	.....
10	90	.....	−	−	.....	−	−
11	50	.....	−	−	.....	−	−
12	.....	.....	.....	.....	.....	.....	.....
13	71	.....	−	−	.....	−	+
14	42	.....	−	−	.....	−	−
15	73	.....	−	−	.....	−	−
16	31	.....	−	−	.....	−	−
17	90	.....	−	.....	.....	−	+
18	120	.....	−	−	.....	−	−
19	.....	.....	.....	.....	.....	.....	.....
20	32	.....	−	−	.....	−	−
21	45	.....	−	−	.....	−	−
22	42	.....	−	−	.....	−	−
23	44	.....	−	−	.....	−	−
24	260	.....	−	.....	.....	−	+
25	50	.....	−	−	.....	−	−
26	.....	.....	.....	.....	.....	.....	.....
27	20	.....	−	+	.....	−	+
28	28	.....	−	+	.....	−	+
29	22	.....	−	−	.....	−	−
30	32	.....	−	−	.....	−	−
31	20	.....	−	−	.....	−	−
Average	56						

FILTERED WATER—Continued.

AUGUST, 1908.

Day of month.	Bacteria per c. c.	B. coli in—			Fermentation in lactose broth.		
		0.1 c. c.	1 c. c.	10 c. c.	0.1 c. c.	1 c. c.	10 c. c.
1	48	.....	−	−	.....	−	−
2	75	.....	−	−	.....	−	−
3	76	.....	+	+	.....	+	+
4	44	.....	−	.....	.....	−	+
5	19	.....	−	−	.....	−	−
6	.....	.....	.....	.....	.....	.....	.....
7	20	.....	−	+	.....	−	+
8	.....	.....	.....	.....	.....	.....	.....
9	37	.....	−	.....	.....	−	+
10	.....	.....	−	−	.....	−	−
11	23	.....	−	−	.....	−	−
12	36	.....	−	−	.....	−	−
13	20	.....	−	−	.....	−	−
14	11	.....	−	−	.....	−	−
15	33	.....	−	−	.....	−	−
16	.....	.....	.....	.....	.....	.....	.....
17	29	.....	−	−	.....	−	−
18	23	.....	−	−	.....	−	−
19	40	.....	−	−	.....	−	−
20	33	.....	−	−	.....	−	−
21	22	.....	−	−	.....	−	−
22	44	.....	−	−	.....	−	−
23	.....	.....	.....	.....	.....	.....	.....
24	25	.....	−	−	.....	−	−
25	22	.....	−	−	.....	−	−
26	62	.....	−	−	.....	−	−
27	54	.....	−	−	.....	−	−
28	.....	.....	.....	.....	.....	.....	.....
29	19	.....	−	−	.....	−	−
30	.....	.....	.....	.....	.....	.....	.....
31	50	.....	−	−	.....	−	−
Average	36						

FILTERED WATER—Continued.

SEPTEMBER, 1908.

Day of month.	Bacteria per c. c.	B. coli in—			Fermentation in lactose broth.		
		0.1 c. c.	1 c. c.	10 c. c.	0.1 c. c.	1 c. c.	10 c. c.
1	32		−			−	+
2	50		−	+		−	+
3	16		−	−		−	−
4							
5							
6							
7							
8	18		−	−		−	−
9	86		−	−		−	−
10	68		−	−		−	−
11	23		−	−		−	−
12	52		−	−		−	−
13							
14	35		−	−		−	−
15	19		−	−		−	−
16	37		−	−		−	−
17	59		−	−		−	−
18	15		−	−		−	−
19	17		−	−		−	−
20							
21	36		−	−		−	−
22							
23							
24	27		−	−		−	−
25	34		−	−		−	+
26			−			−	+
27							
28	11		−	−		−	−
29	42		−	−		−	−
30	45		−	−		−	−
Average	36						

FILTERED WATER—Continued.

OCTOBER, 1908.

Day of month.	Bacteria per c. c.	B. coli in—			Fermentation in lactose broth.		
		0.1 c. c.	1 c. c.	10 c. c.	0.1 c. c.	1 c. c.	10 c. c.
1	25	.....	−	−	.....	−	−
2	32	.....	−	−	.....	−	−
3	30	.....	−	−	.....	−	−
4	.....	.....	.....	.....	.....	.....	.....
5	21	.....	−	−	.....	−	−
6	9	.....	−	−	.....	−	−
7	13	.....	−	−	.....	−	+
8	22	.....	−	−	.....	−	−
9	Lost.	.....	−	−	.....	−	−
10	32	.....	−	−	.....	−	−
11	.....	.....	.....	.....	.....	.....	.....
12	28	.....	−	−	.....	−	−
13	31	.....	−	−	.....	−	−
14	65	.....	−	−	.....	−	−
15	95	.....	−	+	.....	−	+
16	29	.....	−	−	.....	−	−
17	9	.....	−	−	.....	−	−
18	.....	.....	.....	.....	.....	.....	.....
19	27	.....	−	−	.....	−	−
20	19	.....	−	+	.....	−	+
21	.....	.....	.....	.....	.....	.....	.....
22	17	.....	−	−	.....	−	−
23	23	.....	−	−	.....	−	−
24	35	.....	−	−	.....	−	−
25	.....	.....	.....	.....	.....	.....	.....
26	21	.....	−	−	.....	−	−
27	19	.....	−	−	.....	−	−
28	49	.....	−	−	.....	−	−
29	14	.....	−	−	.....	−	−
30	26	.....	−	−	.....	−	−
31	14	.....	−	−	.....	−	−
Average	36						

FILTERED WATER—Continued.

NOVEMBER, 1908.

Day of month.	Bacteria per c. c.	B. coli in—			Fermentation in lactose broth.		
		0.1 c. c.	1 c. c.	10 c. c.	0.1 c. c.	1 c. c.	10 c. c.
1			—	—		—	—
2	47		—	—		—	—
3	26		—	—		—	—
4	14		—	—		—	—
5	54		—	—		—	—
6	85		—	—		—	—
7	17		—	—		—	—
8							
9	14		—	—		—	—
10	27		—	—		—	—
11	68		—	—		—	—
12	55		—	—		—	—
13	17		—	—		—	—
14	26		—	—		—	—
15							
16	23		—	—		—	—
17	15		—	—		—	—
18	11		—	—		—	—
19	50		—	—		—	—
20	50		—	—		—	—
21	25		—	—		—	—
22							
23	35		—	—		—	—
24	40		—			—	
25	48		—	—		—	—
26							
27	55		—	—		—	—
28	26		—	—		—	—
29							
30	55		—	—		—	—
Average	37						

THE FILTERED RIVER WATER FROM TAPS.

Table showing fermentation in lactose broth of samples taken from constantly flowing hydrant in Lafayette Square.

JUNE, 1908.

Day of month.	Fermentation in lactose broth.		
	0.1 c. c.	1 c. c.	10 c. c.
1			
2			
3			
4			
5			
6			
7			
8			
9		−	−
10			
11			
12		−	−
13			
14			
15			
16		−	+
17			
18			
19		−	−
20			
21			
22			
23			
24			
25			
26		−	+
27			
28			
29			
30		−	−

THE FILTERED RIVER WATER FROM TAPS—Continued.

Table showing fermentation in lactose broth of samples taken from constantly flowing hydrant in Lafayette Square—Continued.

JULY, 1908.

Day of month.	Fermentation in lactose broth.		
	0.1 c. c.	1 c. c.	10 c. c.
1			
2		−	−
3			
4			
5			
6			
7			
8			
9		−	−
10			
11			
12			
13		−	+
14			
15			
16		+	+
17			
18			
19			
20			
21		−	+
22		+	−
23		−	+
24		+	+
25		−	−
26			
27		−	−
28		−	+
29		−	−
30		−	−
31		−	+

THE FILTERED RIVER WATER FROM TAPS—Continued.

Table showing fermentation in lactose broth of samples taken from constantly flowing hydrant in Lafayette Square—Continued.

AUGUST, 1908.

Day of month.	Fermentation in lactose broth.		
	0.1 c. c.	1 c. c.	10 c. c.
1		−	+
2			
3		−	−
4		+	+
5		−	+
6			
7		−	−
8			
9		−	+
10		−	−
11		−	+
12		+	+
13		−	+
14		−	+
15		−	−
16			
17		−	−
18		−	−
19		−	+
20		−	+
21		−	−
22		−	−
23			
24		−	−
25		−	−
26		−	−
27		−	−
28		−	−
29		−	−
30			
31		−	−

THE FILTERED RIVER WATER FROM TAPS—Continued.

Table showing fermentation in lactose broth of samples taken from constantly flowing hydrant in Lafayette Square—Continued.

SEPTEMBER, 1908.

Day of month.	Fermentation in lactose broth.		
	0.1 c. c.	1 c. c.	10 c. c.
1		−	−
2		−	−
3		−	−
4			
5		−	+
6			
7			
8		−	−
9		−	−
10		−	−
11		−	−
12			
13			
14			
15		−	−
16		−	−
17		−	−
18			
19		−	−
20			
21		−	−
22			
23			
24		−	+
25		−	+
26		−	+
27			
28		−	+
29			
30		−	+

THE FILTERED RIVER WATER FROM TAPS—Continued.

Table showing fermentation in lactose broth of samples taken from constantly flowing hydrant in Lafayette Square—Continued.

OCTOBER, 1908.

Day of month.	Fermentation in lactose broth.		
	0.1 c. c.	1 c. c.	10 c. c.
1		−	−
2		−	+
3		−	−
4			
5		−	−
6			
7			
8			
9			
10			
11			
12			
13			
14			
15			
16			
17			
18			
19			
20			
21			
22			
23			
24			
25			
26			
27			
28			
29			
30			
31			

THE FILTERED RIVER WATER FROM TAPS—Continued.

Table showing fermentation in lactose broth of samples taken from hydrant tap at Washington Circle.

JULY, 1908.

Day of month.	Fermentation in lactose broth.		
	0.1 c. c.	1 c. c.	10 c. c.
1			
2			
3			
4			
5			
6			
7			
8			
9			
10			
11			
12			
13			
14			
15			
16			
17			
18			
19			
20			
21			
22			
23			
24		−	−
25		−	−
26			
27		−	−
28		−	+
29			
30		−	−
31			

THE FILTERED RIVER WATER FROM TAPS—Continued.

Table showing fermentation in lactose broth of samples taken from hydrant tap at Washington Circle—Continued.

AUGUST, 1908.

Day of month.	Fermentation in lactose broth.		
	0.1 c. c.	1 c. c.	10 c. c.
1		−	−
2			
3		−	−
4		−	+
5		−	+
6			
7		−	−
8			
9		+	−
10		+	+
11		−	−
12		−	−
13		−	−
14		−	−
15		−	−
16			
17		−	−
18		−	−
19		−	−
20		−	−
21			
22			
23			
24		−	−
25		−	−
26		+	+
27		−	−
28		−	−
29		−	−
30			
31		−	−

THE FILTERED RIVER WATER FROM TAPS—Continued.

Table showing fermentation in lactose broth of samples taken from hydrant tap at Washington Circle—Continued.

SEPTEMBER, 1908.

Day of month.	Fermentation in lactose broth.		
	0.1 c. c.	1 c. c.	10 c. c.
1		−	−
2		−	−
3		−	+
4		+	+
5		−	−
6			
7			
8		−	−
9		−	−
10		+	−
11		+	+
12			
13			
14			
15		−	−
16		−	−
17		−	−
18			
19		−	+
20			
21		−	−
22			
23			
24		+	+
25		−	+
26		−	+
27			
28		−	+
29		−	−
30		−	−

THE FILTERED RIVER WATER FROM TAPS—Continued.

Table showing fermentation in lactose broth of samples taken from hydrant tap at Washington Circle—Continued.

OCTOBER, 1908.

Day of month.	Fermentation in lactose broth.		
	0.1 c. c.	1 c. c.	10 c. c.
1		−	+
2		−	−
3		−	−
4			
5		−	+
6			
7			
8			
9			
10			
11			
12			
13			
14			
15			
16			
17			
18			
19			
20			
21			
22			
23			
24			
25			
26			
27			
28			
29			
30			
31			

THE FILTERED RIVER WATER FROM TAPS—Continued.

Table showing fermentation in lactose broth of samples taken from constantly flowing hydrant at Franklin Square.

JULY, 1908.

Day of month.	Fermentation in lactose broth.		
	0.1 c. c.	1 c. c.	10 c. c.
1			
2			
3			
4			
5			
6			
7			
8			
9			
10			
11			
12			
13			
14			
15			
16			
17			
18			
19			
20			
21			
22			
23			
24		−	−
25		−	−
26			
27		−	−
28		−	+
29		−	−
30		−	+
31		−	−

THE FILTERED RIVER WATER FROM TAPS—Continued.

Table showing fermentation in lactose broth of samples taken from constantly flowing hydrant at Franklin Square—Continued.

AUGUST, 1908.

Day of month.	Fermentation in lactose broth.		
	0.1 c. c.	1 c. c.	10 c. c.
1		−	−
2			
3		−	−
4		−	−
5		−	−
6			
7		−	−
8			
9		−	+
10		−	+
11		−	+
12		−	+
13		−	+
14		−	−
15			
16		−	−
17		−	+
18		+	−
19		−	−
20		−	+
21		−	−
22		−	−
23			
24		−	−
25		−	−
26		−	−
27		−	−
28		−	−
29		−	−
30			
31		−	−

THE FILTERED RIVER WATER FROM TAPS—Continued.

Table showing fermentation in lactose broth of samples taken from constantly flowing hydrant at Franklin Square—Continued.

SEPTEMBER, 1908.

Day of month.	Fermentation in lactose broth.		
	0.1 c. c.	1 c. c.	10 c. c.
1		−	−
2		−	−
3		−	−
4		+	+
5		−	+
6			
7			
8		−	−
9		−	−
10		−	+
11		−	+
12			
13			
14			
15		−	−
16		−	−
17		−	−
18			
19		−	−
20			
21		−	−
22			
23			
24		+	+
25		−	+
26		−	−
27			
28			
29			
30		−	−

THE FILTERED RIVER WATER FROM TAPS—Continued.

Table showing fermentation in lactose broth of samples taken from constantly flowing hydrant at Franklin Square—Continued.

OCTOBER, 1908.

Day of month.	Fermentation in lactose broth.		
	0.1 c. c.	1 c. c.	10 c. c.
1		—	—
2		—	—
3		—	—
4			
5		—	—
6			
7			
8			
9			
10			
11			
12			
13			
14			
15			
16			
17			
18			
19			
20			
21			
22			
23			
24			
25			
26			
27			
28			
29			
30			
31			

THE FILTERED RIVER WATER FROM TAPS—Continued.

Table showing fermentation in lactose broth of samples taken from hydrant in southwest Washington.

JULY, 1908.

Day of month.	Fermentation in lactose broth.		
	0.1 c. c.	1 c. c.	10 c. c.
1			
2			
3			
4			
5			
6			
7			
8			
9			
10			
11			
12			
13			
14			
15			
16			
17			
18			
19			
20			
21			
22			
23			
24			
25			
26			
27		−	+
28			
29		−	−
30			
31		−	−

THE FILTERED RIVER WATER FROM TAPS—Continued.

Table showing fermentation in lactose broth of samples taken from hydrant in southwest Washington—Continued.

AUGUST, 1908.

Day of month.	Fermentation in lactose broth.		
	0.1 c. c.	1 c. c.	10 c. c.
1			
2			
3			
4			
5		−	+
6		+	+
7		−	+
8			
9		−	+
10		−	+
11		−	+
12		−	−
13		−	−
14		−	−
15		−	−
16			
17		−	−
18		−	−
19		+	−
20		−	−
21		−	−
22			
23			
24		−	−
25		−	−
26		−	+
27		−	−
28		−	+
29		−	−
30			
31		−	−

THE FILTERED RIVER WATER FROM TAPS—Continued.

Table showing fermentation in lactose broth of samples taken from hydrant in southwest Washington—Continued.

SEPTEMBER, 1908.

Day of month.	Fermentation in lactose broth.		
	0.1 c. c.	1 c. c.	10 c. c.
1		−	+
2		−	+
3		−	−
4		+	+
5		−	−
6			
7			
8		−	−
9		−	+
10		−	−
11		−	+
12			
13			
14			
15		−	+
16			
17		−	−
18			
19		−	−
20			
21			
22			
23			
24		−	+
25		−	−
26		+	−
27			
28		+	+
29		−	−
30		−	+

THE FILTERED RIVER WATER FROM TAPS—Continued.

Table showing fermentation in lactose broth of samples taken from hydrant in southwest Washington—Continued.

OCTOBER, 1908.

Day of month.	Fermentation in lactose broth.		
	0.1 c. c.	1 c. c.	10 c. c.
1		−	+
2		−	−
3		−	+
4			
5		−	+
6			
7			
8			
9			
10			
11			
12			
13			
14			
15			
16			
17			
18			
19			
20			
21			
22			
23			
24			
25			
26			
27			
28			
29			
30			
31			

THE FILTERED RIVER WATER FROM TAPS—Continued.

Table showing fermentation in lactose broth of samples taken from constantly flowing hydrant at the Hygienic Laboratory.

JULY, 1908.

Day of month.	Fermentation in lactose broth.		
	0.1 c. c.	1 c. c.	10 c. c.
1			
2			
3			
4			
5			
6			
7			
8			
9			
10			
11			
12			
13			
14			
15			
16			
17			
18			
19			
20			
21			
22			
23			
24		−	+
25		−	−
26			
27		−	+
28			
29		−	−
30		−	+
31		+	−

THE FILTERED RIVER WATER FROM TAPS—Continued.

Table showing fermentation in lactose broth of samples taken from constantly flowing hydrant at the Hygienic Laboratory—Continued.

AUGUST, 1908.

Day of month.	Fermentation in lactose broth.		
	0.1 c. c.	1 c. c.	10 c. c.
1		−	−
2			
3			
4		−	−
5		−	−
6		−	+
7		−	−
8			
9		−	−
10		−	+
11			
12		−	−
13		−	−
14		−	−
15		−	−
16			
17			
18		−	−
19		−	−
20		−	−
21		−	+
22			
23			
24		−	−
25		−	−
26		−	+
27		−	−
28		−	−
29		−	−
30			
31		−	−

THE FILTERED RIVER WATER FROM TAPS—Continued.

Table showing fermentation in lactose broth of samples taken from constantly flowing hydrant at the Hygienic Laboratory—Continued.

SEPTEMBER, 1908.

Day of month.	Fermentation in lactose broth.		
	0.1 c. c.	1 c. c.	10 c. c.
1		−	+
2		−	−
3		−	−
4		−	+
5		−	−
6		−	−
7			
8		−	+
9		−	−
10		−	+
11			
12			
13			
14			
15		−	−
16		−	+
17		−	−
18			
19			
20			
21			
22			
23			
24		+	+
25		+	+
26			
27			
28		+	+
29		−	+
30		−	−

THE FILTERED RIVER WATER FROM TAPS—Continued.

Table showing fermentation in lactose broth of samples taken from constantly flowing hydrant at the Hygienic Laboratory—Continued.

OCTOBER, 1908.

Day of month.	Fermentation in lactose broth.		
	0.1 c. c.	1 c. c.	10 c. c.
1		—	—
2		—	—
3		—	—
4			
5		—	—
6			
7			
8			
9			
10			
11			
12			
13			
14			
15			
16			
17			
18			
19			
20			
21			
22			
23			
24			
25			
26			
27			
28			
29			
30			
31			

THE FILTERED RIVER WATER FROM TAPS—Continued.

Table showing fermentation in lactose broth of samples taken from tap at 3211 Thirteenth street NW.

JULY, 1908.

Day of month.	Fermentation in lactose broth.		
	0.1 c. c.	1 c. c.	10 c. c.
1			
2			
3			
4			
5			
6			
7		−	−
8			
9			
10			
11			
12			
13			
14			
15			
16			
17			
18			
19			
20			
21			
22			
23			
24			
25			
26			
27			
28		−	+
29		−	−
30		−	−
31		−	−

THE FILTERED RIVER WATER FROM TAPS—Continued.

Table showing fermentation in lactose broth of samples taken from tap at 3211 Thirteenth street NW.—Continued.

AUGUST, 1908.

Day of month.	Fermentation in lactose broth.		
	0.1 c. c.	1 c. c.	10 c. c.
1		−	+
2			
3		+	+
4		−	+
5		−	−
6		−	+
7		−	+
8			
9		−	+
10		+	+
11		−	−
12			
13			
14		−	−
15			
16			
17		−	−
18		−	−
19		−	−
20			
21			
22			
23			
24			
25			
26		−	−
27		−	+
28			
29			
30			
31			

FILTERED (STORAGE RESERVOIR) WATER.

Percentage of samples showing B. coli and fermentation.

Date.	Number of samples.	B. coli in—		Number of samples.	Fermentation in lactose broth in—	
		1 c. c.	10 c. c.		1 c. c.	10 c. c.
1907.		*Per cent.*	*Per cent.*		*Per cent.*	*Per cent.*
December	25	28.0	76.0	25	28.0	88.0
1908.						
January	26	15.3	50.0	26	19.2	80.8
February	22	0.0	18.2	22	0.0	40.9
March	21	0.0	0.0	24	12.5	41.6
April	25	0.0	4.0	26	3.8	23.1
May	21	0.0	4.8	21	4.8	9.5
June	22	0.0	4.5	24	4.2	20.8
July	22	0.0	9.1	24	0.0	25.0
August	25	4.0	8.7	25	4.0	16.0
September	21	0.0	5.2	21	0.0	19.0
October	26	0.0	7.7	26	0.0	11.5
November	24	0.0	0.0	24	0.0	0.0

LAFAYETTE SQUARE TAP WATER.

Percentage of samples giving fermentation in lactose broth.

Date.	Number of samples.	In 1 c. c.	In 10 c. c.
1908.		*Per cent.*	*Per cent.*
June	6	0	33
July	14	21	50
August	25	8	40
September	18	0	38
October	4	0	25

WASHINGTON CIRCLE TAP WATER.

Percentage of samples giving fermentation in lactose broth.

Date.	Number of samples.	In 1 c. c.	In 10 c. c.
1908.		*Per cent.*	*Per cent.*
June			
July	5	0	20
August	23	13	17
September	20	20	40
October	4	0	50

FRANKLIN SQUARE TAP.

Percentage of samples giving fermentation in lactose broth.

Date.	Number of samples.	In 1 c. c.	In 10 c. c.
1908.		*Per cent.*	*Per cent.*
June			
July	7	0	28
August	25	4	28
September	18	11	33
October	4	0	0

SOUTH WASHINGTON (NEAR WHARF) TAP.

Percentage of samples giving fermentation in lactose broth.

Date.	Number of samples.	In 1 c. c.	In 10 c. c.
1908.		*Per cent.*	*Per cent.*
June			
July	3	0	33
August	22	9	36
September	18	16.6	39.8
October	4	0	75

HYGIENIC LABORATORY TAP.

Percentage of samples giving fermentation in lactose broth.

Date.	Number of samples.	In 1 c. c.	In 10 c. c.
1908.		*Per cent.*	*Per cent.*
June			
July	6	16.6	49.8
August	22	0	18
September	17	17.6	52.9
October	4	0	0

TAP AT 3211 THIRTEENTH STREET NW.

Percentage of samples giving fermentation in lactose broth.

Date.	Number of samples.	In 1 c. c.	In 10 c. c.
1908.		*Per cent.*	*Per cent.*
June			
July	5	0	20
August	15	13	60
September			
October			

Chart No. 10 shows the percentage of 10 c. c. samples of water from the filtered-water reservoir and various taps, giving the presumptive test for *B. coli* (fermentation in lactose broth).

It will be noted that the percentage of samples of water from the storage (filtered) water reservoir giving fermentation is considerably lower than that of the samples taken from the different taps. It is difficult to explain why this should be so. Two possible explanations present themselves.

1. Contamination of the water in the mains by suction or seepage into the mains through loose joints, cracks, etc. This is distinctly a sanitary engineering problem.

2. Continued life and perhaps multiplication in the mains of organisms capable of producing fermentation.

THE RELATION OF TYPHOID FEVER TO POTOMAC RIVER WATER.

In our first report upon typhoid fever in the District of Columbia in 1906 we lacked sufficient data to draw definite conclusions upon the relation of the Potomac River water to the disease. In our second report (for 1907) we concluded that—

The filtered Potomac River water during the typhoid season of 1907 (May to September) was, according to present bacteriologic standards, of good sanitary quality and so far as could be ascertained was not responsible for the spread of the infection.

In the typhoid season of 1907 there were about 200 cases less than in the 1906 period. This improvement in the situation suggests that the diminution of the amount of typhoid fever in the District of Columbia was due to the improvement in the quality of the drinking water as the result of sand filtration. Positive proof of this can not now be established.

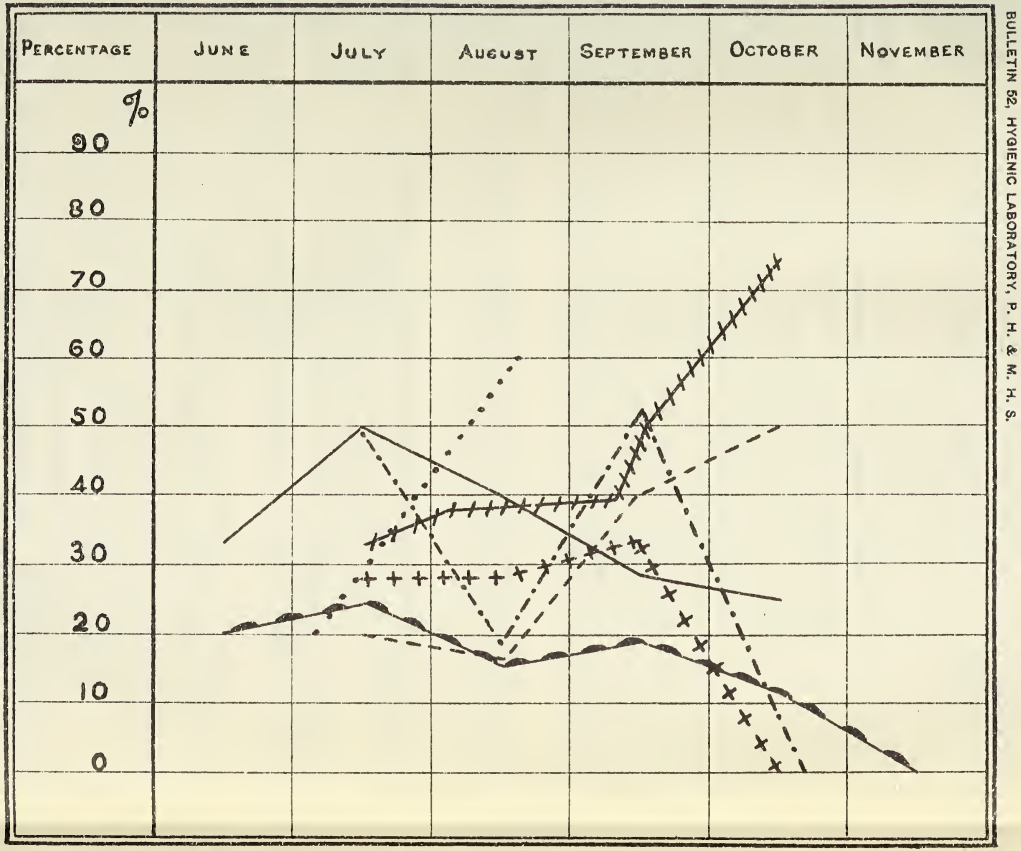
In the 1908 period the filtered water was found to be of about the same quality as it was in the 1907 period, and the rate of prevalence of typhoid fever in the 1908 period was about the same as it was in the 1907 period.

The number of cases occurring in the District of Columbia in the four months June, July, August, and September, of the three years, was as follows: In 1906, 605 cases; in 1907, 418 cases; in 1908, 390 cases.

The results of the bacteriological examination of the filtered water for periods of the three years were as follows:

Date.	Where taken.	Number of samples.	Average number of bacteria per cubic centimeter.	Percentage of samples showing *B. coli*—	
				In 1 c. c.	In 10 c. c.
1906.				*Per cent.*	*Per cent.*
July 16 to August 31, inclusive	Storage reservoir	13	44	7.7	15.4
	Tap	60	67	3.3	26.6
1907.					
May 15 to August 31, inclusive	Storage reservoir	91	31	0	2.2
1908.					
May 1 to August 31, inclusive	Storage reservoir	90	48	1.1	6.6

PERCENTAGE OF 10 C. C. SAMPLES OF WATER FROM THE FILTERED WATER RESERVOIR AND VARIOUS TAPS GIVING FERMENTATION IN SUGAR BROTH.



BULLETIN 52, HYGIENIC LABORATORY, P. H. & M. H. S.

Lafayette Square.
Washington Circle.
Franklin Square.
Southwest Washington.
Hygienic Laboratory.
3211 Thirteenth street NW.
Filtered water reservoir.

Taking the *B. coli* content as an indication, it is evident that the filtered water during the 1907 and 1908 periods was of a better quality than it was in 1906.

In our report for 1907 [a] we stated that—

> The improvement in the water being followed by the lower rate of prevalence of typhoid fever in the summer of 1907 suggests cause and effect. * * * However, it should be borne in mind that the prevalence of typhoid fever in Washington and other communities has varied considerably in different years during which the conditions as to water, etc., have, so far as known, remained approximately the same.

The good results following the purification of the drinking water sometimes appear rather gradually. In some cities the full effects have not been apparent until two or three years following the filtration of the water supply. Washington has been furnished filtered water since October, 1905. In the first year after filtration (1906) there was no lowering of the typhoid death rate coincident with the improvement in the water supply. In fact, the rate was slightly higher than in 1905. In the next two years (1907 and 1908) there was a considerable decrease in the prevalence of the disease during the warm weather season (July, August, and September). The rates for the other (cool) seasons of these three years, however, were almost exactly the same.

The lower prevalence of typhoid fever for 1907 and 1908 was coincident with a still greater improvement in the water than was attained in 1906

Concerning the relation of the Potomac River water to the prevalence of typhoid fever in Washington, there is still some difference of opinion. Three main questions now present themselves:

1. Was much of the typhoid fever in the District of Columbia caused by infection in the water supplied by the Potomac River before the filtration of the water?

2. Was much of the typhoid fever in the District of Columbia in 1906, the first year after filtration, caused by water-borne infection?

3. Was some of the typhoid fever in the District of Columbia in 1907 and in 1908, the second and third years after filtration, caused by infection introduced by the filtered Potomac River water?

The evidence is not yet sufficiently complete to give definite and final answers to the first two of these questions. While much may be said on both sides of these questions, it will be necessary to await the results of still further observations on the typhoid-fever situation and of further bacteriologic studies of the water before arguments sufficiently complete to point to fairly definite conclusions can be presented.

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[a] Hygienic Laboratory Bulletin No. 44, p. 44.

In answer to the third question, we are satisfied that, as the results of our studies, the bulk of the typhoid fever in the District of Columbia during the years 1907 and 1908 was not caused directly by water-borne infection. The facts do not prove, however, that some of the infection during those two years may not have been water borne. The amount of infection so conveyed may have been but an insignificant part of the whole, yet the amount so added may have been sufficient to have accounted for enough typhoid fever to keep the rate higher than it should be for a city under the sanitary and climatic conditions of Washington and having pure water.

During the summer periods of both years the water was, according to the present generally accepted bacteriological standards, of good sanitary quality, and it certainly does not seem probable that such water could have been directly responsible for more than a very insignificant part of the infection. Yet in these summer periods the typhoid-fever rate was much higher than in the other seasons of the two years.

While we advocate still greater improvement of the city's water supply by increased storage or the use of a coagulant, we believe that in order to materially reduce the prevalence of typhoid fever in the District of Columbia measures will have to be directed against other factors concerned in the transmission of the disease, especially contact and milk.

MILK.

The 542 cases gave the following history as to the use of milk during the thirty days prior to onset of illness:

How used:	Number of cases.
As a beverage	228
On fruits or cereals, but not as a beverage	141
In hot coffee or tea only	64
As ice cream only	44
As ice cream and as condensed milk	18
As condensed milk only	12
None in any way	32
Not determined	3
Total	542

Of those using milk as a beverage, one used boiled milk exclusively; and of those using milk on fruits and cereals, one used pasteurized milk exclusively.

The large proportion of the cases which gave a history of having used raw milk during the thirty days previous to illness is striking when considered in connection with the data on milk obtained for the general population of 32 blocks of the city. (See Table No. 1, p. 15.)

Of 1,101 households canvassed, only 614, or about 54 per cent, habitually used raw milk. Of the remainder, 11 per cent used home-heated milk, 7 per cent commercially pasteurized milk, 11 per cent condensed milk, 2 per cent used milk very rarely, and 10 per cent used no milk.

There are now in Washington three dairymen (Nos. 3, 4, and 10, see chart No. 9), each doing a large business, who have installed pasteurizing machines and who claim that all the milk which they supply their customers is pasteurized. In our figures, however, we confine the number of cases as having used pasteurized milk to those who had pasteurized the milk after receiving it from the dairy.

Dairymen Nos. 3 and 4 began pasteurizing their supplies of milk about July 1, 1908. The number of cases of typhoid fever among their customers per 100,000 gallons of milk sold during the typhoid seasons of the two years before pasteurization and the year of pasteurization (1908) was as follows:

Year.	Dairyman No. 3.	Dairyman No. 4.
1906, no pasteurization	16.6	52.5
1907, no pasteurization	7.1	21.6
1908, pasteurization	5.8	10.1

The reduction in the number of cases since pasteurization is marked, especially for dairyman No. 4. This dealer had a pronounced outbreak among his customers from October 2 to October 21, 1906. The outbreak was attributed to infection introduced into the milk from a typhoid-fever patient on one of the farms at that time supplying this dealer with milk. If pasteurization of the milk at his dairy had been done at that time, the outbreak would not have occurred and Washington would have been saved 32 of the cases of typhoid fever which developed in October, 1906.

The following table gives the number of cases of typhoid fever per 100,000 gallons of milk sold by the principal dealers during the three years.

Dealer No.—	Number cases typhoid per 100,000 gallons milk sold.			Remarks.
	1906.	1907.	1908.	
1	18.2	7.1	10.4	
2	21.5	23.4	10.2	
3	16.6	7.1	[a] 5.8	[a] Milk pasteurized summer of 1908.
4	[b] 52.5	21.6	[c] 10.1	[b] Outbreak October 2–21, 1906; 32 cases. [c] Milk pasteurized summer of 1908.
5	22.0	7.2	16.8	
6	23.7	15.6	13.3	
7	35.0	13.6	9.1	
8	36.6	17.1	18.8	
9	35.2	8.9	13.4	
10	6.7	5.4	7.3	Bottles sterilized and milk pasteurized during all three years.
11	18.2	8.2	[d] 35.1	[d] Outbreak September 24 to October 24, 1908; 19 cases.
12	12.7	11.2	2.3	
13	[e] 113.9	6.5	14.0	[e] Outbreak July 6 to August 20, 1906; 35 cases.
14	22.2	.....	8.6	
15	[f] 6.3	.....	.....	[f] Comparatively small dealer.
16	13.7	8.1	9.5	
17	18.4	5.2	0.0	Bottles sterilized and special milk from one farm during all three years.
18	28.2	4.7	3.4	
44	.....	26.7	13.0	
46	.....	29.3	8.3	
59	.....	.....	187.5	Outbreak September 24 to October 22, 1908; 33 cases.
71	.....	.....	5.7	
163	.....	[g] 282.9	25.0	[g] Outbreak June 8 to August 17, 1907; 28 cases.

It should be noted that the ratio for dealer No. 10, who has sterilized bottles and pasteurized milk during all three years, has been consistently low.

One distinct milk outbreak [a] occurred this year (1908). This was among the customers of milk dealers Nos. 11 and 59 (see Chart No. 9). There were in the course of this outbreak 54 cases with onsets of illness from September 24 to October 24. Two of these cases were reported subsequent to October 31 and, therefore, are not included in the number covered by our period of investigation (cases reported from May 1 to November 1).

Fifty-two, or about 10 per cent of the 542 cases investigated occurred in the course of this one milk outbreak and were attributed to infected milk.

Thirty-three of the cases were among the customers of milk dealer No. 59 and 21 among the customers of milk dealer No. 11.

[a] "A milk-borne outbreak of typhoid fever traced to a bacillus carrier," by L. L. Lumsden and William C. Woodward, Journ. Am. Med. Assn., vol. 52, March 6, 1909, pp. 749–752.

TOMERS OF THE F

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TIN 52, HYGIENIC LABORATORY,

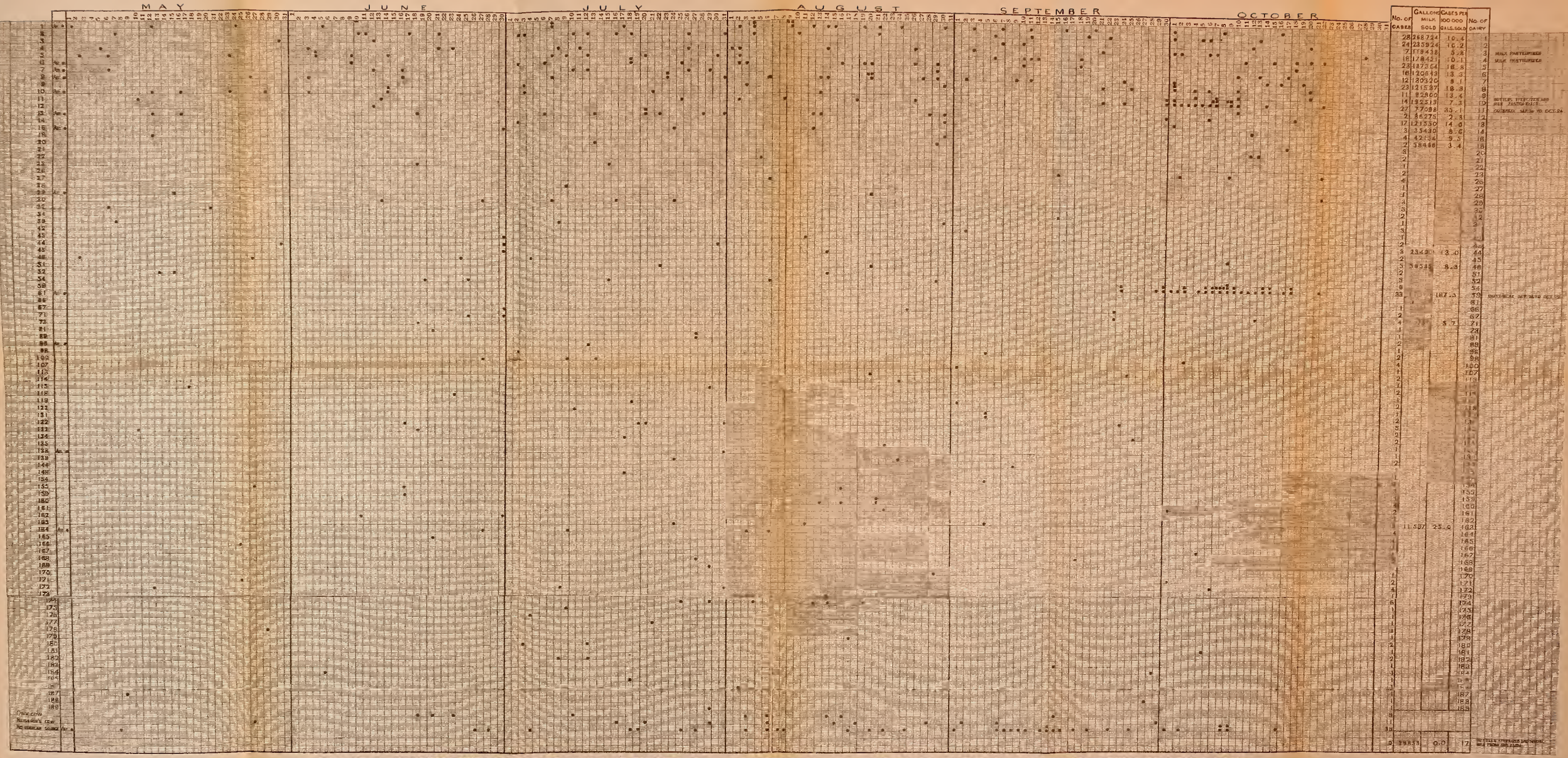

Chart No. 9.—SHOWING THE NUMBER OF CASES OF TYPHOID FEVER, ACCORDING TO DATE OF ONSET, AMONG THE CUSTOMERS OF THE PRINCIPAL MILK DEALERS IN THE DISTRICT OF COLUMBIA, 1908.

The number of the dairy corresponds to the number of the dairy on Chart No. 3 of Bulletin No. 35, and Chart No. 4 of Bulletin No. 44, Hygienic Laboratory, P. H. & M. H. S.

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BULLETIN 52, HYGIENIC LABORATORY, P. H. & M. H. S.

The following table gives the cases by date of onset of definite symptoms:

Date of onset.	Number of cases.		
	Among customers of dealer No. 59.	Among customers of dealer No. 11.	Total.
Sept. 24	2	1	3
29	1	0	1
30	2	2	4
Oct. 1	1	2	3
2	1	1	2
3	2	1	3
4	0	1	1
5	1	1	2
6	2	1	3
7	2	3	5
8	3	0	3
9	3	3	6
10	2	1	3
11	1	0	1
12	1	0	1
13	1	0	1
14	2	1	3
15	2	0	2
16	0	1	1
17	1	0	1
18	2	0	2
19	0	0	0
20	0	0	0
21	0	0	0
22	1	1	2
23	0	0	0
24	0	1	1
Total	33	21	54

The records of the health department showed that during the entire twelve months preceding this outbreak no cases had occurred in households supplied by dairyman No. 59 and only 7 in households supplied by dairyman No. 11. It was evident, therefore, that there had occurred among persons supplied with milk by these two dealers an unusually large number of cases.

At the time of the beginning of this outbreak the prevalence of typhoid fever generally in the District of Columbia was on the decline. (See Chart No. 1.)

The sudden and marked increase in cases was confined to sections of the city supplied with milk by these two dealers and in those sections the unusual number of cases were confined to persons using milk from these two dealers. Most of the cases were in the western section of the city commonly known as Georgetown. Here cer-

tainly over thirty other dairymen sold milk and the routes of some of them ramified through the same sections as those of Nos. 59 and 11; but there was no unusual number of cases among the customers of any of these other dairymen.

The following table gives the age and sex of the cases attributed to infection in milk during this outbreak:

Age in years.	Number of cases.		
	Male.	Female.	Total.
0 to 4	4	1	5
5 to 9	5	8	13
10 to 14	5	3	8
15 to 19	2	2	4
20 to 24	3	3	6
25 to 29	1	6	7
30 to 34	3	1	4
35 to 39	1	2	3
40 to 44	0	1	1
45 to 49	0	0	0
50 to 54	0	0	0
55 to 59	0	0	0
60 to 64	1	1	2
65 to 69	0	1	1
Total	25	29	54

Thus the majority of the cases were among children, about 50 per cent of the cases being in persons under 15 years of age.

The history of the cases in regard to the use of milk previous to illness was as follows:

How used:	Number of cases.
As a beverage	30
On fruits or cereals, but not as a beverage	20
In coffee only	2
As ice cream only	2
Total	54

The source of water used by the cases for drinking during the thirty days previous to illness was as follows:

Unboiled tap solely	28
Unboiled tap principally and outside of the District of Columbia	7
Boiled tap solely	1
Boiled tap principally and outside of the District of Columbia occasionally	7
Boiled tap principally, unboiled tap, and outside of the District of Columbia occasionally	5
Boiled tap principally, but unboiled tap, bottled, and outside of the District of Columbia also	1
Boiled tap principally, unboiled tap occasionally	2
Boiled tap principally, bottled water occasionally	1
Filtered tap principally and boiled tap occasionally	1
Private well solely	1
Total	54

The various drinking waters used by these cases, as compared with those used by all the cases of the 1908 period (see table, p. 47), offers an interesting field for speculation as to the part played by drinking water in constituting susceptibility to typhoid fever infection.

The 54 cases were distributed among the members of 45 households. There were eight instances in which two or more members of the same household were affected. The largest number of cases in one household was three. There were fifteen instances in which the members of the household who used milk freely were affected, while those who used none or used it more sparingly escaped.

Most of the cases were among well-to-do persons.

The sanitary conditions of the residences at which the cases occurred were as follows:

Sanitary condition:	Number of cases.
Good	25
Fairly good	23
Rather bad	6
Bad	0
Total	54

Many of the cases had a sudden onset of definite symptoms without recognizable prodromes and many ran a mild course.

Among the 54 cases there were eight fatalities—mortality rate of 14.8 per cent, which is rather high for cases due to milk-borne infection.

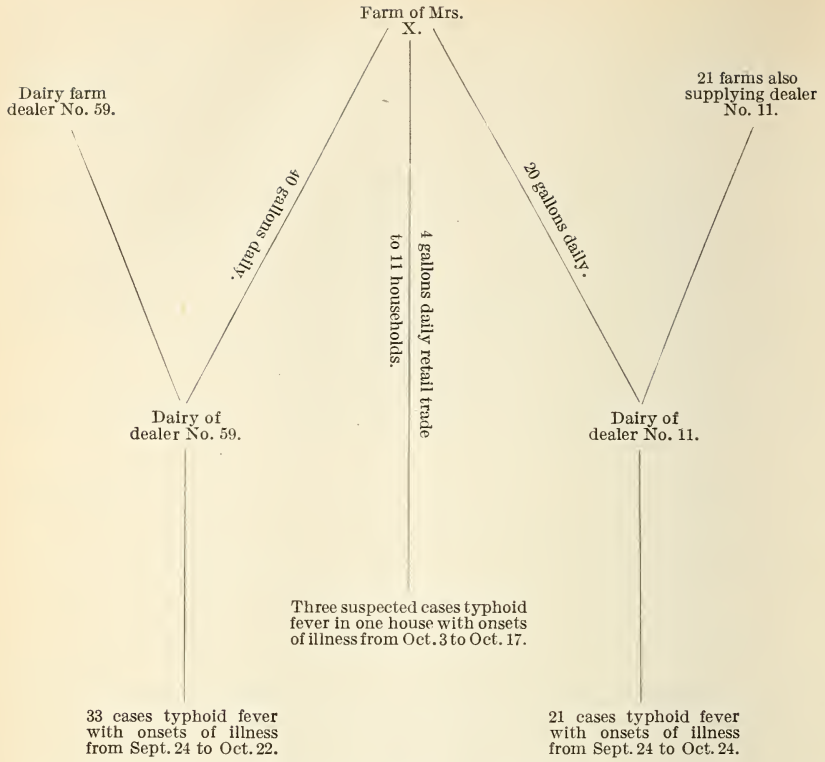
Among the persons associated with the cases attributed to milk infection there were six secondary cases attributed to infection by contact with milk cases.

All but one of these secondary cases were reported subsequent to October 31 and are not included in our figures on contact infection.

The synchronous outbreaks among the customers of these two dairymen at once suggested a common source of infection.

Dairyman No. 59 received milk from two farms. One of these, in the District of Columbia, was owned by him and was the location of his dairy. The other was the farm of Mrs. X, in Maryland, from which he received about 40 gallons of milk daily.

Dairyman No. 11 received milk from about 22 different farms located in Virginia and Maryland. One of the farms supplying this dairyman was that of Mrs. X, the same farm which furnished a part of No. 59's supply. From this farm dairyman No. 11 received about 20 gallons of milk daily. Diagrammatically the situation was as follows.



A most careful investigation was made by the health department of the District of Columbia and by ourselves, but no suspicious illness could be learned of among those concerned in handling the milk at the dairy of dealer No. 59 or at the dairy of dealer No. 11, or at any of the farms supplying either of these dealers with milk. All the evidence, however, pointed very strongly to the farm of Mrs. X as the source of the infection, and as there had been no suspicious illness on this farm or in the neighborhood thereof to account for the infection the possibility of a bacillus carrier being the source of the infection presented itself.

Specimens of stools and urine from all the thirteen persons on the farm were obtained and examined at the Hygienic Laboratory for the typhoid bacillus. All were negative except the specimen of feces from Mrs. X; this specimen contained a large number of typhoid bacilli.

Mrs. X had had typhoid fever about eighteen years before and had been in robust health since then. She had more to do with handling the milk on her farm than any of the others there so concerned. There were a number of ways to account for the infection getting into the milk, since infected excreta were being deposited in an open privy on the place. Among the many possible ways were

the hands of persons, flies, and barely possibly from contamination of the water supply used for washing the milk cans.

Neither dairymen No. 59 nor No. 11 mixed the milk from the various farms supplying them before bottling and delivering it to their customers. Had they followed this custom, which is rather general at the other dairies in Washington, there might have been a much larger number of cases in this outbreak. They both bottled directly from the cans as the cans came from the different farms.

Dairyman No. 11 stated that he was quite sure that all the milk which he received from Mrs. X he sold to a bakery, where it was cooked. However, in view of all the evidence, it seems probable that he was mistaken and that some of the milk from the farm of Mrs. X was bottled at his dairy and delivered to his retail customers. As he acknowledged that he used the same scouring cloths and brushes on the milk cans which had contained the milk from the farm of Mrs. X, and on those in which the milk he bottled and retailed was stored, it is possible that the infection was so transferred from the cans of Mrs. X to the other cans.

The positive finding of typhoid bacilli in the feces of Mrs. X was determined on October 16, and on that date the sale of milk from her farm was discontinued by order of the health officer. Eight days later—October 24—the last cases occurring among the customers of dairymen Nos. 59 and 11 had their definite onset of typhoid fever.

In each of the three years in which we have studied the typhoid-fever situation in Washington one or more pronounced and easily recognized milk outbreaks have occurred. The number of cases definitely traced to milk infection has amounted to about 10 per cent of the cases originating in the District of Columbia during the periods (May 1 to November 1) of all three years.

Besides the cases occurring in the course of the pronounced outbreaks there were very probably scattering cases due to infected milk which could not be traced. This surmise is supported by the fact that, coincident with the periods of these milk outbreaks, there have been increases in the number of cases not definitely attributable to any of the known factors. (See Chart No. 2.)

A pernicious custom among the milk dealers of Washington is the exchange and use of unsterilized milk bottles. Bottles used for serving milk by a certain dealer on one day may be used by other dealers on the next day. Only two or three of the milk dealers make any pretense of sterilizing the bottles after collecting them from the various households and before refilling them with milk. It is readily conceivable, therefore, how bottles which have contained the infected milk of a certain dairy may carry infection to milk supplied by other dairies and so be responsible for scattering cases of typhoid fever among persons receiving milk from these other dairies.

ICE CREAM.

The history of the cases in regard to the eating of ice cream within the thirty days prior to onset of illness was as follows: Yes, 395; no, 138; not determined, 9.

Eighty-six of the cases gave a history of having eaten ice cream which was purchased from street vendors. Ten other cases, with onsets of illness distributed over a period of about two months, ate ice cream at a certain bakery. Among the persons who work at this bakery a case of typhoid fever developed in the summer of 1907 and two cases in the summer of 1908. This bakery does quite a large business in a section of the city in which typhoid fever each year is somewhat disproportionately prevalent. The possibility of some one at this bakery being a bacillus carrier occurred to us and specimens of stools and urine from all persons at the bakery were examined bacteriologically; but all were negative for the typhoid bacillus.

No cases this year could be traced to ice cream, but in view of the bad sanitary conditions under which much of the ice cream in Washington is handled (particularly by street vendors) this year, as last, a certain number of scattering cases probably were caused by infection in ice cream.

RAW SHELLFISH.

The following table gives the history of the cases in regard to the eating of raw oysters and clams from time to time during the thirty days prior to the onset of illness:

Shellfish	Yes	No.	Not determined.
Oysters	33	495	14
Clams	18	510	14

The small number of cases giving a history of having eaten uncooked shellfish shows that shellfish this year, as was the case in 1906 and 1907, could not have been the means of transmitting the infection to any considerable number of cases. Furthermore, during the season—June, July, August, and September—when typhoid fever is most prevalent in the District of Columbia comparatively few raw oysters or clams are eaten.

OCCUPATION.

In the following table are given the occupations of the cases, along with the number of persons engaged in such occupations, in the District of Columbia, according to the United States census of 1900:

Occupation.	Number of cases.			Number of persons engaged in occupation, census of 1900.		
	Male	Female.	Total.	Male.	Female	Total.
Professional pursuits:						
Electricians	1	0	1	461	0	461
Journalists	2	0	2	398	0	398
Lawyers	1	0	1	1,445	0	1,445
Officials (government)	1	0	1	900	0	900
Officials (bank)	1	0	1	.....	.....	.....
Chemists	1	0	1	.....	.....	.....
Artists	1	0	1	164	146	310
Clergymen	2	0	2	504	0	504
Physicians	1	0	1	881	0	881
School-teachers	0	3	3	.....	1,598	1,598
Architects	1	0	1	478	0	478
Total	12	3	15	.....	.....	.....
Domestic and personal service:						
Barbers and hairdressers	1	1	2	860	0	860
Bartenders	3	0	3	591	0	591
Housekeepers, stewardesses	0	1	1	0	529	529
Laborers, not specified	27	0	27	12,476	263	12,739
Launderers, laundresses	2	13	15	474	7,194	7,666
Nurses and midwives	3	3	6	231	1,311	1,542
Restaurant and saloon keepers	1	1	2	612	112	724
Servants and waiters	9	10	19	2,898	15,231	20,129
Soldiers, sailors, marines (United States)	2	0	2	745	0	745
Watchmen, policemen, firemen, etc	4	0	4	1,667	0	1,667
Total	52	29	81	.....	.....	.....
Trade and transportation:						
Agents (real estate)	4	0	4	.....	.....	.....
Bookkeepers and accountants	1	2	3	837	482	1,319
Clerks, copyists	11	0	11	11,523	4,697	14,220
Draymen, hackmen, teamsters	13	0	13	3,994	0	3,994
Hostlers	2	0	2	410	0	410
Hucksters, peddlers	7	0	7	526	0	526
Merchants, dealers	5	0	5	3,945	418	4,363
Messengers, errand and office boys	9	0	9	1,345	0	1,345
Porters, helpers in stores	4	0	4	921	0	931
Salesmen and saleswomen	9	8	17	2,644	1,320	3,964
Steam-railroad employees	5	1	6	1,185	0	1,185
Stenographers and typewriters	6	3	9	521	708	1,229
Street-railway employees	4	0	4	817	0	817
Telegraph and telephone operators	0	3	3	307	(?)	307
Total	28	15	43	.....	.....	.....

Occupation.	Number of cases.			Number of persons engaged in occupation, census of 1900.		
	Male.	Female.	Total.	Male.	Female.	Total.
Mechanical and manufacturing:						
Bakers	2	0	2	622	(?)	622
Blacksmiths	1	0	1	775	0	775
Boot and shoe makers, repairers	2	0	2	496	0	496
Carpenters, joiners	2	0	2	2,298	0	2,298
Dressmakers	0	5	5	0	2,993	2,993
Engineers, firemen (not locomotive)	3	0	3	1,116	0	1,116
Iron and steel workers	2	0	2	300	0	300
Machinists	4	0	4	1,392	0	1,392
Masons, brick and stone	2	0	2	1,153	0	1,153
Paper hangers	1	0	1	363	0	363
Plumbers, gas and steam fitters	5	0	5	1,074	0	1,074
Printers, lithographers, pressmen	6	2	8	2,942	481	3,323
Tailors	2	0	2	616	0	616
Total	32	7	39	.....	.....	.....
Miscellaneous:						
Persons attending school	88	86	174	18,788	21,116	39,904
Infants and small children	19	13	32	(?)	(?)	(?)
Housewives	.....	46	46	0	35,000	35,000
Inmates of institutions	9	3	12	.....	.....	.....
No occupation	4	22	26	.....	.....	.....
Tinners and stove fitters	1	0	1	.....	.....	.....
Inspector, city water department	1	0	1	.....	.....	.....
Elevator boys	2	0	2	.....	.....	.....
Chauffeurs	1	0	1	.....	.....	.....
Charwomen	0	2	2	.....	.....	.....
Gardeners, truck	1	0	1	.....	.....	.....
Gardeners, landscape	1	0	1	.....	.....	.....
Butchers	1	0	1	.....	.....	.....
Draftsmen	1	0	1	.....	.....	.....
Hotel clerks	1	0	1	.....	.....	.....
Clothing collector, philanthrophy	1	0	1	.....	.....	.....
Commercial travelers	1	0	1	.....	.....	.....
Newsboys	5	0	5	.....	.....	.....
Photo-engravers	1	0	1	.....	.....	.....
Total	138	172	310	.....	.....	.....
Grand total	314	228	542	.....	.....	.....

This year, as in 1907 and 1906, the disease was fairly uniformly distributed among persons regardless of occupations.

INFECTION BY CONTACT.

Of the 542 cases, 66, or about 12 per cent, gave a history of having had, within the thirty days prior to onset of illness, more or less free and intimate association with typhoid patients in the febrile stage of the disease, and were attributable to infection by direct contact.

Of the 523 cases studied in 1907, about 13 per cent, and of the 747 cases in 1906 only about 4 per cent gave a history of association with previous cases in the febrile stage and were attributed to infection by direct contact.

In the number of cases attributed to infection by direct contact are not included cases giving a history of association with previous cases but which, on account of the precautions being taken and the conditions generally, were considered more probably attributable to some other factor, such as milk.

The following table gives the intervals in days elapsing between the definite onset of illness of the 66 secondary cases and the definite onset of the primary cases from which these 66 cases were considered to have contracted the infection, the figures for the cases studied in 1907 being placed in a parallel column:

Number of days elapsing between onset of primary and secondary cases.	Number of secondary cases.	
	1908.	1907.
7	0	2
8	3	3
9	2	4
10	1	3
11	0	3
12	1	2
13	3	0
14	1	1
15	1	0
16	1	1
17	0	1
18	3	2
19	0	2
20	3	1
21	1	1
22	3	1
24	0	2
25	0	3
27	0	1
28	0	2
29	2	2
30	1	2
31	1	1
32	1	0
33	2	1
34	1	0
35	3	1
36	0	1
37	2	0
38	1	0
39	2	1
40	3	1
41	0	1
44	1	1

Number of days elapsing between onset of primary and secondary cases.	Number of secondary cases.	
	1908.	1907.
45	2	0
46	1	1
47	1	0
48	1	1
49	1	0
50	2	0
51	0	1
52	1	0
53	0	1
54	0	1
64	1	0
70	1	0
80	1	0
84	1	0
Not accurately determined	0	6
Interval not determined, secondary cases being among physicians, nurses, helpers, or inmates of hospitals, who were more or less constantly exposed	10	12
Total	66	70

These figures do not support the view held by H. Conradi[a] that the infection is transmissible most often during the earliest days of the disease and frequently in the incubation period; but they do emphasize the importance of exercising the utmost precautions to prevent the spread of infection from typhoid-fever patients from the onset to the end of illness.

The cases attributed to direct contact with previous cases are indicated in red on Chart No. 2 and by chromes on maps Nos. 8, 9, and 10. In some instances the location of the residence of the secondary case was some distance from that of the primary case, the infection presumably having been contracted during visits to the home of the primary case.

Among the cases occurring in the 1908 period not definitely attributable to a known factor there were 15 which gave a history of association with febrile cases suspected to be, but not reported as, typhoid fever.

There was strongly suggestive evidence that 78 of the cases were due to infection indirectly conveyed by persons or flies from previously existing known cases.

In determining that conveyance of infection by flies was probable, the proximity of the house occupied by the primary case to that occu-

[a] Deut. med. Woch., Oct. 10, 1907.

pied by the secondary case, the number of flies in the neighborhood and the readiness with which they could pass from the infected excreta of the known case to the food of the persons subsequently attacked, and all other conditions thought to have any bearing on the situation, were taken into consideration.

Four cases gave a history of association with persons convalescent from typhoid fever.

Estimating the chances of these 97 cases having become infected by contact at about 50 per cent, and adding 48 cases so deduced to the 66 cases considered as almost certainly having been infected by contact, there were 114, or about 21 per cent, of the 542 cases attributable to contact infection.

These 114 cases comprise about 17 per cent of the total 665 cases investigated, as against about 15 per cent (estimated on the same basis of probability) of the 670 cases investigated in 1907 and about 6 per cent of the 866 cases investigated in 1906.

BACILLUS CARRIERS.

In consideration of the likelihood of chronic bacillus carriers playing a part in the dissemination of typhoid fever in the District of Columbia the following data in regard to known association of the 542 cases with persons who, within the five years previous, had had typhoid fever were obtained:

Association 30 days prior with persons who had had typhoid fever within—	Number of cases and amount of association.			
	Intimate.	Fairly intimate.	Slight.	Total cases.
6 months	1	1	2	4
6 months to 1 year	9	0	2	11
1 to 2 years	8	4	3	15
2 to 3 years	3	0	3	6
3 to 4 years	3	2	0	5
4 to 5 years	8	0	0	8
Total	32	7	10	49
No known association				493
Grand total				542

For people living in a large city there is, of course, much association with persons which would be difficult, if not impossible, to trace. Therefore the data in the above table presented for the cases studied in 1908, like those for the cases studied in 1907, refer almost entirely to association with members of the family or with intimate acquaintances. The number of cases giving a history of such association is, therefore, far below the actual.

Effort was made to obtain from persons who had had typhoid in previous years, and with whom the cases had been in association during the thirty days prior to onset of illness, specimens of feces for bacteriological examination. Thirty-two such specimens, obtained from persons who had had typhoid from six months to twenty years previous, were examined and all were negative for the typhoid bacillus.

Beside these there were examined in the course of the intensive study in special districts specimens of feces from 71 persons who had had typhoid fever from one to ten years previous and all were negative for the typhoid bacillus. (See Table No. 3, page 16.)

With a view to forming some idea of how many persons in Washington during the typhoid fever season are bacillus carriers we had collected and examined, from July 16 to November 8, 1,014 specimens of feces and 26 specimens of urine from 993 persons living in 32 blocks of the city. The method followed in making these examinations was as follows:

METHOD OF EXAMINING FECES FOR THE TYPHOID BACILLUS.

The specimens are collected in 2-ounce wide-mouth cork-stoppered bottles. Each bottle is accompanied by a piece of wood to be used as a spatula. The bottles and spatulas are sterilized before distribution. In collecting the specimens instructions are given to place a small quantity of feces about the size of a walnut into the bottle with the wooden spatula, replace the cork, and label at once with the name and date. The bottles are distributed throughout the day and collected next morning and brought to the laboratory as soon as practicable.

About 10 c. c. of sterile bouillon is poured into each bottle and agitated. This bouillon is now poured off into a conical graduate and allowed to stand one-half to one hour, during which time heavy particles settle, and it is supposed the upper layers of such a fluid will be more apt to contain motile organisms like the typhoid bacillus.

A bent glass rod is now dipped into the upper layer of the bouillon and used to streak five Endo plates. These are incubated 24 hours, at the end of which time the small transparent colorless dewdroplike colonies are fished and planted into bouillon These bouillon tubes are incubated 24 hours. A drop or two of horse serum of high agglutinating power for *B. typhosus* is then added to the 24-hour old bouillon growth.

With a serum of very high agglutinating power definite clumping is evident to the naked eye usually within 15 to 20 minutes and almost invariably within an hour. Positive growths which fail to agglutinate with typhoid agglutinin should be tested with paratyphoid serums.

If agglutination occurs with either the typhoid or paratyphoid serum, agitate the tube to break up clumps and plate out some of the growth either on Endo or lactose-litmus agar plates. Note the appearance of the colonies after 24 hours. Fish one or more of these colonies and so obtain the organism in pure culture. Then carry the organism through the various cultural and other tests required to establish positively its identity.

DIRECTIONS FOR MAKING ENDO'S MEDIUM.[a]

Make 4 per cent neutral agar as follows: Take 1 liter distilled water, add 5 grams sodium chloride, 10 grams Liebig's extract meat, 10 grams peptone.

Dissolve by heating, allow to cool, and place on surface 40 grams powdered agar. When this has settled, place in Arnold sterilizer, covering beaker with paper, and cook three hours.

Filter through cotton on a perforated funnel (Buchnerfilter) by the aid of a vacuum or allow to settle while slowly cooling and discard the bottom turbid stratum.

Neutralize to litmus paper with Na_2CO_3 solution.

Add 10 c. c. sterile 10 per cent Na_2CO_3 solution.

It is convenient at this point to put up the medium in 100, 200, and 400 c. c. quantities in flasks, the flasks being large enough to provide room for the other ingredients. These can be stored until needed for use; then the agar is melted and the other ingredients added in the proper proportion.

Add 10 grams chemically pure lactose. It is important that this should be pure.

Add 5 c. c. alcoholic fuchsin solution. This is prepared as follows: To 10 grams fuchsin (not acid fuchsin) add 100 c. c. 96 per cent alcohol, shake, allow to stand 20 hours, and filter the supernatant fluid, which is used. It should be filtered each time before using.

Add 25 c. c. freshly made and sterilized 10 per cent sodium sulphite solution.

Sterilize for a few minutes in the Arnold and pour plates while the medium is steaming hot.

The medium after cooling should be nearly colorless to transmitted light, and rose or flesh colored to reflected light.

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[a] References.

Endo, S. Centblt. f. Bakt., vol. 35, No. 1, 1903–4, p. 109.
Klinger. Arb. a. d. Kais. Ges.-Amt., 1906, p. 52. See also Herford.
Willson. Jour. hyg., 1905, p. 429.
Scheller. Centblt. f. Bakt., Sept. 15, 1906, p. 62.
Clauditz. Hyg. runds., vol. 14, 1904, p. 718.
Herford. Arb. a. d. Kais. Ges.-Amt., 1906, p. 62.
Kayser. Munch. med. Woch., 1906, p. 17–18.

The lactose, fuchsin and sodium sulphite solutions must be added to the melted agar just before it is to be used. The plates are flown and allowed to stand 20 minutes uncovered in the incubator in order to do away with water of condensation and to obtain a good surface.

Organisms which split lactose restore the red fuchsin and appear as deep red sharply limited opaque colonies with a greenish surface sheen.

The typhoid organism produces smaller transparent colonies, resembling a small drop of water.

Specimens of feces and urine examined for typhoid bacilli July 16 to November 8, 1908.

Specimen No.	Name of person.	Street address.	Age.	Color.	Sex.	Previous typhoid.	Present health.	Date of examination.	Result.	Examiner.	Remarks.
			Years.								
1	A. O.....	2133 F nw...	9	W	F	0	OK	7/16	—	L	Slight bowel trouble July 4.
2	G. H.....	2109 F nw...	22 m.	W	F	0		7/16	—	L	Teething.
3	J. T.....	.....do.....	5	W	M	0	OK	7/16	—	L	
4	R. L. S.....	2116 G nw...	?30	W	F	0		7/21	—	L	Acute indigestion from fruit.
5	F. S.....	.....do.....	2	W	F	0	OK	7/22	—	L	Recent acute indigestion.
6	F. K.....	.....do.....	4	W	F	0		7/18	—	L	Acute indigestion, castor oil.
7	G. W. A. W.	2120 G nw, A203	?40	W	F	0	OK	7/19	—	L	Slight occasional diarrheas.
8	C. H. J.....	2120 G nw, A103	?50	W	M	0		7/19	+	L	Recurrent gastritis, an attack last night. (See Nos. 27A and 1013.)
9	E. H.....	2120 G nw, A104	?30	W	F	?		7/19	—	L	"Chronic intestinal catarrh."
10	J. S.....	604 22d nw...	4	W	M	0	OK	7/23	—	M	Often gets stomach trouble from overeating.
11	W. H. J.....	608 22d nw...	?30	W	F	5	OK	7/22	—	M	
12	W. J.....	.....do.....	15 m.	W	F	0	OK	7/22	—	M	Occasional stomach attacks.
13	I. A.....	.....do.....	?30	W	F	III	OK	7/22	—	M	
14	L. V. McG..	610 22d nw...	?25	W	F	4	OK	7/23	—	M	
15	G. R. M.....	2138 G nw...	21	W	M	0		7/24	—	M	Slight transient undiagnosed illness.
16	L. O'N.....	616 22d nw...	1½	W	M	0		7/24	—	M	Teething.
17	C. T.....	622 22d nw...	?25	W	F	3	OK	7/23	—	M	
18	U. B.....	621 23d nw...	?30	W	M	0	OK	7/24	—	M	Has had gall-bladder attacks.
19	C. Y., jr.....	611 23d nw...	12	W	M	5		7/24	—	M	
20	F. M.....	2127 G nw...	19	W	M	0		7/25	—	M	Slight general debility.
21	F. J.....	2124 Daly's ct	?40	C	M	?	OK	7/27	—	R	
22	H. I. S.....	702 22d nw, A12	?25	W	M	0	OK	7/27	—	R	Wife now has typhoid.

Specimens of feces and urine examined for typhoid bacilli July 16 to November 8, 1908—Continued.

Specimen No.	Name of person.	Street address.	Age.	Color.	Sex.	Previous typhoid.	Present health.	Date of examination.	Result.	Examiner.	Remarks.
			Years.								
23	E. I.	720 21st nw..	6	W	M	¼	OK	7/28	—	R	
24	W. S.	730 21st nw..	?65	W	M	0	OK	7/28	—	R	Recent slight heat exhaustion.
25	F. P.	2104 H nw...	?20	W	M	4	OK	7/29	—	L	Urine.
26	R. G.	716 22d nw...	7 m.	W	F	0	OK	7/29	—	L	Digestive disturbance 6 weeks ago.
27	M. G.	.....do.....	4	W	F	0	OK	7/29	—	L	
27A	C. H. J.	2120 G nw, A103	.....	.....	.....	.....	.....	7/29	—	L	Somewhat improved. (See Nos. 8 and 1013.)
28	H. McC.	700 22d nw...	2	W	F	0	OK	7/29	—	L	
29	H. McC.	.....do.....	8	W	M	0	OK	7/29	—	L	
30	W. B. S.	2120 G nw, A302	?30	W	M	4		7/29	—	L	Chronic dyspesia.
31	G. H. M.	603 22d nw...	19	W	M	7		7/29	—	L	Early typhoid. (See Nos. 239 and 1003.)
32	W. S.	2200 H nw...	11	W	M	0	OK	7/29	—	L	
33	L. J.	2208 H nw...	7	C	M	0	OK	7/30	—	L	
34	M. M. K.	2218 H nw...	?55	W	F	0	OK	7/30	—	L	
35	M. T. K.	.....do.....	?30	W	F	0	OK	7/30	—	L	
36	L. McC.	700 22d nw...	5	W	M	0	OK	7/30	—	L	
37	L. McC.	.....do.....	12	W	M	0		7/30	—	L	Fever and headache 3 weeks ago.
38	C. B.	2216 H nw...	10	W	F	0	OK	7/30	—	L	
39	B. B.	.....do.....	12	W	M	0	OK	8/3	—	L	
40	Z. B.	.....do.....	16	W	F	0	OK	8/3	—	L	
41	R. B.	.....do.....	19	W	F	0		8/3	—	L	Recent appendicitis
42	E. B.	.....do.....	14	W	F	0	OK	8/3	—	L	
43	M. B.	.....do.....	8	W	M	0	OK	8/3	—	L	
44	K. G.	716 22d nw...	5½	W	M	0	OK	7/30	—	L	
45	V. I.	720 21st nw..	13	W	F	0	OK	7/31	—	L	
46	F. I.	.....do.....	15	W	M	0	OK	7/31	—	L	
47	D. I.	.....do.....	11	W	F	0	OK	7/31	—	L	
48	L. I.	.....do.....	17	W	F	0	OK	8/1	—	L	
49	T. I.	.....do.....	9	W	M	0	OK	8/1	—	L	
50	G. I.	.....do.....	3	W	M	0	OK	8/1	—	L	
51	A. I.	.....do.....	11 m.	W	M	0	OK	8/1	—	L	
52	A. B.	735 23d nw...	6	W	M	0	OK	7/31	—	L	
53	A. B.	.....do.....	8	W	F	0	OK	8/1	—	L	
54	B. B.	.....do.....	10	W	M	0	OK	8/3	—	L	
55	G. C.	.....do.....	3	W	F	0	OK	7/31	—	L	
56	A. G.	2206 H nw...	15	C	M	4	OK	7/31	—	L	Recent slight summer diarrhea.
57	W. S.	1 Bessell's ct.	15	C	M	0	OK	7/29	—	M	Urine.
58	A. S.	.....do.....	11	C	M	0	OK	7/29	—	M	Do.
59	T. S.	.....do.....	10	C	M	0	OK	7/29	—	M	Do.
60	R. L.	717 23d nw...	?35	C	F	0		8/1	—	L	Chronic dyspepsia.
61	T. L.	.....do.....	11	C	M	0	OK	8/1	—	L	
62	A. T.	2 Bessell's ct.	6	C	F	0	OK	8/1	—	L	

Specimens of feces and urine examined for typhoid bacilli July 16 to November 8, 1908—Continued.

Specimen No.	Name of person.	Street address.	Age.	Color.	Sex.	Previous typhoid.	Present health.	Date of examination.	Result.	Examiner.	Remarks.
			Years.								
63	I. T.....	2 Bessell's ct.	13	C	F	0	OK	8/1	—	L	
64	W. T.....	.....do.....	15 m.	C	M	0	OK	8/1	—	L	
65	P. J.....	2214 H nw...	? 25	W	F	0	OK	8/1	—	L	Chills 3 months ago.
66	M. D.....	3 Bessell's ct.	13	C	F	0	OK	8/3	0	L	
67	M. D.....	.....do.....	11	C	F	0	OK	8/5	—	M	
68	I. D.....	.....do.....	9	C	M	0	OK	8/3	—	L	
69	L. D.....	.....do.....	5	C	M	0	OK	8/1	—	L	
70	B. D.....	.....do.....	2	C	F	0	OK	8/1	—	L	
71	M. T.....	2 Bessell's ct.	10	C	F	0	OK	8/3	—	L	
72	N. S.....	717 23d nw...	? 55	C	F	0		8/3	—	L	Chronic dyspepsia.
73	H. T., jr....	2214 H nw...	2½	W	M	0	OK	8/3	—	L	
74	E. McQ.....	.....do.....	? 30	W	M	0		8/3	—	L	Recovering from "generalized tuberculosis." (?!)
75	E. L.....	717 23d nw...	9	C	F	0	OK	8/4	—	M	
76	F. A. M.....	2125 H nw...	2	W	F	0	OK	8/4	—	M	
77	J. T.....	2 Bessell's ct.	3	C	M	0	OK	8/4	—	M	
78	L. S.....	2218 G nw...	10	W	M	0		8/5	—	M	Transient diarrhea and chills.
79	W. S	1 Bessell's ct.	.....	C	M	.....	.....	.....	—	M	Urines. (See Nos. 57, 58, 59.)
80	A. S			C	M				—	M	
81	T. S			C	M				—	M	
82	S. B.....	2216 H nw...	18	W	F	0	OK	8/5	—	M	Dysentery 1 month ago.
83	T. B.....	2125 H nw...	13	W	F	2	OK	8/5	—	M	
84	L. R.....	2143 H nw...	5	W	F	0	OK	8/5	—	M	
85	C. R., jr.....	.....do.....	1	W	M	0	OK	8/5	—	M	
86	E. R.....	.....do.....	9	W	F	0	OK	8/5	—	M	
87	R. K.....	2209 G nw...	6	C	M	0	OK	8/6	—	M	
88	L. K.....	.....do.....	4	C	F	0	OK	8/6	—	M	
89	G. K.....	.....do.....	5	C	M	0	OK	8/6	—	M	
90	N. J.....	.....do.....	11	C	M	0	OK	8/6	—	M	
91	A. V.....	2147 H nw...	9	W	F	0	OK	8/6	—	M	
92	H. V.....	.....do.....	12	W	M	0	OK	8/6	—	M	
93	E. V.....	.....do.....	13	W	F	0	OK	8/6	—	M	
94	M. V.....	.....do.....	7	W	F	0	OK	8/6	—	M	
95	E. R.....	2143 H nw...	2	W	F	0	OK	8/6	—	M	
96	A. R.....	.....do.....	7	W	F	0	OK	8/6	—	M	
97	F. M.....	2125 H nw...	? 30	W	F	4		8/6	—	M	Summer complaint yesterday.
98	J. B. McC...	821 22d nw...	? 45	W	M	4	OK	8/7	—	M	
99	E. McC.....	.....do.....	12	W	F	0	OK	8/7	—	M	
100	F. McC.....	.....do.....	10	W	M	0	OK	8/7	—	M	
101	J. M.....	604 21st nw..	13	W	M	0	OK	8/8	—	M	
102	R. H.....	610 21st nw..	9	W	F	0	OK	8/8	—	M	
103	F. H.....	.....do.....	2½	W	M	0	OK	8/8	—	M	
104	P. O.....	2106 G nw...	12	W	M	0	OK	8/8	—	M	
105	U. O.....	.....do.....	6	W	F	0	OK	8/8	—	M	
106	J. P.....	621 22d nw...	12	W	F	0	OK	8/8	—	M	
107	M. P.....	.....do.....	11	W	F	0	OK	8/8	—	M	

Specimens of feces and urine examined for typhoid bacilli July 16 to November 8, 1908—Continued.

Specimen No.	Name of person.	Street address.	Age.	Color.	Sex.	Previous typhoid.	Present health.	Date of examination.	Result.	Examiner.	Remarks.
			Years.								
108	I. B.....	619 22d nw...	12	W	F	0	OK	8/8	—	M	
109	N. McF. A..	617 22d nw...	? 30	W	F	6	OK	8/8	—	M	
110	A. M.....	603 22d nw...	? 20	W	F	0	OK	8/8	—	M	Typhoid in house.
111	E. B.....	2135 F nw...	5	W	F	0	OK	8/8	—	M	
112	H. B.....	.....do.....	3	W	M	0	OK	8/8	—	M	
113	N. C.....	2123 F nw...	9	W	M	0	OK	8/8	—	M	
114	F. K.....	2144 I nw....	11	W	M	0	OK	8/8	—	M	
115	L. K.....	2116 G nw...	1½	W	M	0	OK	8/8	—	M	
116	A. S.....	608 22d nw...	6	W	F	0	OK	8/10	—	M	
117	C. S.....	.....do.....	5	W	M	0	OK	8/10	—	M	
118	A. D.....	612½ 22d nw..	1½	W	F	0	OK	8/10	—	M	
119	R. K.....	614 22d nw...	4	W	F	0	OK	8/10	—	M	
120	M. C.....	2123 F nw...	12	W	F	0	OK	8/10	—	M	
121	O. L.....	622 22d nw...	12	W	F	0	OK	8/10	—	M	
122	G. C.....	607 22d nw...	14	W	F	9	OK	8/10	—	M	
123	A. B.....	619 22d nw...	8	W	F	0	OK	8/10	—	M	
124	N. B. (M.?).	.....do.....	3	W	M	0	OK	8/10	—	M	
125	E. C.....	829 22d nw...	9	W	F	0	OK	8/10	—	M	
126	R. C.....	.....do.....	6	W	M	0	OK	8/10	—	M	
127	B. G.....	825 22d nw...	7	W	M	0	OK	8/10	—	M	
128	R. G.....	.....do.....	9	W	F	0	OK	8/10	—	M	
129	C. G.....	.....do.....	4	W	F	0	OK	8/10	—	M	
130	E. W.....	823 22d nw...	? 35	W	F	?		8/10	—	M	Chronic "sewer-gas poisoning?"
131	S. W.....	.....do.....	? 40	W	M	?*	OK	8/17	—	M	Do.
132	J. J.....	2144 I nw....	14	W	M	0	OK	8/10	—	M	
133	M. L.....	2119 H nw...	9	C	F	0	OK	8/10	—	M	
134	B. S.....	2218 G nw...	19 M.	W	M	0	OK	8/11	—	M	
135	M. C.....	2228 G nw...	13	W	F	0	OK	8/11	—	M	
136	K. C.....	.....do.....	8	W	F	0	OK	8/11	—	M	
137	E. C.....	.....do.....	7	W	F	0	OK	8/11	—	M	
138	L. R.....	2144 I nw....	13	W	F	0	OK	8/11	—	M	
139	F. S.....	616 22d nw...	3	W	M	0	OK	8/11	—	M	
140	M. D.....	624 22d nw...	4	W	F	0	OK	8/11	—	M	
141	G. M. (P.?)..	2137 F nw...	10	W	M	0	OK	8/11	—	M	
142	O. P.....	612 22d nw...	9	W	F	0	OK	8/11	—	M	
143	L. P.....	.....do.....	3	W	M	0	OK	8/11	—	M	
144	J. E.....	607 23d nw...	4	C	M	0	OK	8/11	—	M	
145	S. S.....	2211 F nw...	9	C	F	0	OK	8/11	—	M	
146	H. S.....	.....do.....	5	C	M	0	OK	8/11	—	M	
147	C. S.....	.....do.....	4	C	M?	0	OK	8/11	—	M	
148	L. R.....	2221 F nw...	6	W	M	0	OK	8/11	—	M	
149	G. S.....	2130 H nw...	11	W	F	0	OK	8/11	—	M	
150	F. K.....	733 22d nw...	21 m.	W	M	0	OK	8/11	—	M	
151	G. K.....	.....do.....	6	W	F	0	OK	8/11	—	M	
152	A. K.....	.....do.....	4	W	M	0	OK	8/11	—	M	
153	J. B.....	831 22d nw...	? 55	W	M	0		8/11	—	M	Recent summer complaint.
154	M. R.....	707 22d nw...	4	W	F	0	OK	8/11	—	M	

Specimens of feces and urine examined for typhoid bacilli July 16 to November 8, 1908—Continued.

Specimen No.	Name of person.	Street address.	Age.	Color.	Sex.	Previous typhoid.	Present health.	Date of examination.	Result.	Examiner.	Remarks.
			Years.								
155	J. G.	2130 I nw.	16 m.	W	M	0	OK	8/11	—	M	
156	J. D.	2226 G nw.	12	W	M	0	OK	8/12	—	M	
157	E. D.	do.	8	W	F	0	OK	8/12	—	M	
158	A. F.	621 23d nw.	6	W	M	0	OK	8/12	—	M	
159	A. D.	609½ 23d nw.	1½	C	M	0	OK	8/12	—	M	
160	M. S.	2211 F nw.	2	C	F	0	OK	8/12	—	M	
161	C. C.	603 23d nw.	4	C	F	0	OK	8/12	—	M	
162	H. S.	2102 H nw.	14	W	F	0	OK	8/12	—	M	
163	G. R.	713½ 23d nw.	8	C	M	0	OK	8/12	—	M	
164	I. M.	713 23d nw.	?35	C	F	0		8/12	+	M	Now has typhoid.
165	R. E.	2205 H nw.	6	W	M	0	OK	8/12	—	M	
166	J. G.	813 23d nw.	3	C	F?	0	OK	8/12	—	M	
167	M. G.	do.	5	C	F	0	OK	8/12	—	M	
168	N. C.	2120 H nw.	9	W	M	0	OK	8/13	—	M	
169	A. C.	do.	7	W	M	0	OK	8/13	—	M	
170	L. C.	do.	4	W	M	0	OK	8/13	—	M	
171	T. R.	2221 F nw.	8	W	F	0	OK	8/13	—	M	
172	C. E.	2124½ Daly's court.	7	C	M	0	OK	8/13	—	M	
173	D. K.	2116 G nw.	10	W	F	0	OK	8/13	—	M	
174	N. H.	2104 I nw.	6	W	F	0	OK	8/13	—	M	
175	W. H.	do.	13	W	M	0	OK	8/13	—	M	
176	U. S. K.	2225 H nw.	?50	W	F	1½	OK	8/13	—	M	
177	W. K.	2117 H nw.	10	C	M	0		8/13	—	M	Whooping cough now.
178	F. C.	810 22d nw.	3	W	M	0	OK	8/13	—	M	
179	W. C.	do.	6	W	M	0	OK	8/13	—	M	
180	S. B. R.	812 21st nw.	?25	W	M	2	OK	8/13	—	M	
181	T. P.	2213 H nw.	6	W	F	0	OK	8/13	—	M	
182	M. P.	do.	2	W	F	0	OK	8/13	—	M	
183	A. P., jr.	do.	4	W	M	0	OK	8/13	—	M	
184	M. M.	do.	14	W	F	0	OK	8/13	—	M	
185	R. L.	715 22d nw.	13	C	M	0	OK	8/14	—	M	
186	A. C.	do.	16	C	F	0	OK	8/14	—	M	
187	M. J.	734 22d nw.	5	W	F	0	OK	8/14	—	M	
188	F. J.	do.	3	W	M	0	OK	8/14	—	M	
189	F. K.	736 22d nw.	2	W	M	0	OK	8/14	—	M	
190	H. K.	2225 H nw.	7	W	F	0	OK	8/14	—	M	
191	C. N.	815 23d nw.	12	C	M	0	OK	8/14	—	M	
192	J. M.	2202 I nw.	14	W	F	0	OK	8/14	—	M	
193	J. S.	818 22d nw.	12	C	F	0	OK	8/14	—	M	
194	H. C.	810 22d nw.	14	W	F	0	OK	8/14	—	M	
195	E. C.	821 23d nw.	5	C	F	0	OK	8/14	—	M	
196	A. K.	2207 H nw.	12	W	F	8	OK	8/14	—	M	
197	N. K.	do.	7	W	M	0	OK	8/14	—	M	
198	E. K.	2117 H nw.	7	C	F	0		8/14	—	M	Whooping cough.
199	M. I. S.	2107 I nw.	10	W	F	0	OK	8/14	—	M	
200	F. W. K.	2105 I nw.	?30	W	M	5*	OK	8/14	—	M	"Paratyphoid" 5 years ago.
201	E. L.	2119 H nw.	13	C	F	0	OK	8/15	—	M	

Specimens of feces and urine examined for typhoid bacilli July 16 to November 8, 1908—Continued.

Specimen No.	Name of person.	Street address.	Age.	Color.	Sex.	Previous typhoid.	Present health.	Date of examination.	Result.	Examiner.	Remarks.
			Years.								
202	A. L.....	818 22d nw...	14	C	M	1	OK	8/15	—	M	Probably typhoid, fall, 1907.
203	R. W.....	2125 I nw....	3	C	M	0	OK	8/15	—	M	
204	P. S.....	2130 H nw	13	W	F	0	OK	8/15	—	M	
205	M. R.....	2145 I nw....	9	W	F	0	OK	8/15	—	M	
206	E. W.....	2125 I nw....	2	C	M	0	OK	8/15	—	M	
207	I. H.....	2205 I nw....	15	W	M	0	OK	8/15	—	M	
208	H. D.....	2146 I nw....	12	W	M	0	OK	8/15	—	M	
209	R. T	823 23d nw...	14	C	F	0	OK	8/15	—	M	
210	F. R.....	713 23d nw...	?30	C	F	20		8/15	—	M	Recurrent "indigestion," recent attack.
211	M. B.....	2211 I nw....	4	W	F	0	OK	8/15	—	M	
212	E. R.....	823 23d nw...	12	C	F	0	OK	8/15	—	M	
213	E. H.....	2205 I nw....	10	W	F	0	OK	8/15	—	M	
214	C. W.....	2211 I nw....	17 m.	W	F	0	OK	8/15	—	M	
215	T. S.....	2116 G nw...	5	W	M	0	OK	8/15	—	M	
216	H. W.....	2211 I nw....	12	W	F	0	OK	8/15	—	M	
217	F. B.....	733 23d nw...	7	W	F	0	OK	8/17	—	M	
218	W. B.....	.....do.....	4	W	M	0	OK	8/17	—	M	
219	A. C.....	810 22d nw...	9	W	M	0	OK	8/17	—	M	
220	T. E. B.....	2122 H nw...	?30	W	M	3	OK	8/17	—	M	
221	L. W.....	2211 I nw....	8	W	M	0	OK	8/17	—	M	
222	D. S.....	707 22d nw, A3.	13	W	F	0	OK	8/17	—	M	
223	E. L.....	901 22d nw...	2	W	F	0	OK	8/17	—	M	
224	A. L.....	.....do.....	6	W	M	0	OK	8/17	—	M	
225	I. F.....	2125 I nw....	13	C	F	0	OK	8/17	—	M	
226	M. R., jr	2145 I nw....	7	W	M	0	OK	8/17	—	M	
227	G. R.....	.....do.....	10	W	M	0	OK	8/17	—	M	
228	E. L.....	901 22d nw...	4	W	M	0	OK	8/17	—	M	
229	A. P.....	928 22d nw...	6	W	M	0	OK	8/18	—	M	
230	J. P.....	.....do.....	8	W	F	0	OK	8/18	—	M	
231	R. E.....	706 22d nw...	14	W	M	0	OK	8/18	—	M	
232	A. E.....	.....do.....	12	W	F	0	OK	8/18	—	M	
233	F. L.....	908 22d nw...	9	W	F	0	OK	8/18	—	M	
234	L. L.....	.....do.....	12	W	F	0	OK	8/18	—	M	
235	M. K	2145 I nw....	14	W	F	0	OK	8/18	—	M	
236	C. S.....	2216 G nw...	3	W	M	0	OK	8/18	—	M	
237	J. S.....	.....do.....	6	W	M	0	OK	8/18	—	M	
238	G. S.....	.....do.....	2	W	M	0	OK	8/18	—	M	
239	G. M.....	.....	.....	...	...	...		8/18	+	M	Now has typhoid. (See Nos. 31 and 1003.)
240	V. C.....	2122 Daly's court.	3	C	F	0	OK	8/18	—	M	
241	C. L.....	908 22d nw...	14	W	M	0	OK	8/18	—	M	
242	L. K.....	2145 I nw....	11	W	M	0	OK	8/18	—	M	
243	D. H.....	2101 F nw...	9	W	F	0	OK	8/18	—	M	
244	L. H.....	.....do.....	7	W	F	0	OK	8/18	—	M	
245	H. H.....	.....do.....	6	W	F	0	OK	8/18	—	M	

Specimens of feces and urine examined for typhoid bacilli July 16 to November 8, 1908—Continued.

Specimen No.	Name of person.	Street address.	Age.	Color.	Sex.	Previous typhoid.	Present health.	Date of examination.	Result.	Examiner.	Remarks.
			Years.								
246	W. S. L.....	2106 Pa. ave., nw.	17 m.	W	M	0	OK	8/19	–	M	
247	H. L.....	2104 Pa. ave., nw.	8	W	M	0	OK	8/19	–	M	
248	N. T.....	2140 Pa. ave., nw.	7	W	M	0	OK	8/20	–	M	
249	R. T.....	.....do.....	10	W	F	2	OK	8/20	–	M	
250	G. T.....	.....do.....	13	W	F	0	OK	8/20	–	M	
251	J. G.....	2144 Pa. ave., nw.	3	W	M	0	OK	8/20	–	M	
252	L. G.....	.....do.....	10 m.	W	M	0	OK	8/20	–	M	
253	E. A.....	2118 Pa. ave., nw.	5	W	F	0	OK	8/20	–	M	
254	J. A.....	.....do.....	10	W	M	0	OK	8/20	–	M	
255	L. A.....	.....do.....	15	W	F	0	OK	8/20	–	M	
256	M. A.....	.....do.....	14	W	F	0	OK	8/20	–	M	
257	C. A. M.....	.....do.....	?35	W	F	0		8/20	–	M	Gynecologic.
258	G. S.....	2130 Pa. ave., nw.	7 m.	W	F	0	OK	8/20	–	M	
259	E. S.....	.....do.....	2	W	F	0	OK	8/20	–	M	
260	R. W.....	2122 Pa. ave., nw.	15	C	M	0	OK	8/20	–	M	
261	E. C.....	2100 Pa. ave. nw.	4	W	M	0	OK	8/20	–	M	
262	A. T.....	2124 Pa. ave. nw.	14	W	M	0	OK	8/20	–	M	
263	F. B.....	2221 St. Paul's ct.	9 m.	C	M	0	OK	8/20	–	R	
264	L. C.....	916 St. Paul's ct.	9	C	F	0		8/22	–	R	Worms.
265	M. C.....	.....do.....	11	C	F	0	OK	8/22	–	R	
266	M. G.....	2225 St. Paul's ct.	9	C	F	5	OK	8/22	–	R	
267	M. L.....	2104 Pa. ave. nw.	10	W	M	0	OK	8/22	–	R	
268	J. P. S.....	2108 Pa. ave. nw.	?35	W	F	0		8/22	–	R	Now has typhoid.
269	C. L.....	902 22d nw...	7	W	M	0	OK	8/22	–	R	
270	P. L.....	.....do.....	4	W	M	0	OK	8/22	–	R	
271	L. G.....	920 St. Paul's ct.	4 m.	C	M	0	OK	8/22	–	R, C	
272	F. C.....	918 St. Paul's ct.	12	C	F	0	OK	8/22	–	R, C	
273	L. D.....	2223 St. Paul's ct.	3	C	F	0	OK	8/22	–	R, C	
274	A. C.....	701 24th nw..	11	W	M	0	OK	8/22	–	R, C	See Nos. 381 and 611.
275	W. P. C.....	.....do.....	9	W	M	....	OK	8/22	+	R, C	Typhoid onset 7 weeks ago. (See Nos. 303 and 463.)

Specimens of feces and urine examined for typhoid bacilli July 16 to November 8, 1908—Continued.

Specimen No.	Name of person.	Street address.	Age.	Color.	Sex.	Previous typhoid.	Present health.	Date of examination.	Result.	Examiner.	Remarks.
			Years.								
276	J. C.	701 24th nw.	6	W	F	0	OK	8/22	–	R, C	See Nos. 396 and 616.
277	S. M.	2327 G nw	13	C	F	0	OK	8/22	–	R, C	
278	L. S.	2301 G nw.	3	C	M	0	OK	8/22	–	R, C	
279	F. F.	2319 G nw.	5	C	M	0	OK	8/22	–	R, C	
280	E. J.	2301 G nw.	15	C	F	0		8/24	+	R, C	Probably has typhoid. (See Nos. 372 and 945.)
280a	E. W.	2204 Pa. ave. nw.	5	C	F	0	OK	8/24	–	R, C	
281	L. V.	907 23d nw.	16	W	M	2	OK	8/24	–	R, C	
282	T. R.	2331 G nw.	10	C	M	0	OK	8/24	–	R, C	
283	H. S.	2325 G nw.	1	C	M	0	OK	8/24	–	R, C	
284	M. N.	706 23d nw.	9	C	F	0	OK	8/24	–	R, C	
285	J. S.	2325 G nw.	7	C	M	0	OK	8/24	–	R, C	
286	G. V. P.	718 23d nw.	13	C	M	8	OK	8/24	–	R, C	
287	F. W.	2204 Pa. ave. nw.	2	C	F	0	OK	8/24	–	R, C	
288	M. P.	718 23d nw.	10	C	F	0	OK	8/25	–	M	
289	C. B.	716 23d nw.	10	C	M	0	OK	8/25	–	M	
290	A. N.	2312 H nw.	8	C	F	0	OK	8/25	–	M	
291	A. B.	716 23d nw.	12	C	F	0	OK	8/25	–	M	
292	M. P.	718 23d nw.	14	C	F	7	OK	8/25	–	M	
293	R. B.	716 23d nw.	14	C	M	0	OK	8/25	–	M	
294	J. G.	2127 H nw.	1	W	M	0	OK	8/25	–	M	Measles July, 1908.
295	R. C.	707 24th nw.						8/25	–	M	
296	F. D.	719 24th nw.	2	W	M	0	OK	8/25	–	M	
297	L. D.	do	5	W	M	0	OK	8/25	–	M	
298	B. T.	739 24th nw.	8	C	F	0	OK	8/25	–	M	
299	C. S.	2301 G nw.	2	C	M	0	OK	8/25	–	M	
300	J. H.	2303 G nw.	8	C	M	0	OK	8/25	–	M	
301	R. B.	2312 H nw.	12	C	M	0	OK	8/25	–	M	
302	J. T.	739 24th nw.	5	C	M	0	OK	8/25	–	M	
303	W. P. C.	701 24th nw.	16	W	M		OK	8/25	+	M	Typhoid in July. (See Nos. 275 and 463.)
304	R. B.	718 St. Mary's ct.	10 m.	C	M	0	OK	8/25	–	M	Subsequent typhoid. (See No. 1040.)
305	T. B.	do	4	C	M	0	OK	8/25	–	M	Subsequent typhoid. (See No. 1038.)
306	B.	do	? 25	C	F	10	OK	8/25	–	M	Typhoid in family. (See No. 1036.)
307	M. C.	707 24th nw.						8/25	–	M	
308	M. B.	733 24th nw.	14	W	F	0	OK	8/26	–	M,H,C	
309	C. B.	718 St. Mary's ct.	5	C	M	0	OK	8/26	–	M,H,C	Subsequent typhoid. (See No. 1037.)
310	W. K.	703 24th nw.	8	W	M	0	OK	8/26	–	M,H,C	
311	K. K.	do	13	W	F	0	OK	8/26	–	M,H,C	
312	W. D.	733 24th nw.	2	W	M	0	OK	8/26	–	M,H,C	
313	M. M.	722 St. Mary's ct.	10	C	F	0		8/26	–	M,H,C	Malaria.
314	M. T.	707 24th nw.	4	W	F	0	OK	8/26	–	M,H,C	

Specimens of feces and urine examined for typhoid bacilli July 16 to November 8, 1908—Continued.

Specimen No.	Name of person.	Street address.	Age.	Color.	Sex.	Previous typhoid.	Present health.	Date of examination.	Result.	Examiner.	Remarks.
			Years.								
315	E. W.....	721 24th nw..	12	W	F	0	OK	8/26	—	M,H,C	
316	M. W.....	.....do.....	14	W	M	0	OK	8/26	—	M,H,C	
317	E. S.....	938 Va. ave. sw.	6	W	M	0	OK	8/26	—	M,H,C	
318	W. L. F.....	920 Va. ave. sw.	?30	W	M	12	OK	8/26	—	M,H,C	
319	R. P.....	916 Va. ave. sw.	1	W	M	0	OK	8/26	—	M,H,C	
320	R. M.....	944 Va. ave. sw.	7 m.	W	M	0	OK	8/26	—	M,H,C	
321	A. S.....	927 C sw.....	?	W	F	0		8/26	—	M,H,C	Torpid liver and kidney trouble.
322	E. M.....	944 Va. ave. sw.	3	W	M	0	OK	8/26	—	M,H,C	
323	J. O'B.....	941 C sw.....	13	W	M	0	OK	8/26	—	M,H,C	Subsequent typhoid.
324	J. K.....	703 24th nw..	6	W	M	0	OK	8/26	—	M,H,C	
325	E. L.....	418 5th se....	4	C	F	0	OK	8/27	—	M,H,C	
326	M. S.....	407 5th se....	?	C	F	0	OK	8/27	—	M,H,C	
327	W. W. H ...	.....do.....	3½	C	M	0	OK	8/27	—	M,H,C	
328	H. H.....	.....do.....	6	C	M	0	OK	8/27	—	M,H,C	
329	M. M.....	413 5th se	7	C	F	0	OK	8/27	—	M,H C	
330	M. M.....	.....do.....	4	C	F	0	OK	8/27	—	M,H,C	
331	W. B. M....	.....do.....	2	C	M	0	OK	8/27	—	M,H,C	
332	A. A.....	417 5th se....	7	C	F	0	OK	8/27	—	M,H,C	
333	N. A.....	.....do.....	10	C	F	0	OK	8/27	—	M,H,C	
334	A. A.....	.....do.....	2½	C	F	0	OK	8/27	—	M,H,C	
335	M. D.....	924 Va. ave. sw.	3	W	F	0	OK	8/27	—	M,H,C	
336	M. F.....	920 Va. ave. sw.	2½	W	M	0	OK	8/27	—	M,H,C	
337	J. L. B.....	928 Va. ave. sw.	8	W	M	0	OK	8/27	—	M,H,C	
338	M. T.....	219 9th sw...	10	W	M	0	OK	8/27	—	M,H,C	
339	H. B.....	930 Va. ave. sw.	10	W	F	3	OK	8/27	—	M,H,C	
340	J. M. H.....	932 Va. ave. sw.	?35	W	M	0	OK	8/27	—	M,H,C	Malaria last spring.
341	A. T.....	219 9th sw...	14	W	F	0	OK	8/27	—	M,H,C	
342	A. Y.....	940½ Va. ave. sw.	7 m.	W	M	0	OK	8/27	—	M,H,C	
343	A. W.....	708 23d nw ..	?35	C	M	*	OK	8/27	—	M,H,C	Typhoid pneumonia last spring.
344	J. R. McD..	934 Va. ave. sw.	2	W	M	0	OK	8/27	—	M,H,C	
345	H. M.....	944 Va. ave. sw.	?35	W	F	0	OK	8/27	—	M,H,C	Chronic dyspepsia.
346	F. S.....	938 Va. ave. sw.	3	W	M	0	OK	8/27	—	M,H,C	
347	J. M.....	927 C sw.....	?	W	F	0	OK	8/27	—	M,F,C	

Specimens of feces and urine examined for typhoid bacilli July 16 to November 8, 1908—Continued.

Specimen No.	Name of person.	Street address.	Age.	Color.	Sex.	Previous typhoid.	Present health.	Date of examination.	Result.	Examiner.	Remarks.
			Years.								
348	G. S.....	938 Va. ave. sw.	5	W	M	0	OK	8/27	–	M,H,C	
349	A. S.....	2322 H nw...	6	C	M	0	OK	8/27	–	M,H,C	
350	D. W.....	422 5th se....	20 m.	C	F	0	OK	8/28	–	M, C	
351	L. W.....	.....do.....	?30	C	F	7	OK	8/28	–	M, C	
352	W. C.....	402 4th se....	4	W	M	0	OK	8/28	–	M, C	
353	F. D.....	414 5th se....	11	W	F	0	OK	8/28	–	M, C	
354	E. H.....	407 5th se....	1	C	F	0	OK	8/28	–	M, C	Summer complaint 2 weeks ago.
355	E. W.....	420 E se.....	7	C	M	0	OK	8/28	–	M, C	
356	M. W.....	.....do.....	4	C	F	0	OK	8/28	–	M, C	
357	C. B.....	422 5th se....	7	C	M	0	OK	8/28	–	M, C	
358	I. A.....	417 5th se....	4	C	F	0	OK	8/28	–	M, C	
359	G. J.....	425 5th se....	4	C	F	0	OK	8/28	–	M, C	Summer complaint recently.
360	L. B.....	422 5th se....	9	C	F	0	OK	8/28	–	M, C	
361	E. N.....	410 E se.....	14	C	F	0	OK	8/28	–	M, C	
362	J. A. O'C...	424 4th se....	3	W	M	0	OK	8/28	–	M, C	
363	R. E. C.....	402 4th se....	3	W	M	0	OK	8/28	–	M, C	
364	L. C.....	2316 I nw....	4	W	M	0	OK	8/28	–	M, C	
365	A. R.....	2324 I nw....	10	C	M	0	OK	8/28	–	M, C	
366	A. M.....	.....do.....	4	C	F	0	OK	8/28	–	M, C	
367	R. J.....	2318 I nw....	6	W	M	0	OK	8/28	–	M, C	
368	M. G.....	823 24th nw .	11	C	F	0	OK	8/28	–	M, C	
369	S. C.....	2316 I nw....	6	W	F	0	OK	8/28	–	M, C	
370	C. D.....	714 St.Mary's ct.	9	C	M	....	OK	8/28	–	M, C	
371	T. C.....	2316 I nw....	2	W	M	0	OK	8/28	–	M, C	
372	E. J.....	2315 G nw....	.....	....	....	....	.....	8/28	–	M, C	See Nos. 280 and 945.
373	C. P.....	2319 H nw....	12	C	M	0	OK	8/28	–	M, C	
374	W. McG....	2315 H nw....	2	W	M	0	OK	8/28	–	M, C	
375	J. McG.....	.....do.....	5	W	M	0	OK	8/28	–	M, C	
376	M. C.....	2317 H nw....	10	C	F	0	OK	8/28	–	M, C	
377	S. P.....	2321 H nw....	21 m.	C	M	0	OK	8/28	–	M, C	
378	M. P.....	.....do.....	?20	C	F	9	OK	8/29	–	M, C	
379	L. W.....	712 St.Mary's ct.	?20	C	F	7	OK	8/29	–	M, C	
380	J. C.....	2314 I nw....	20 m.	W	M	0	OK	8/29	–	M, C	Malaria last spring.
381	A. C.....	701 24th nw..	.....	....	....	....	OK	8/29	–	M, C	See Nos. 274 and 611.
382	W. W.....	712 St.Mary's ct.	?	C	M	7	OK	8/29	–	M, C	
383	J. S.....	816 23d nw...	1 m.	W	M	0	OK	8/29	–	M, C	
384	H. J.....	2318 I nw....	12	W	M	0	OK	8/29	–	M, C	
385	E. C. B.....	928 Va. ave. sw.	16 m.	W	F	0	OK	8/29	–	M, C	
386	L. R.....	834 23d nw...	10	C	M	0	OK	8/29	–	M, C	
387	M. W.....	419 D se.....	9	C	M	0	OK	8/29	–	M, C	
388	A. W.....	.....do.....	3	C	M	0	OK	8/29	–	M, C	
389	H. D.....	714 St.Mary's ct.	7	C	M	....	OK	8/29	–	M, C	

Specimens of feces and urine examined for typhoid bacilli July 16 to November 8, 1908—Continued.

Specimen No.	Name of person.	Street address.	Age.	Color.	Sex.	Previous typhoid.	Present health.	Date of examination.	Result.	Examiner.	Remarks.
			Years.								
390	H. J.....	425 5th se ...	17	C	F	6	OK	8/29	–	M, C	
391	E. C.....	2314 I nw....	3	W	F	0	OK	8/29	–	M, C	Recent malaria.
392	J. W.....	419 D se.....	7	C	F	0	OK	8/29	–	M, C	
393	M. T.....	408 4th se....	5	W	M	0	OK	8/29	–	M, C	
394	M. H.....	412 E se.....	10	C	M	0	OK	8/29	–	M, C	
395	N. W.....	419 D se.....	5	C	M	0	OK	8/29	–	M, C	
396	J. C.....	701 24th nw..	.....	.....	.....	.....	OK	8/29	–	M, C	See Nos. 276 and 616.
397	F. J.....	2318 I nw....	10	W	M	0	OK	8/29	–	M, C	
398	R. L.....	412 E se.....	11	C	M	0	OK	8/29	–	M, C	
399	H. J.....	2318 I nw....	15 m.	W	F	0	OK	8/29	–	M, C	
400	L. G.....	420 E se.....	6 m.	C	F	0	OK	8/29	–	M, C	
401	C. J.....	416 4th se....	5	W	F	0	OK	8/29	–	M, C	
402	V. J.....	.....do.....	7	W	M	0	OK	8/29	–	M, C	
403	C. F.....	303 Mass. ave. ne.	9½	W	M	0	OK	8/31	–	M, C	
404	W.....	419 D se.....	6 m.	C	F	0	OK	8/31	–	M, C	Recent summer complaint.
405	M. B.....	718 St. Marys ct.	3	C	F	0	.....	8/31	+	M, C	Now has typhoid. (See No. 1039.)
406	L. H.....	903 24th nw..	?35	C	F	3	OK	8/31	–	M, C	
407	M. N.....	926 Va. ave. sw.	5	W	F	0	OK	8/31	–	M, C	
408	M. J.....	2318 I nw....	7	W	M	0	OK	8/31	–	M, C	
409	H. McC.....	2313 I nw....	6	W	F	0	OK	8/31	–	M, C	
410	A. W.....	832 23d nw...	11	C	M	0	OK	8/31	–	M, C	
411	R. H.....	2305 I nw....	4	W	M	0	OK	8/31	–	M, C	
412	T. R. C.....	701 24th nw..	16	W	M	0	OK	8/31	–	M, C	See No. 737; recent typhoid in family.
413	F. M.....	2321 I nw....	3	W	M	0	OK	8/31	–	M, C	
414	E. McC.....	2313 I nw....	11	W	M	0	OK	8/31	–	M, C	
415	P. McC.....	.....do.....	9	W	M	0	OK	8/31	–	M, C	
416	L. J.....	806 23d nw...	14	C	F	0	OK	8/31	–	M, C	
417	R. N.....	926 Va. ave. sw.	3	W	F	0	OK	8/31	–	M, C	
418	L. McC.....	2313 I nw....	4	W	F	0	OK	8/31	–	M, C	
419	M. K.....	812 23d nw...	?	W	F	0	OK	8/31	–	M, C	
420	A. N.....	410 E se.....	1	C	F	0	OK	8/31	–	M, C	
421	L. C.....	325 Mass. ave ne.	?30	W	M	3	OK	8/31	–	M, C	
422	W. W.....	832 23d nw...	12	C	M	0	OK	8/31	–	M, C	
423	L. F.....	325 Mass. ave. ne.	2	W	M	0	OK	8/31	–	M, C	
424	D. K.....	812 23d nw...	?	W	F	0	OK	8/31	–	M, C	
425	G. E. W....	2315 I nw....	2 m.	W	M	0	OK	8/31	–	M, C	
426	E. R.....	327 Mass. ave. ne.	6	W	F	0	OK	9/1	–	M, C	
427	M. E.....	301 Mass. ave. ne.	12	W	F	0	OK	9/1	–	M, C	
428	F. E.....	.....do.....	6	W	F	0	OK	9/1	–	M, C	

Specimens of feces and urine examined for typhoid bacilli July 16 to November 8, 1908—Continued.

Specimen No.	Name of person.	Street address.	Age.	Color.	Sex.	Previous typhoid.	Present health.	Date of examination.	Result.	Examiner.	Remarks.
			Years.								
429	F. M.....	923 N. H. ave. nw.	6	C	F	0	OK	9/1	—	M, C	
430	E. F.....	810 23d nw...	9	C	M		OK	9/1	—	M, C	
431	A. F.....	822 23d nw...	3	W	F	0	OK	9/1	—	M, C	Measles recently.
432	A. M.....	305 Mass. ave. ne.	12	W	M	0	OK	9/1	—	M, C	
433	C. P.....	931 N. H. ave. nw.	22 m.	W	F	0	OK	9/1	—	M, C	
434	O. S., jr.....	930 23d nw...	21	W	M	*	OK	9/1	—	M, C	Typhoid, May, 1908.
435	L. H.....	903 24th nw..	13	C	F	0	OK	9/1	—	M, C	
436	W. O'C.....	909 N. H. ave. nw.	7	W	M	0	OK	9/1	—	M, C	
437	A. O'C.....	.....do.....	55	W	M	0	OK	9/1	—	M, C	
438	C. H.....	903 24th nw..	10	C	M	0	OK	9/1	—	M, C	
439	H O'C.....	909 N. H. ave. nw.	9	W	F	0	OK	9/1	—	M, C	
440	O. T. O'C...	.....do.....	2	W	M	0	OK	9/1	—	M, C	
441	S. K.....	812 23d nw...	?	W	M	0	OK	9/1	—	M, C	
442	P. K.....	.....do.....	?	W	F	0	OK	9/1	—	M, C	
443	H. E.....	301 Mass. ave. ne.	10	W	F	0	OK	9/1	—	M, C	
444	H. F.....	927 N. H. ave. nw.	5	W	F	0	OK	9/1	—	M, C	
445	E. W.....	307 Mass. ave. ne.	2	W	M	0	OK	9/1	—	M, C	Recent summer complaint.
446	C. W.....	.....do.....	8	W	F	0	OK	9/1	—	M, C	Do.
447	J. W., jr....	.....do.....	6	W	M	0	OK	9/1	—	M, C	
448	E. E.....	620 23d nw...	16 m.	W	F	0	OK	9/2	—	M, C	
449	P. S.....	332 C ne.....	11	W	F	0	OK	9/2	—	M, C	
450	G. W.....	818 23d nw...	8	W	F	0		9/2	—	M, C	Cervical adenitis.
451	L. E. B.....	302 C ne.....	? 25	W	M	....	OK	9/2	—	M, C	Typhoid, Jan., 1908.
452	J. F.....	927 N. H. ave. nw.	3	W	M	0	OK	9/2	—	M, C	
453	F. V. L.....	626 23d nw...	? 25	W	M	*	OK	9/2	—	M, C	Typhoid, July, 1908.
454	F. G.....	2314 G nw...	12	C	F	0	OK	9/2	—	M, C	
455	P. H.....	2306 G nw...	5	C	M	0	OK	9/2	—	M, C	
456	S. W.....	608 23d nw...	4	C	M	0	OK	9/2	—	M, C	
457	A. McE.....	923 N.H. ave. nw.	4	C	M	0	OK	9/2	—	M, C	
458	E. H.....	2305 I nw....	2	W	F	0	OK	9/2	—	M, C	
459	M. G.....	600 23d nw...	9	W	F	0	OK	9/2	—	M, C	
460	J. H. C.....	2316 G nw...	3	W	M	0	OK	9/2	—	M, C	
461	P. K.....	2308 G nw...	10	W	M	0	OK	9/2	—	M, C	
462	M. J. O'C...	909 N.H. ave. nw.	12	W	M	0	OK	9/2	—	M, C	
463	W. P. C.....	701 24th nw..	.....	.....	.....	.....	OK	9/3	—	M, C	See Nos. 275 and 303.
464	A. D.....	300 C ne.....	14	W	M	0	OK	9/3	—	M, C	
465	C. W.....	306 C ne.....	11	W	M	0	OK	9/3	—	M, C	
466	E. W.....	.....do.....	9	W	F	0	OK	9/3	—	M, C	

Specimens of feces and urine examined for typhoid bacilli July 16 to November 8, 1908—Continued.

Specimen No.	Name of person.	Street address.	Age.	Color.	Sex.	Previous typhoid.	Present health.	Date of examination.	Result.	Examiner.	Remarks.
			Years.								
467	M. P.....	308 C ne.....	9	W	F	0	OK	9/3	–	M, C	
468	A. P.....	316 C ne.....	12	W	M	0	OK	9/3	–	M, C	
469	L. P.....	.....do.....	10	W	F	0	OK	9/3	–	M, C	
470	L. P.....	.....do.....	7	W	F	0	OK	9/3	–	M, C	
471	G. B.....	330 C ne.....	6	W	M	0	OK	9/3	–	M, C	
472	E. B.....	.....do.....	3	W	M	0	OK	9/3	–	M, C	
473	T. E. W....	323 Mass ave. ne.	13 m.	W	M	0	OK	9/3	–	M, C	
474	E. F. J.....	312 C ne.....	12	W	M	0	OK	9/3	–	M, C	
475	N. C.....	608 23d nw...	4	C	M	0	OK	9/3	–	M, C	
476	E. P.....	903 N.H. ave. nw.	13	W	M	0	OK	9/3	–	M, C	
477	C. P.....	2322 G nw...	9	C	M	0	OK	9/3	–	M, C	
478	E. J.....	616 23d nw...	13	C	F	0	OK	9/3	–	M, C	
479	H. P.....	903 N.H. ave. nw.	2	W	F	0	OK	9/3	–	M, C	
480	M. H.....	2306 G nw...	7	C	F	0	OK	9/3	–	M, C	
481	E. G.....	2314 G nw...	6	C	F	0	OK	9/3	–	M, C	
482	L. H.....	602 23d nw...	9	W	F	0	OK	9/3	–	M, C	Scarlet fever last winter.
483	J. F.....	2320 G nw...	10	W	M	0	OK	9/3	–	M, C	
484	W. P.....	2322 G nw...	7	C	M	0	OK	9/3	–	M, C	
485	M. C.....	2316 G nw...	7	W	F	0	OK	9/3	–	M, C	
486	J. J.....	616 23d nw...	5	C	F	0	OK	9/3	–	M, C	Measles last winter.
487	L. P.....	903 N.H. ave. nw.	6	W	F	0	OK	9/3	–	M, C	
488	R. M.....	923 N.H. ave. nw.	9	C	F	0	OK	9/3	–	M, C	
489	A. B.....	302 C ne.....	?20	W	F	....	OK	9/3	–	M, C	Typhoid, March, 1908.
490	B. L. P.....	308 C ne.....	15	W	F	0	OK	9/3	–	M, C	
491	C. C. P.....	316 C ne.....	5	W	M	3	OK	9/3	–	M, C	
492	E. M.....	320 C ne.....	2	W	F	0	OK	9/3	–	M, C	
493	E. M.....	.....do.....	4	W	M	0	OK	9/3	–	M, C	
494	V. M.....	.....do.....	1	W	F	0	OK	9/3	–	M, C	
495	R. T.....	322 C ne.....	6	W	F	0	OK	9/3	–	M, C	
496	I. T.....	.....do.....	3	W	F	0	OK	9/3	–	M, C	
497	C. E.....	301 Mass. ave. ne.	14	W	M	0	OK	9/3	+	M, C	See Nos. 687 and 1021.
498	L. B.....	524 2d ne....	2½	W	M	0	OK	9/3	–	M, C	Recent summer complaint.
499	N. F.....	2320 G nw...	13	W	F	0	OK	9/3	–	M, C	
500	B. C.....	2309 Va. ave. nw.	5	W	F	0	OK	9/3	–	M, C	
501	E. G.....	600 23d nw...	9	W	F	0	OK	9/3	–	M, C	
502	J. H. S.....	2311 Va. ave. nw.	3	W	M	0	OK	9/3	–	M, C	
503	K. C.....	2309 Va. ave. nw.	14	W	F	0	OK	9/3	–	M, C	
504	B. C.....	.....do.....	11	W	M	0	OK	9/3	–	M, C	

Specimens of feces and urine examined for typhoid bacilli July 16 to November 8, 1908—Continued.

Specimen No.	Name of person.	Street address.	Age.	Color.	Sex.	Previous typhoid.	Present health.	Date of examination.	Result.	Examiner.	Remarks.
			Years.								
505	S. S.....	2315 Va. ave. nw.	8	C	M	0	OK	9/4	—	M, C	
506	M. C.....	2309 Va. ave. nw.	9	W	F	0	OK	9/4	—	M, C	
507	S. S.....	2315 Va. ave. nw.	13	C	F	0	OK	9/4	—	M, C	
508	C. H.....	2329 Va. ave. nw.	8	W	M	0	OK	9/4	—	M, C	
509	A. B.....	2319 Va. ave. nw.	4	W	F	0	OK	9/4	—	M, C	
510	I. H.....	2329 Va. ave. nw.	2	W	F	0	OK	9/4	—	M, C	
511	G. S.....	2315 Va. ave. nw.	3	C	F	0	OK	9/4	—	M, C	
512	E. B.....	2319 Va. ave. nw.	7	W	F	0	OK	9/4	—	M, C	
513	R. H.....	2329 Va. ave. nw.	9	W	M	0	OK	9/4	—	M, C	
514	R. C.....	2316 G nw....	10	W	F	0	OK	9/4	—	M, C	
515	R. S.....	2315 Va. ave. nw.	14	C	M	0	OK	9/4	—	M, C	
516	C. S.....	2311 Va. ave. nw.	18 m.	W	M	0	OK	9/4	—	M, C	
517	J. B.....	2319 Va. ave. nw.	9	W	F	0	OK	9/4	—	M, C	
518	E. C.....	2323 Va. ave. nw.	?	C	F	0	OK	9/4	—	M, C	
519	M. S.....	2315 Va. ave. nw.	10	C	F	0	OK	9/4	—	M, C	
520	G. S.....	2311 Va. ave. nw.	?	W	F	4	OK	9/5	—	M, C	
521	J. S.....	2311 Va. ave. nw.	? 25	W	M	0	OK	9/5	—	M, C	
522	M. H.....	2329 Va. ave. nw.	12	W	F	0	OK	9/5	—	M, C	
523	G. B.....	2319 Va. ave. nw.	15	W	M	0	OK	9/5	—	M, C	
524	A. B.....	.....do.....	? 50	W	F	0		9/5	—	M, C	Dyspepsia.
525	P. D.....	2325 E nw...	13	C	M	0	OK	9/5	—	M, C	
526	M. Y.....	2327 E nw...	10	W	F	0	OK	9/5	—	M, C	
527	M. Y.....	.....do.....	8	W	F	0	OK	9/5	—	M, C	
528	E. L.....	2313 E nw...	6	C	M	0	OK	9/5	—	M, C	
529	L. H.....	2329 Va. ave. nw.	14	W	F	0	OK	9/5	—	M, C	
530	F. H.....	.....do.....	5	W	F	0	OK	9/5	—	M, C	
531	M. S.....	2315 Va. ave. nw.	15	C	F	0	OK	9/5	—	M, C	
532	N. A.....	2325 E nw...	15 m.	C	F	0	OK	9/5	—	M, C	
533	R. L.....	2313 E nw...	11	C	M	0	OK	9/5	—	M, C	

Specimens of feces and urine examined for typhoid bacilli July 16 to November 8, 1908—Continued.

Specimen No.	Name of person.	Street address.	Age.	Color.	Sex.	Previous typhoid.	Present health.	Date of examination.	Result.	Examiner.	Remarks.
			Years.								
534	J. W. (C.?)..	500 23d nw...	14	W	F	0	OK	9/5	—	M, C	
535	P. A.....	502 23d nw...	3	W	M	0	OK	9/5	—	M, C	
536	J. C.....	514 23d nw...	6	W	M	0	OK	9/5	—	M, C	
537	L. D.....	522 23d nw...	?	W	F	0	?	9/5	—	M, C	Grip last winter.
538	L. E.....	620 23d nw...	6	W	M	0	OK	9/5	—	M, C	
539	H. S.....	506 23d nw...	7	W	M	0	OK	9/5	—	M, C	
540	R. S.....	.....do.....	12	W	F	0	OK	9/5	—	M, C	
541	E. A.....	502 23d nw...	5	W	F	0	OK	9/5	—	M, C	
542	M. C.....	514 23d nw...	8	W	F	0	OK	9/5	—	M, C	
543	J. D.....	516 23d nw...	8	W	M	0	OK	9/5	—	M, C	
544	P. S.....	610 23d nw...	4	C	F	0	OK	9/5	—	M, C	
545	E. C.....	514 23d nw...	13	W	F	0	OK	9/5	—	M, C	
546	E. D.....	516 23d nw...	12	W	F	0	OK	9/5	—	M, C	
547	E. R.....	512 23d nw...	15	W	M	0	OK	9/5	—	M, C	
548	F. D.....	516 23d nw...	7	W	F	0	OK	9/5	—	M, C	
549	C. S.....	610 23d nw...	10	C	M	0	OK	9/5	—	M, C	
550	C. D.....	516 23d nw...	3	W	F	0	OK	9/5	—	M, C	
551	I. S.....	610 23d nw...	9	C	F	0	OK	9/5	—	M, C	
552	W. S.....	.....do.....	8	C	M	0	OK	9/5	—	M, C	
553	E. M.....	.....do.....	18 m.	C	M	0	OK	9/5	—	M, C	
554	M. C.....	514 23d nw...	11	W	F	0	OK	9/5	—	M, C	
555	C. C.....	.....do.....	3	W	F	0	OK	9/5	—	M, C	
556	E. D.....	516 23d nw...	10	W	F	0	OK	9/5	—	M, C	
557	B. D.....	.....do.....	5	W	M	0		9/5	—	M, C	Has chicken pox now.
558	C. S.....	506 23d nw...	5	W	F	0	OK	9/5	—	M, C	
559	M. A.....	502 23d nw...	8	W	F	0	OK	9/5	—	M, C	
560	H. D.....	520 23d nw...	9	W	F	0	OK	9/8	—	M, C	
561	E. M.....	610 23d nw...	11	C	F	0	OK	9/8	—	M, C	
562	P. S.....	.....do.....	6	C	M	0	OK	9/8	—	M, C	
563	M. McC.....	2330 G nw...	?	W	F	0	OK	9/8	—	M, C	
564	J. H., jr.....	2306 G nw...	12	C	M	0	OK	9/8	—	M, C	
565	M. S.....	610 23d nw...	12	C	F	0	OK	9/8	—	M, C	
566	S. Y.....	2327 E nw...	12	W	F	0	OK	9/8	—	M, C	
567	H. S.....	506 23d nw...	2	W	F	0	OK	9/8	—	M, C	
568	J. E. B.....	505 24th nw..	11	C	M	0	OK	9/8	—	M, C	
569	R. Y.....	2327 E nw...	14	W	F	0	OK	9/8	—	M, C	
570	M. D.....	520 23d nw...	12	W	F	0	OK	9/8	—	M, C	
571	J. F.....	522 22d nw...	11	W	F	0	OK	9/9	—	M, C	
572	J. M.....	524 22d nw...	8	W	M	0	OK	9/9	—	M, C	Recent slight heat affection.
573	J. F.....	2203 E nw...	7	C	F	0	OK	9/9	—	M, C	
574	L. D.....	508 23d nw...	2	W	F	0	OK	9/9	—	M, C	
575	L. D.....	.....do.....	? 30	W	F	7	OK	9/9	—	M, C	
576	R. B.....	2215 D nw...	3	C	M	0	OK	9/9	—	M, C	
577	W. B.....	.....do.....	7	C	M	0	OK	9/9	—	M, C	
578	L. Y.....	412 22d nw...	11	C	M	0	OK	9/9	—	M, C	
579	M. F.....	532 23d nw...	? 30	W	F	2	OK	9/9	—	M, C	
580	E. P. C.....	519 24th nw..	18 m.	C	F	0	OK	9/9	—	M, C	
581	G. B.....	2215 D nw...	15 m.	C	F	0	OK	9/9	—	M, C	

Specimens of feces and urine examined for typhoid bacilli July 16 to November 8, 1908—Continued.

Specimen No.	Name of person.	Street address.	Age.	Color.	Sex.	Previous typhoid.	Present health.	Date of examination.	Result.	Examiner.	Remarks.
			Years.								
582	D. T.	512 Rickett's ct.	11	C	M	1	OK	9/9	—	M, C	
583	G. H.	2224 E nw.	20 m.	C	M	0	OK	9/9	—	M, C	
584	P. W.	519 Rickett's ct.	6	C	F	0	OK	9/9	—	M, C	
585	M. B.	2215 D nw.	5	C	F	0	OK	9/9	—	M, C	
586	M. B.	2125 D nw.	10	C	F	0	OK	9/9	—	M, C	
587	J. B.	do.	6	C	F	0	OK	9/9	—	M, C	
588	E. H.	2224 E nw.	3	C	F	0	OK	9/9	—	M, C	
589	M. N.	512 Rickett's ct.	12	C	F	0	OK	9/9	—	M, C	
590	M. L.	525 Rickett's ct.	4	C	M	0	OK	9/9	—	M, C	
591	M. K.	510 23d nw.	14	W	F	0	OK	9/9	—	M, C	
592	C. E. C.	519 24th nw.	6	C	M	0	OK	9/9	—	M, C	
593	L. B.	529 24th nw.	4	C	F	0	OK	9/9	—	M, C	
594	W. A.	515 24th nw.	6	C	M	0	OK	9/9	—	M, C	
595	H. A.	do.	2	C	F	0	OK	9/9	—	M, C	
596	I. J.	2321 Va. ave. nw.	10	C	F	*		9/9	—	M, C	Typhoid convalescent.
597	H. A.	515 24th nw.	18 m.	C	M	0	OK	9/9	—	M, C	
598	A. C.	525 24th nw.	5	C	M	0	OK	9/9	—	M, C	
599	I. J.	2321 Va. ave. nw.	2	C	F	0	OK	9/9	—	M, C	
600	L. A.	515 24th nw.	4	C	F	0	OK	9/9	—	M, C	
601	P. S.	506 23d nw.	7	W	M	0	OK	9/9	—	M, C	
602	L. D.	410 22d nw.	12	C	F	0	OK	9/10	—	M, C	
603	J. S.	2217 D nw.	4	C	F	0	OK	9/10	—	M, C	
604	M. B.	521 Rickett's ct.	4	C	F	0	OK	9/10	—	M, C	
605	V. H.	do.	12	C	F	0	OK	9/10	—	M, C	
606	E. B.	do.	9	C	M	0	OK	9/10	—	M, C	
607	J. J.	517 Rickett's ct.	10	C	M	0	OK	9/10	—	M, C	
608	D. B.	516 Rickett's ct.	4	C	M	0	OK	9/10	—	M, C	
609	A. B.	do.	6	C	M	0	OK	9/10	—	M, C	
610	M. L.	525 Rickett's ct.	? 18	C	F	4	OK	9/10	—	M, C	
611	A. C.	701 24th nw.					OK	9/10	—	M, C	See Nos. 274 and 381.
612	E. L.	513 24th nw.	9	C	M	2	OK	9/10	—	M, C	
613	E. L.	525 Rickett's ct.	8	C	F	0	OK	9/10	—	M, C	
614	M. B.	516 Rickett's ct.	13	C	F	0	OK	9/10	—	M, C	
615	E. J.	517 Rickett's ct.	4	C	M	0	OK	9/10	—	M, C	
616	J. C.	701 24th nw.					OK	9/10	—	M, C	See Nos. 276 and 396.
617	J. L.	2222 E nw.	4	C	M	0	OK	9/10	—	M, C	

Specimens of feces and urine examined for typhoid bacilli July 16 to November 8, 1908—Continued.

Specimen No.	Name of person.	Street address.	Age.	Color.	Sex.	Previous typhoid.	Present health.	Date of examination.	Result.	Examiner.	Remarks.
			Years.								
618	J. J.....	517 Rickett's ct.	8	C	F	0	OK	9/10	—	M, C	
619	F. S.....	2217 D nw...	13	C	M	2	OK	9/10	—	M, C	
620	J. H.....	2224 E nw...	? 25	C	F	4	OK	9/10	—	M, C	
621	W. J.....	521 Rickett's ct.	3	C	M	0	OK	9/10	—	M, C	
622	L. J.....	517 Rickett's ct.	7	C	F	0	OK	9/10	—	M, C	
623	I. P.....	518 Rickett's ct.	6	C	F	0	OK	9/10	—	M, C	
624	C. P.....	.....do.....	4	C	M	0	OK	9/10	—	M, C	
625	E. F.....	522 22d nw...	4	W	F	0	OK	9/10	—	M, C	
626	H. W.....	511 23d nw...	8	C	F	0	OK	9/10	—	M, C	Urine.
627	M. L.....	2203 E nw...	19 m.	C	F	0	OK	9/11	—	M, C	Recent summer complaint.
628	M. F.....	.....do.....	14	C	F	0	OK	9/11	—	M, C	
629	D. S.....	2224 F nw, A40.	13	W	F	0	OK	9/11	—	M, C	
630	W. W.....	2224 F nw, A42.	2	W	M	0	OK	9/11	—	M, C	
631	M. B.....	2148 D nw...	12	C	F	0	OK	9/11	—	M, C	
632	H. L.....	2222 E nw...	17 m.	C	F	0	OK	9/11	—	M, C	Pneumonia February, 1908.
633	U. W.....	2224 F nw, A21.	23 m.	W	F	0	OK	9/11	—	M, C	
634	D. M.....	353 22d nw...	11	C	F	0	OK	9/11	—	M, C	
635	M. P.....	2148 D nw...	11	C	F	0	OK	9/11	—	M, C	
636	M. A.....	349 22d nw...	15	C	F	0	OK	9/11	—	M, C	
637	R. M.....	353 22d nw...	13	C	M	0	OK	9/11	—	M, C	
638	L. T.....	349 22d nw...	3	C	M	0	OK	9/11	—	M, C	
639	F. W.....	334 22d nw...	? 40	C	F	0		9/11	—	M, C	Stomach trouble.
640	E. P.....	2148 D nw...	9	C	F	0	OK	9/11	—	M, C	
641	D. T.....	349 22d nw...	3	C	F	0	OK	9/11	—	M, C	
642	R. L. T.....	.....do.....	? 30	C	M	2	OK	9/11	—	M, C	
643	R. L. T.....	.....do.....	? 25	C	F	0		9/11	—	M, C	Indefinite ill health.
644	A. W.....	2212 D nw...	12	C	M	1	OK	9/11	—	M, C	
645	J. J.....	2218 D nw...	9	C	F	0	OK	9/11	—	M, C	
646	L. L.....	2222 E nw...	6	C	F	0	OK	9/11	—	M, C	
647	A. S.....	2220 E nw...	7	C	F	0	OK	9/11	—	M, C	
648	B. C.....	505 23d nw...	1	W	M	0	OK	9/11	—	M, C	Recent summer complaint.
649	M. L.....	2216 D nw...	15	C	F	0	OK	9/11	—	M, C	
650	M. M.....	2152 D nw...	4	C	F	0	OK	9/11	—	M, C	
651	L. L.....	525 Rickett's ct.	13	C	M	6	OK	9/11	—	M, C	
652	P. L.....	2222 E nw..	9	C	F	0	OK	9/11	—	M, C	
653	M. F.....	521 Rickett's ct.	?	C	F	0		9/11	—	M, C	"Bowel trouble."
654	J. R.....	624 Pa. ave. se.	? 10	W	M	....	OK	9/11	—	M, C	Typhoid in August, 1908.

Specimens of feces and urine examined for typhoid bacilli July 16 to November 8, 1908—Continued.

Specimen No.	Name of person.	Street address.	Age.	Color.	Sex.	Previous typhoid.	Present health.	Date of examination.	Result.	Examiner.	Remarks.
			Years.								
655	C. F.	2204 Va. ave. nw.	6	C	M	0	OK	9/14	–	M, C	
656	E. A.	2202 Va. ave. nw.	4	C	F	0	OK	9/14	–	M, C	
657	D. F.	2204 Va. ave. nw.	3	C	F	0	OK	9/14	–	M, C	
658	I. F.	do	15 m.	C	F	0	OK	9/14	–	M, C	
659	E. B.	502 22d nw.	7	C	M	0	OK	9/14	–	M, C	
660	J. A.	2210 Va. ave. nw.	10	C	M	0	OK	9/14	–	M, C	
661	F. F.	2214 Va. ave. nw.	11	C	F	0	OK	9/14	–	M, C	
662	D. W.	2208 Va. ave. nw.	4	C	F	0		9/14	–	M, C	Summer complaint.
663	E. W.	do	10	C	F	0	OK	9/14	–	M, C	
664	S. A.	332 22d nw.	3	C	F	0	OK	9/14	–	M, C	
665	R. A.	do	7	C	M	0	OK	9/14	–	M, C	
666	J. A.	do	1	C	M	0	OK	9/14	–	M, C	
667	A. S.	312 21st nw.	?35	C	F	0	OK	9/14	–	M, C	
668	L. R.	624 Pa. ave. se.	?25	W	F	0	OK	9/14	–	M, C	Succession of typhoid in family; 3 cases in 7 years.
669	J. R.	do	?25	W	F	*	OK	9/14	–	M, C	Succession of cases in family; 3 cases in 7 years; typhoid in 1901.
670	M. S.	312 21st nw.	6	C	M	0	OK	9/14	–	M, C	
671	A. S.	do	11	C	M	0	OK	9/14	–	M, C	
672	A. S.	do	5	C	M	0	OK	9/14	–	M, C	
673	R. A.	2150 D nw.	2	C	M	0	OK	9/14	–	M, C	
674	C. A.	2115 D nw.	1½	C	M	0	OK	9/14	–	M, C	
675	C.	701 24th nw.	?40	W	F	0	OK	9/14	–	M, C	Case and + findings in family.
676	F. W.	334 22d nw.	?40	C	F	0		9/14	–	M, C	Stomach trouble.
677	J. H. P.	2107 C nw.	2	C	M	0	OK	9/14	–	M, C	
678	M. L.	326 22d nw.	9	C	F	0	OK	9/14	–	M, C	Measles in April.
679	E. H.	2150 D nw.	11	C	F	0	OK	9/14	–	M, C	
680	J. H.	2114 N. Y. ave. nw.	6	C	F	0	OK	9/15	–	M, C	
681	G. H.	do	10	C	M	0	OK	9/15	–	M, C	
682	R. H.	do	2	C	F	0	OK	9/15	–	M, C	
683	P. H.	do	14	C	F	0	OK	9/15	–	M, C	
684	I. H.	do	12	C	F	0	OK	9/15	–	M, C	
685	J. H.	do	4	C	M	0	OK	9/15	–	M, C	
686	A. S.	2124 N. Y. ave. nw.	4	C	F	0	OK	9/15	–	M, C	
687	C. E.	301 Mass. ave. ne.					OK	9/15	–	M, C	See Nos. 497 and 1021.

Specimens of feces and urine examined for typhoid bacilli July 16 to November 8, 1908—Continued.

Specimen No.	Name of person.	Street address.	Age.	Color.	Sex.	Previous typhoid.	Present health.	Date of examination.	Result.	Examiner.	Remarks.
			Years.								
688	A. J.	2114 N. Y. ave. nw.	9	C	M	0	OK	9/15	—	M, C	
689	S. M.	2139 D nw.	7	C	F	0	OK	9/15	—	M, C	
690	J. H.	2150 D nw.	10	C	M	0	OK	9/15	—	M, C	
691	F. G.	2102 N. Y. ave. nw.	7	C	M	0	OK	9/15	—	M, C	
692	M. J.	301 22d nw.	10	C	F	0	OK	9/15	—	M, C	
693	J. J.	do.	6	C	F	0	OK	9/15	—	M, C	
694	L. M.	2224 Va. ave. nw.	12	C	M	0	OK	9/15	—	M, C	
695	M. G.	2206 Va. ave. nw.	12	C	F	0	OK	9/15	—	M, C	
696	H. G.	504 22d nw.	3¼	C	F	0	OK	9/15	—	M, C	
697	C. B.	2118 Va. ave. nw.	11	C	M	0	OK	9/16	—	M, C	
698	J. W., jr.	328 22d nw.	3	C	M	0	OK	9/16	—	M, C	
699	C. W.	do.	11	C	F	0	OK	9/16	—	M, C	
700	C. W.	do.	5	C	F	0	OK	9/16	—	M, C	
701	J. C.	413 22d nw.	?35	C	F	6	OK	9/16	—	M, C	
702	I. C.	do.	10	C	F	0	OK	9/16	—	M, C	
703	M. C.	do.	12	C	F	0	OK	9/16	—	M, C	
704	G. C.	do.	4	C	F	0	OK	9/16	—	M, C	
705	L. C.	do.	8	C	M	0	OK	9/16	—	M, C	
706	G. H.	2427 I nw.	11	W	F	0	OK	9/16	—	M, C	
707	R. H.	do.	7	W	F	0	OK	9/16	—	M, C	
708	C. H.	do.	15	W	F	0	OK	9/16	—	M, C	
709	W. S.	2429 I nw.	8	W	M	0	OK	9/16	—	M, C	Measles in July.
710	R. S.	do.	11	W	M	0	OK	9/16	—	M, C	
711	C. S.	do.	1½	W	F	0	OK	9/16	—	M, C	Do.
712	L. S.	do.	5	W	M	0	OK	9/16	—	M, C	Do.
713	E. G.	301 22d nw.	12	C	F	0	OK	9/16	—	M, C	
714	T. G.	do.	3	C	F	0	OK	9/16	—	M, C	
715	R. S.	2100 N. Y. ave. nw.	?	C	M	0	OK	9/16	—	M, C	
716	R. S.	do.	?	C	F	0	OK	9/16	—	M, C	
717	A. W. (R.?).	2425 I nw.	(?16)	(W)	(F)	(0)	(OK)	9/16	—	M, C	No A. W.; A. R. lives at 2425 I.
718	E. R.	2120 N. Y. ave. nw.	?35	C	F	0	OK	9/16	—	M, C	Malaria in April.
719	E. B.	914 N. H. ave. nw.	8	C	F	0	OK	9/16	—	M, C	
720	L. A.	332 22d nw.	4	C	F	0	OK	9/16	—	M, C	
721	R. B.	343 22d nw.	?	C	F	0		9/16	—	M, C	Stomach trouble.
722	J. F.	912 N. H. ave. nw.	13	W	F	0	OK	9/16	—	M, C	
723	O. J.	2114 N. Y. ave. nw.	4	C	F	0	OK	9/16	—	M, C	
724	R. E. A.	707 22d nw, A1.	?35	W	F	0	OK	9/16	—	M, C	

Specimens of feces and urine examined for typhoid bacilli July 16 to November 8, 1908—Continued.

Specimen No.	Name of person.	Street address.	Age.	Color.	Sex.	Previous typhoid.	Present health.	Date of examination.	Result.	Examiner.	Remarks.
			Years.								
725	R. E. A.....	707 22d nw, A1.	? 35	W	M	0	OK	9/16	—	M, C	
726	P. A.....	2210 Va. ave. nw.	13	C	F	2	OK	9/16	—	M, C	
727	F. V. R.....	2132 F nw, A1E.	9	W	M	0	OK	9/17	—	M, C	
728	M. V. R.....	.....do.....	7	W	F	0	OK	9/17	—	M, C	
729	I. G.....	2102 N. Y. ave. nw.	12	C	F	0	OK	9/17	—	M, C	
730	A. B.....	927 25th nw..	7	W	F	0	OK	9/17	—	M, C	
731	E. B.....	.....do.....	5	W	M	0	OK	9/17	—	M, C	
732	S. F.....	912 N. H. ave. nw.	11	W	F	0	OK	9/17	—	M, C	
733	E. R.....	2114 Va. ave. nw.	10	C	F	0	OK	9/17	—	M, C	
734	L. B.....	2116 Va. ave. nw.	6	C	F	0	OK	9/17	—	M, C	
735	J. L. C.....	904 N. H. ave. nw.	2	W	M	0	OK	9/17	—	M, C	
736	W. B.....	2433 I nw....	? 14	W	M	1	OK	9/17	—	M, C	
737	T. C.....	701 24th nw..	.....	....	....	....	OK	9/17	—	M, C	See No. 412.
738	L. B. (L. ?).	322½ 22d nw..	3	C	M	0	OK	9/17	—	M, C	
739	F. R.....	2425 I nw....	3	W	M	0	OK	9/17	—	M, C	
740	B. F.....	2141 D nw...	12	C	M	0	OK	9/17	—	M, C	
741	E. W.....	2423 I nw....	10	W	M	*	OK	9/17	—	M, C	Typhoid, May, 1908.
742	F. W.....	.....do.....	12	W	M	0	OK	9/17	—	M, C	
743	S. D.....	913 25th nw..	12	C	M	0	OK	9/17	—	M, C	
744	C. B.....	.....do.....	6	C	F	0	OK	9/17	—	M, C	
745	S. K.....	2108 N. Y. ave. nw.	10	C	M	0	OK	9/17	—	M, C	
746	A. K.....	.....do.....	11	C	M	0	OK	9/17	—	M, C	
747	F. K.....	.....do.....	13	C	M	0	OK	9/17	—	M, C	
748	C. J.....	.....do.....	11	C	F	0	OK	9/17	—	M, C	
749	T. J.....	.....do.....	4	C	M	0	OK	9/17	—	M, C	
750	K. J.....	.....do.....	8	C	F	0	OK	9/17	—	M, C	
751	M. W.....	2423 I nw....	5	W	F	0	OK	9/17	—	M, C	
752	V. M.....	908 N. H. ave. nw.	5	C	F	0	OK	9/17	—	M, C	
753	H. M.....	.....do.....	3	C	F	0	OK	9/17	—	M, C	
754	G. N.....	2437 I nw....	9	W	M	0	OK	9/17	—	M, C	
755	A. N.....	.....do.....	3	W	M	0	OK	9/17	—	M, C	
756	E. N.....	.....do.....	7	W	M	0	OK	9/17	—	M, C	
757	E. N.....	.....do.....	11	W	M	0	OK	9/17	—	M, C	
758	R. S.....	2124 N. Y. ave. nw.	9	C	M	0	OK	9/17	—	M, C	
759	J. A.....	707 22d nw, A1.	5	W	F	0	OK	9/17	—	M, C	
760	R. A., jr....	.....do.....	7	W	M	0	OK	9/17	—	M, C	

Specimens of feces and urine examined for typhoid bacilli July 16 to November 8, 1908—Continued.

Specimen No.	Name of person.	Street address.	Age.	Color.	Sex.	Previous typhoid.	Present health.	Date of examination.	Result.	Examiner.	Remarks.
			Years.								
761	H. Z.....	2205 Va. ave. nw.	4	W	M	0	OK	9/17	—	M, C	
762	J. Z.....	.....do.....	13	W	M	0	OK	9/17	—	M, C	
763	J. M. M.....	2140 F nw...	16 m.	C	M	0	OK	9/17	—	M, C	
764	M. E. M.....	.....do.....	4	C	F	0	OK	9/17	—	M, C	
765	V. E. M.....	.....do.....	7½	C	F	0	OK	9/17	—	M, C	
766	P. B.....	509 23d nw...	8	C	F	0	OK	9/17	—	M, C	
767	K. H.....	2214 F nw...	2	C	M	0	OK	9/17	—	M, C	Recent summer complaint.
768	L. P.....	2216 F nw...	? 60	C	F	0	OK	9/17	—	M, C	Recent obscure abdominal trouble.
769	D. C.....	517 23d nw...	5	W	F	0	OK	9/17	—	M, C	
770	F. D.....	921 25th nw..	12	W	M	0	OK	9/18	—	M, C	
771	W. W.....	2423 I nw....	8	W	M	0	OK	9/18	—	M, C	
772	C. M.....	2436 K nw...	15	W	M	0	OK	9/18	—	M, C	
773	M. W.....	936 24th nw..	7	C	F	0	OK	9/18	—	M, C	
774	L. W.....	.....do.....	4	C	F	0	OK	9/18	—	M, C	
775	E. N.....	2437 I nw....	4	W	F	0	OK	9/18	—	M, C	
776	Dr. R. P. C.	2132 F nw, A2W.	?	W	M	4	OK	9/18	—	M, C	
777	R. J.....	301 22d nw...	1	C	M	0	OK	9/18	—	M, C	
778	J. S.....	405 22d nw...	7	C	M	0	OK	9/18	—	M, C	
779	C. H.....	2106 Va. ave. nw.	7	C	M	0	OK	9/18	—	M, C	
780	N. M.....	2436 K nw...	7	W	F	0	OK	9/18	—	M, C	
781	E. S.....	405 22d nw...	8	C	F	0	OK	9/18	—	M, C	
782	E. W.....	2431 I nw....	3	W	F	0	OK	9/18	—	M, C	
783	L. McK.....	940 24th nw..	13	C	F	0	OK	9/18	—	M, C	
784	M. L.....	2428 K nw...	10	W	F	0	OK	9/18	—	M, C	
785	M. Lee.....	2110 Va. ave. nw.	6	C	F	0	OK	9/18	—	M, C	
786	C. A.....	2115 D nw...	2	C	M	0	OK	9/18	—	M, C	
787	E. A.....	.....do.....	4	C	M	0	OK	9/18	—	M, C	
788	J. M.....	517 22d nw...	2	C	F	0	OK	9/18	—	M, C	
789	A. B.....	2148 F nw...	86	W	F	0	OK	9/18	—	M, C	Recent severe summer diarrhea.
790	L. H.....	.....do	6	W	F	2	OK	9/18	—	M, C	
791	F. M. M.....	2140 F nw...	6	C	F	0	OK	9/18	—	M, C	
792	N. A.....	707 22d nw...	8 m.	W	M	0	OK	9/19	—	M, C	
793	W. W.....	2431 I nw....	12	W	M	0	OK	9/21	—	R, C	
794	L. W.....	.....do.....	14	W	F	0	OK	9/21	—	R, C	
795	E. P. H.....	934 24th nw..	6	C	M	0	OK	9/21	—	R, C	
796	E. R.....	922 24th nw..	5	C	M	2	OK	9/21	—	R, C	
797	J. K.....	933 25th nw..	9	W	M	0	OK	9/21	+	R, C	Urine; several reexaminations all negative.
798	M. H.....	922 24th nw..	2	C	F	0	OK	9/21	—	R, C	
799	M. K.....	933 25th nw..	13	W	M	0	OK	9/21	—	R, C	Do.
800	M. O'N.....	935 25th nw..	2	W	F	0	OK	9/21	—	R, C	
801	A. M. R.....	940 24th nw..	1½	C	F	0	OK	9/21	—	R, C	

Specimens of feces and urine examined for typhoid bacilli July 16 to November 8, 1908—Continued.

Specimen No.	Name of person.	Street address.	Age.	Color.	Sex.	Previous typhoid.	Present health.	Date of examination.	Result.	Examiner.	Remarks.
			Years.								
802	E. G. M.....	1347 Girard nw.	?	W	M	2	OK	9/21	—	R, C	
803	F. F.....	1327 Girard nw.	11	W	M	0	OK	9/21	—	R, C	
804	W. E. S.....	507 22d nw...	? 45	W	M	20	OK	9/21	—	R, C	Recent summer diarrhea.
805	M. L. W.....	2135 Va. ave. nw.	2	C	F	0	OK	9/21	—	R, C	
806	H. W.....	2112 Va. ave. nw.	16	C	F	0	OK	9/21	—	R, C	
807	B. B. W.....	2135 Va. ave. nw.	4	C	F	0	OK	9/21	—	R, C	
808	C. P.....	2141 Va. ave. nw.	16	C	M	6	OK	9/21	—	R, C	
809	D. W.....	2112 Va. ave. nw.	15 m.	C	F	0	OK	9/21	—	R, C	
810	G. S.....	2124 N. Y. ave. nw.	12	C	F	0	OK	9/21	—	R, C	
811	R. B.....	502 22d nw...	15	C	M	0	OK	9/21	—	R, C	
812	R. R.....	922 24th nw..	8	? C	M	0	OK	9/21	—	R, C	
813	O. K.....	515½ 23d nw..	? 25	W	M	0	OK	9/21	—	R, C	Recent summer complaint.
814	R. W.....	519 22d nw...	4	W	M	0	OK	9/22	—	R, C	
815	W. G.....	.....do.....	6	W	M	0	OK	9/22	—	R, C	
816	B. G.....	.....do.....	4	W	M	0	OK	9/22	—	R, C	
817	I. F.....	503 22d nw...	6	W	F	0	OK	9/22	—	R, C	Recent summer diarrhea.
818	R. F.....	.....do.....	3	W	F	0	OK	9/22	—	R, C	Do.
819	E. V. W.....	2135 Va. ave. nw.	9	C	F	0	OK	9/22	—	R, C	
820	R. V. W.....	.....do.....	12	C	F	0	OK	9/22	—	R, C	
821	N. L.....	2127 Va. ave. nw.	10	C	F	0	OK	9/22	—	R, C	
822	S. L.....	.....do.....	1½	C	M	0	OK	9/22	—	R, C	
823	P. L.....	.....do.....	7	C	M	0	OK	9/22	—	R, C	
824	R. B.....	2109 E nw...	3	C	M	0	OK	9/22	—	R, C	
825	C. B.....	.....do.....	9 m.	C	M	0	OK	9/22	—	R, C	
826	F. B.....	.....do.....	1½	C	M	0	OK	9/22	—	R, C	
827	A. B.....	.....do.....	6	C	F	0	OK	9/22	—	R, C	
828	W. B.....	.....do.....	5	C	M	0	OK	9/22	—	R, C	Now has malaria.
829	L. R.....	2114 Va. ave. nw.	8	C	F	0	OK	9/22	—	R, C	
830	E. L.....	2206 Va. ave. nw.	3	C	F	0	OK	9/22	—	R, C	
831	J. W.....	936 24th nw..	11	C	M	0	OK	9/22	—	R, C	
832	F. W.....	.....do.....	? 35	C	F	2	OK	9/22	—	R, C	
833	R. W.....	.....do.....	13	C	M	0	OK	9/22	—	R, C	
834	G. L. R.....	940 24th nw..	3	C	F	0	OK	9/22	—	R, C	
835	S. (?) B.....	947 25th nw..	12	C	F	0	OK	9/22	—	R, C	

Specimens of feces and urine examined for typhoid bacilli July 16 to November 8, 1908—Continued.

Specimen No.	Name of person.	Street address.	Age.	Color.	Sex.	Previous typhoid.	Present health.	Date of examination.	Result.	Examiner.	Remarks.
			Years.								
836	F. B.....	947 25th nw..	10	C	F	0	OK	9/22	–	R, C	
837	C. O'N.....	935 25th nw..	4	W	M	0	OK	9/22	–	R, C	
838	A. K.....	933 25th nw..	8 m.	W	M	0	OK	9/22	–	R, C	Several reexaminations all negative.
839	F. F.....	923 25th nw..	13	W	M	0	OK	9/22	–	R, C	
840	W. F.....	.....do.....	7	W	M	0	OK	9/22	–	R, C	
841	A. D.....	1330 Harvard nw.	?	? C	F	4	OK	9/22	–	R, C	
842	C. W.....	1368 Harvard nw.	15	W	M	0	OK	9/22	–	R, C	
843	J. S., jr.....	2812 13th nw.	5	W	M	0	OK	9/22	–	R, C	
844	F. W.....	1374 Harvard nw.	11	W	M	6	OK	9/22	–	R, C	
845	E. K.....	1328 Harvard nw.	11	W	M	0	OK	9/22	–	R, C	
846	A. K.....	1351 Girard nw.	7	W	F	0	OK	9/22	–	R, C	
847	M. W.....	1374 Harvard nw.	5	W	F	0	OK	9/22	–	R, C	
848	G. M. G.....	519 22d nw...	2	W	M	0	OK	9/23	–	H, C	
849	V. K.....	1351 Girard nw.	12	W	M	3	OK	9/23	–	H, C	
850	M. K.....	1328 Harvard nw.	7	W	M	0	OK	9/23	–	H, C	
851	D. W.....	1374 Harvard nw.	13	W	F	0	OK	9/23	–	H, C	
852	A. G.....	2823 14th nw.	5	W	F	0	OK	9/23	–	H, C	
853	M. E. E.....	2817 14th nw.	13	W	F	0	OK	9/23	–	H, C	
854	T. E.....	.....do.....	6	W	M	0	OK	9/23	–	H, C	
855	B. E.....	.....do.....	12	W	M	0	OK	9/23	–	H, C	
856	F. E.....	2815 14th nw.	3	W	F	0	OK	9/23	–	H, C	
857	M. H.....	1336 Harvard nw.	14	W	M	2	OK	9/23	–	H, C	
858	Rhett. P....	928 24th nw..	12	C	M	0	OK	9/23	–	H, C	
859	R. P.....	.....do.....	15	C	M	0	OK	9/23	–	H, C	
860	E. W.....	936 24th nw..	9	C	F	0	OK	9/23	–	H, C	
861	M. E.....	2507 I nw....	9	W	F	0	OK	9/23	–	H, C	
862	K. E.....	.....do.....	2	W	F	0	OK	9/23	–	H, C	
863	A. F.....	925 26th nw..	11	W	M	1	OK	9/23	–	H, C	
864	W. E.....	2511 I nw....	2	W	M	0	.....	9/23	–	H, C	Whooping cough.
865	O. D.....	2115 E nw....	14	C	F	0	OK	9/23	–	H, C	
866	W. H.....	2123 E nw....	? 10	C	M	0	OK	9/23	–	H, C	
867	A. L.....	2127 Va. ave. nw.	3	C	F	0	OK	9/23	–	H, C	
868	J. R. F.....	503 22d nw...	9	W	M	0	OK	9/23	–	H, C	Recent summer complaint.
869	J. G.....	519 22d nw...	? 35	W	M	8	OK	9/23	–	H, C	
870	F. C.....	413 22d nw...	2	C	M	0	OK	9/23	–	H, C	
871	M. K.....	515½ 23d nw..	? 20	W	F	0	OK	9/23	–	H, C	Do.

Specimens of feces and urine examined for typhoid bacilli July 16 to November 8, 1908—Continued.

Specimen No.	Name of person.	Street address.	Age.	Color.	Sex.	Previous typhoid.	Present health.	Date of examination.	Result.	Examiner.	Remarks.
			Years.								
872	G. C.....	1353 Girard nw.	5	W	M	0	OK	9/24	–	H, C	
873	W. K.....	1328 Harvard nw.	5	W	M	0	OK	9/24	–	H, C	
874	E. L.....	2817 14th nw.	?	C	F	1	OK	9/24	–	H, C	
875	L. E.....	2815 14th nw.	17 m.	W	F	0	OK	9/24	–	H, C	
876	C. S.....	943 25th nw..	9	C	F	0	OK	9/24	–	H, C	
877	A. S.....	.....do.....	6	C	F	0	OK	9/24	–	H, C	
878	M. D.....	921 25th nw..	8	W	F	0	OK	9/24	–	H, C	
879	E. F.....	923 25th nw..	12	W	M	0	OK	9/24	–	H, C	
880	M. E.....	2511 I nw....	5	W	M	0	.....	9/24	–	H, C	Whooping cough.
881	N. S.....	2515 I nw....	13	C	F	0	OK	9/24	–	H, C	
882	G. W.....	935 26th nw..	4	C	M	0	OK	9/24	–	H, C	
883	M. W.....	.....do.....	10	C	F	0	OK	9/24	–	H, C	
884	W. W.....	.....do.....	6	C	M	0	OK	9/24	–	H, C	
885	A. W.....	.....do.....	1½	C	M	0	OK	9/24	–	H, C	
886	Earl K.....	834 N.H.ave. nw.	7	W	M	*1	OK	9/24	–	H, C	Doubtful case of typhoid, summer, 1907.
887	E. K.....	.....do.....	4	W	F	0	OK	9/24	–	H, C	
888	M. S.....	818 N.H.ave. nw.	16 m.	W	F	0	OK	9/24	–	H, C	
889	T. S.....	.....do.....	2	W	F	0	OK	9/24	–	H, C	
890	A. L. C.....	816 N.H.ave. nw.	10	W	F	0	OK	9/24	–	H, C	
891	W. J. C.....	.....do.....	14	W	M	0	OK	9/24	–	H, C	
892	T. C.....	.....do.....	4	W	F	0	OK	9/24	–	H, C	
893	C. G.....	814 N.H.ave. nw.	14	W	F	0	OK	9/24	–	H, C	
894	M. G.....	.....do.....	10	W	F	0	OK	9/24	–	H, C	
895	J. McG.....	810 N.H.ave. nw.	12	W	M	0	OK	9/24	–	H, C	
896	A. McG.....	.....do.....	7	W	F	0	OK	9/24	–	H, C	
897	E. McG.....	.....do.....	3	W	M	0	OK	9/24	–	H, C	
898	N. S.....	512 21st nw...	13	C	M	0	OK	9/24	–	H, C	
899	W. S.....	.....do.....	15	C	M	0	OK	9/24	–	H, C	
900	L. H.....	2113 E nw....	2	C	F	0	OK	9/24	–	H, C	
901	L. L.....	2127 Va. ave. nw.	13	C	F	0	OK	9/24	–	H, C	
902	A. R.....	507 22d nw...	?21	W	M	0	OK	9/24	–	H, C	Recent summer complaint.
903	E. S.....	2818 N nw...	7	W	F	0	OK	9/25	–	H, C	
904	L. P.....	1230 28th nw.	15	W	F	0	OK	9/25	–	H, C	
905	J. P.....	.....do.....	12	W	M	0	OK	9/25	–	H, C	
906	K. O'B.....	2436 K nw...	2	W	F	0	OK	9/25	–	H, C	
907	C. W.....	935 26th nw..	2	C	F	0	OK	9/25	–	H, C	
908	J. K.....	933 25th nw..	9	W	M	0	OK	9/25	–	H, C	Several examinations. See No. 797.
909	.....do.....	.....do	.....	.....	.....	.....	.....	9/25	–	H, C	Urine.
910	W. D.....	2426 I nw....	11	W	M	0	OK	9/25	–	H, C	

Specimens of feces and urine examined for typhoid bacilli July 16 to November 8, 1908.—Continued.

Specimen No.	Name of person.	Street address.	Age.	Color.	Sex.	Previous typhoid.	Present health.	Date of examination.	Result.	Examiner.	Remarks.
			Years.								
911	W. M. L.	2424 I nw.	19 m.	C	M	0	OK	9/25	—	H, C	
912	N. F.	2422 I nw.	13	C	M	0	OK	9/25	—	H, C	
913	M. H.	2416 I nw.	9	W	F	0	OK	9/25	—	H, C	
914	T. F. McC.	2412 I nw.	14	W	M	0	OK	9/25	—	H, C	
915	I. W.	930 24th nw.	12	C	F	0	OK	9/25	—	H, C	
916	M. C.	828 N.H.ave. nw.	2	W	F	0	OK	9/25	—	H, C	
917	F. C.	816 N.H,ave. nw.	8	W	M	0	OK	9/25	—	H, C	
918	T. G.	814 N.H.ave. nw.	8	W	M	0	OK	9/25	—	H, C	
919	K. B.	2433 H nw.	12	C	F	0	OK	9/25	—	H, C	
920	E. P.	803 25th nw.	3	C	F	0	OK	9/25	—	H, C	
921	R. P.	do.	7	W	M	0	OK	9/25	—	H, C	
922	G. O.	829 25th nw.	14	W	M	0	OK	9/25	—	H, C	
923	P. O.	do.	13	W	F	0	OK	9/25	—	H, C	
924	G. R.	506 21st nw.	14	C	F	0	OK	9/25	—	H, C	
925	M. S.	2818 N nw.	12	W	F	0	OK	9/26	—	H, C	
926	C. L.	2820 N nw.	? 18	W	F	7	OK	9/26	—	H, C	
927	L. W.	do.	? 30	W	F	7	OK	9/26	—	H, C	
928	T. B.	2504 I nw.	5	W	M	0	OK	9/26	—	H, C	
929	W. T.	808 25th nw.	2	C	M	0	OK	9/26	—	H, C	
930	A. D.	do.	3	C	M	0	OK	9/26	—	H, C	
931	E. D.	do.	20 m.	C	F	0	OK	9/26	—	H, C	
932	E. D.	do.	11	C	F	0	OK	9/26	—	H, C	
933	H. D.	do.	5	C	M	0	OK	9/26	—	H, C	
934	E. L.	812 25th nw.	8	C	F	0	OK	9/26	—	H, C	
935	P. L.	do.	?	C	F	0		9/26	—	H, C	Stomach trouble.
936	S. O.	829 25th nw.	10	W	F	0	OK	9/26	—	H, C	
937	A. B.	815 25th nw.	2	W	M	0	OK	9/26	—	H, C	
938	R. C.	803 25th nw.	11	C	F	0	OK	9/26	—	H, C	
939	C. B.	807 26th nw.	10	C	M	0	OK	9/26	—	H, C	
940	E. B.	do	12	C	M	0	OK	9/26	—	H, C	
941	E. T.	823½ 26th nw.	7	C	F	....	OK	9/26	—	H, C	Typhoid June, 1908.
942	M. T.	do.	6	C	F	....	OK	9/26	—	H, C	Do.
943	A. H.	825 26th nw.	5	C	M	0	OK	9/26	—	H, C	Scarlet fever 6 weeks ago.
944	M. H.	do.	8	C	M	0	OK	9/26	—	H, C	
945	E. J.	.....	.....	....	....	....		9/18	—	.....	Improving. (See Nos. 280 and 372.)
946	J. Q., jr.	837 26th nw.	11	W	M	0	OK	9/26	—	H, C	
947	M. H.	2416 I nw.	4	W	M	0	OK	9/26	—	H, C	
948	J. C.	2111 E nw.	3	C	M	0	OK	9/26	—	H, C	
949	J. M. B.	532 21st nw.	11	W	F	0	OK	9/26	—	H, C	
950	L. M. B.	do.	10	W	F	0	OK	9/26	—	H, C	
951	M. L. B.	do.	4	W	F	0	OK	9/26	—	H, C	
952	M. E. B.	do.	6	W	F	0	OK	9/26	—	H, C	
953	J. H.	2123 E nw.	? 55	C	M	0	OK	9/28	—	H, C	
954	M. H.	2416 I nw.	7	W	M	0	OK	9/28	—	H, C	

Specimens of feces and urine examined for typhoid bacilli July 16 to November 8, 1908—Continued.

Specimen No.	Name of person.	Street address.	Age.	Color.	Sex.	Previous typhoid.	Present health.	Date of examination.	Result.	Examiner.	Remarks.
			Years.								
955	F. L. C.....	532 21st nw..	12	W	F	0	OK	9/28	—	H, C	
956	F. S.....	804 24th nw..	7	W	M	0	OK	9/28	—	H, C	
957	W. S.....	.....do.....	5	W	M	0	OK	9/28	—	H, C	
958	A. O.....	829 25th nw..	6	W	F	0	OK	9/28	—	H, C	
959	W. W.....	813 25th nw..	? 10	C	M	0	OK	9/28	—	H, C	
960	P. J.....	528 21st nw..	12	W	F	0	OK	9/28	—	H, C	
961	O. W.....	813 25th nw..	? 10	C	M	0	OK	9/28	—	H, C	
962	M. T.....	829 26th nw..	4	W	F	0	OK	9/28	—	H, C	
963	C. S.....	818 26th nw..	12	W	M	0	OK	9/28	—	H, C	
964	N. L.....	2820 N nw...	14	W	M	0	OK	9/28	—	H, C	
965	J. McG.....	810 N.H.ave. nw.	6	W	M	0	OK	9/28	—	H, C	
966	C. C.....	2415 H nw...	4	W	F	0	OK	9/28	—	H, C	
967	M. G.....	2519 H nw...	11	W	F	0	OK	9/28	—	H, C	
968	D. A.....	2417 H nw...	10	C	F	5	OK	9/28	—	H, C	
969	E. H.....	2600 I nw....	15	W	F	0	OK	9/28	—	H, C	
970	L. S.....	818 26th nw..	8	W	M	0	OK	9/28	—	H, C	
971	A. McD.....	2428 I nw....	11	W	F	0	OK	9/28	—	H, C	
972	A. W.....	2820 N nw...	? 10	W	F	0	OK	9/28	—	H, C	
973	M. I.....	827 26th nw..	14	W	F	0	OK	9/28	—	H, C	
974	J. G.....	2519 H nw...	14	W	M	0	OK	9/28	—	H, C	
975	A. K.....	831 26th nw..	14	W	F	0	OK	9/28	—	H, C	
976	D. G.....	2524 K nw...	? 40	W	F	0	OK	9/28	—	H, C	
977	I. H.....	2600 I nw....	11	W	F	0	OK	9/28	—	H, C	
978	F. N.....	2602 I nw....	13	W	M	0	OK	9/28	—	H, C	
979	F. C.....	2415 H nw...	2	W	M	0	OK	9/28	—	H, C	
980	A. C.....	.....do.....	13	W	M	0	OK	9/28	—	H, C	
981	W. W.....	822 24th nw..	5	C	M	0	OK	9/28	—	H, C	
982	J. D.....	813 25th nw..	10	C	M	?	OK	9/28	—	H, C	
983	A. T.....	801 N.H.ave. nw.	5	C	F	0	OK	9/29	—	C, M	
984	J. T.....	.....do.....	10	C	M	0	OK	9/29	—	C, M	
985	F. T.....	.....do.....	1	C	M	0	OK	9/29	—	C, M	
986	L. T.....	.....do.....	4	C	F	0	OK	9/29	—	C, M	
987	J. T.....	.....do.....	8	C	F	0	OK	9/29	—	C, M	
988	C. F., jr.....	830 24th nw..	20 m.	C	M	0	OK	9/29	—	C, M	
989	M. J. B	820 24th nw..	13	C	F	0	OK	9/29	—	C, M	
990	N. Y.....	806 24th nw..	2½	W	F	0	OK	9/29	—	C, M	
991	R. F.....	830 24th nw..	3	C	M	0	OK	9/29	—	C, M	
992	R. A.....	832 24th nw..	7	C	M	0	OK	9/29	—	C, M	
993	E. G.....	2510 I nw....	6	C	F	0	OK	9/29	—	C, M	
994	W. B.....	815 25th nw..	5	W	M	0	OK	9/29	—	C, M	
995	S. A.....	2503 I nw....	? 35	W	F	3	OK	9/29	—	C, M	
996	C. H.....	2502 I nw....	8	W	M	0	OK	9/29	—	C, M	
997	M. L.....	2424 I nw....	6	C	F	0	OK	9/29	—	C, M	
998	M. E.....	524 21st nw..	14 m.	W	F	0	OK	9/29	—	C. M	
999	F. G.....	2519 H nw...	5	W	M	0	OK	9/29	—	C, M	
1000	P. W.....	2820 N nw...	2	W	F	0	OK	9/29	—	C, M	
1001	R. T.....	829 26th nw..	13	W	F	0	OK	9/29	—	C, M	

Specimens of feces and urine examined for typhoid bacilli July 16 to November 8, 1908—Continued.

Specimen No.	Name of person.	Street address.	Age.	Color.	Sex.	Previous typhoid.	Present health.	Date of examination.	Result.	Examiner.	Remarks.
			Years.								
1002	L. B.	1236 28th nw.	9	W	F	0	OK	9/29	—	C, M	
1003	G. M.	603 22d nw.						10/8	—	C, H	Convalescent. (See Nos. 31 and 239.)
1004	D. K.	933 25th nw.	? 12	W	F	0	OK	10/8	—	C, H	See Table III, page 144.
1005	R. K.	do	? 40	W	F	0	OK	10/8	—	C, H	Do.
1006	J. K.	do	9	W	M	0	OK	10/8	—	C, H	Do.
1007	A. K.	do	8 m.	W	M	0	OK	10/8	—	C, H	Do.
1008	do	do					OK	10/8	—	C, H	Urine. (See Table III, page 144.)
1009	D. K.	do					OK	10/8	—	C, H	Do.
1010	R. K.	do					OK	10/8	—	C, H	Do.
1011	R. K.	do					OK	10/8	—	C, H	Do.
1012	J. K.	do					OK	10/8	—	C, H	Do.
1013	C. J.	2120 G nw, A103.					OK	10/8	—	C, H	See Nos. 8 and 27A.
1014	L. K.	933 25th nw.	? 50	W	M	0	OK	10/9	—	C	See Table III, page 144.
1015	M. K.	do	13	W	M	0	OK	10/9	—	C	Urine. (See Table III, page 144.)
1016	I. K.	do	? 18	W	F	0	OK	10/9	—	C	Do.
1017	R. K.	do					OK	10/9	—	C	
1018	I. K.	do					OK	10/9	—	C	
1019	M. K.	do					OK	10/9	—	C	
1020	L. K.	do					OK	10/9	—	C	Do.
1021	C. E.	301 Mass. ave. ne.					OK	10/24	—	C	See Nos. 497 and 687.
1022	J. K.	933 25th nw.					OK	10/24	—	C	Urine—feces. (See Table III, page 144.)
1023											
1024	M. K.	do					OK	10/24	—	C	Do.
1025											
1026	L. K.	do					OK	10/24	—	C	Do.
1027											
1028	R. K.	do					OK	10/24	—	C	Do.
1029											
1030	D. K.	do					OK	10/24	—	C	Do.
1031											
1032	A. K.	do					OK	10/24	—	C	Do.
1033											
1034	I. K.	do					OK	10/24	—	C	Urine. (See Table III, page 144.
1035	H. B.	718 St. Mary's ct.	? 25	C	M	10	.....	11/7	—	L, C	Convalescing from typhoid.
1036	H. B.	do	? 25	C	F	10	OK	11/7	—	L, C	All others in family had typhoid recently.
1037	C. B.	do	5	C	M	0	.....	11/7	+	L, C	Convalescing from typhoid.
1038	T. B.	do	4	C	M	0	OK	11/7	—	L, C	Have had typhoid this season.
1039	M. B.	do	3	C	F	0	OK	11/7	—	L, C	
1040	R. B.	do	10 m.	C	M	0	OK	11/7	—	L, C	

I.—*List of controls.*

Number.	Date.	Drops of typhoid bouillon.	Period of contact.	Result.	Number.	Date.	Drops of typhoid bouillon.	Period of contact.	Result.
1	8/12	5	Short	−	54	9/11	15	24 hours	+
2	8/12	0	do	−	55	9/11	3	do	−
3	8/13	5	Short	−	56	9/11	1	Short	+
4	8/13	0	do	−	57	9/14	5	do	+
5	8/15	5	Short	+	58	9/14	3	24 hours	+
6	8/15	5	do	−	59	9/14	1	Short	+
7	8/15	5	do	+	60	9/14	15	24 hours	+
8	8/19	5	do	+	61	9/15	1	Short	+
9	8/20	5	do	+	62	9/15	3	24 hours	+
10	8/20	0	do	−	63	9/15	15	do	+
11	8/22	5	Short	+	64	9/15	5	Short	+
12	8/24	5	do	−	65	9/16	3	24 hours	+
13	8/25	5	do	−	66	9/16	1	Short	+
14	8/25	2	do	−	67	9/16	15	24 hours	+
15	8/26	5	do	−	68	9/16	5	Short	+
16	8/26	2	do	+	69	9/17	5	do	+
17	8/27	2	do	+	70	9/17	15	24 hours	+
18	8/27	5	do	+	71	9/17	3	do	+
19	8/28	5	do	+	72	9/17	1	Short	+
20	8/28	2	do	+	73	9/18	3	24 hours	+
21	8/29	5	do	+	74	9/18	15	do	+
22	8/29	2	do	+	75	9/18	5	Short	+
23	8/31	5	do	+	76	9/18	1	do	+
24	8/31	2	do	+	77	9/21	5	do	+
25	9/1	2	do	+	78	9/21	1	do	+
26	9/1	5	do	+	79	9/21	15	48 hours	+
27	9/2	2	do	+	80	9/21	3	do	+
28	9/2	5	do	+	81	9/22	3	24 hours	+
29	9/3	3	24 hours	+	82	9/22	5	Short	+
30	9/3	15	do	+	83	9/22	1	do	+
31	9/3	1	Short	+	84	9/22	15	24 hours	+
32	9/3	5	do	−	85	9/23	5	Short	+
33	9/4	5	24 hours	+	86	9/23	1	do	+
34	9/4	20	do	−	87	9/23	15	24 hours	+
35	9/4	1	Short	+	88	9/23	3	do	+
36	9/4	5	do	−	89	9/24	5	Short	+
37	9/5	1	do	+	90	9/24	1	do	+
38	9/5	5	do	+	91	9/24	3	24 hours	+
39	9/5	15	24 hours	−	92	9/24	15	do	+
40	9/5	3	do	−	93	9/25	3	do	+
41	9/8	3	72 hours	−	94	9/25	5	Short	+
42	9/8	15	do	−	95	9/25	1	do	+
43	9/8	1	Short	+	96	9/25	15	24 hours	+
44	9/8	5	do	+	97	9/26	3	do	+
45	9/9	3	24 hours	−	98	9/26	1	Short	+
46	9/9	15	do	−	99	9/26	5	do	+
47	9/9	1	Short	−	100	9/26	15	24 hours	+
48	9/9	5	do	−	101	9/28	1	Short	+
49	9/10	3	24 hours	−	102	9/28	5	do	+
50	9/10	15	do	−	103	9/28	3	24 hours	+
51	9/10	1	Short	+	104	9/28	15	do	+
52	9/10	5	do	+	105	9/29	3	do	+
53	9/11	5	do	+	106	9/29	15	do	+

II.—*Summary of controls.*

Result.	Contact of culture in the feces.				Normal feces.	Total.
	Short.	24 hours.	48 hours.	72 hours.		
Total	63	36	2	2	3	106
Positive	52	28	2	0	0	82
Negative	11	8	0	2	3	24
Per cent positive	84	78	100	0	0	[a] 80

[a] Specimens containing no typhoid culture and those having culture added 48 and 72 hours before examination not counted in calculating this percentage.

III.—*Persons furnishing two or more specimens.*

C. H. J. ..... 8, +; 27A, and 1013, −.
G. M. ..... 31, −; 239, +; 1003, −.
W. S. ..... 57 and 79, −.
A. S. ..... 58 and 80, −.
T. S. ..... 59 and 81, −.
A. C. ..... 274, 381, and 611, −.
W. C. ..... 275, +; 303, +; 463, −.
J. C. ..... 276, 396, and 616, −.
T. C. ..... 412 and 737, −.
E. J. ..... 280, +; 372, −; 945, −.
R. B. ..... 304 and 1040, −.
T. B. ..... 305 and 1038, −.
M. B. ..... 405, +; 1039, −.
C. B. ..... 309, −; 1037, +.
B. ..... 306 and 1036, −.
C. E. ..... 479, +; 687, −; 1021, −.
L. K. ..... 1014 and 1020, −; 1026 and 1027, −.
L. K. ..... 1005 and 1010, −; 1028 and 1029, −.
I. K. ..... 1016 and 1018, −; 1034, −.
D. K. ..... 1004 and 1009, −; 1030 and 1031, −.
R. K. ..... 1011 and 1017, −.
M. K. ..... 799, 1015, 1019, 1024, and 1025, −.
A. K. ..... 838, 1007, 1032, and 1033, −.
J. K. ..... 797, +; 908, 1006, 1012, 1022, and 1023, −

IV.—*Bacteriologists.*—Rosenau=R; Lumsden=L; Miller=M; Hart=H; Cannon=C. These initials appear in the table under the column "examiner."

The laboratory work referred to in the above tables was controlled by two methods. The first, followed only for about one week, was to submit to the examiners at the same time that the collected specimens were delivered specimens of known normal feces. To some of these control specimens 5 drops of a 24-hour broth culture of *B. typhosus* were added an hour or two before the examination was begun. To others no typhoid culture was added. The examiners were not told which of the control specimens contained typhoid culture until after their report on the results of the examinations was made. The control specimens, however, were kept separate from the collected specimens.

By this method of three control specimens containing no typhoid culture all were reported negative. Of 7 control specimens containing typhoid culture, 4 were reported positive and 3 negative. The

rather large percentage of negative results from the examination of the control specimens containing typhoid culture which were obtained in the early part of the work was discovered to have been due to a large extent to the fact that a culture of *B. typhosus* was used which grew poorly and agglutinated feebly.

The second method of controlling the laboratory examinations, followed the greater part of the time, was as follows:

The bottles containing the known normal feces plus typhoid culture were labeled with a fictitious name to make them appear as if they were collected specimens, and placed on the laboratory table along with the collected specimens. The amount of broth culture added to the 20 to 30 grams of feces was from 1 to 20 drops, and the culture was added to the feces from 1 to 72 hours before delivery for examination. The mixture of feces and typhoid culture was kept at room temperature from the time of preparation to examination unless the culture was exposed to the feces for 48 or 72 hours, in which instances the mixture was kept in the cool room at a temperature of 20° C.

This second method had the advantage of being a more exact check on the efficiency of the work, but required the greatest possible care in the handling and labeling of the series of plates made from each specimen to prevent a plate made from a control specimen from being confused with one made from a collected specimen.

By this second method 96 control specimens were examined and 78, or about 81 per cent, were reported positive. It should be noted on the table that the 50 control specimens examined on and subsequent to September 14 were all reported positive. From that date a fresh culture from a known vigorous strain of typhoid was used invariably, which had not been the case prior to that date.

Another and more convincing check on the efficiency of the examinations was the examinations of the feces of ten typhoid-fever patients or early convalescents. Positive results were obtained in seven.

Ten specimens, 9 of feces and 1 of urine, out of the 1,040 examined gave a positive result for *B. typhosus*. Seven of the specimens of feces giving positive results were obtained from persons who at the time of the examination had typhoid fever clinically. The remaining two specimens of feces and the one of urine giving positive results were obtained from three persons who did not have clinically recognized typhoid fever. The histories of these three persons were as follows:

1. C. H. J. Male; white; æt. 50 years. Occupation, clerk Post-Office Department for the past eight years. Born in New Orleans, La., and lived there until about 30 years of age; since then lived in Washington and in New England (continuously in Washington for the past nine years). For the past three years has lived in an apart-

ment house at 2120 G street NW. Occupants of apartment, himself and wife. During six years before going to this apartment, lived at different boarding houses in Washington. He has never had typhoid fever. His wife had typhoid fever about thirty-eight years ago. Mr. J. does not know of any case of typhoid fever having developed at any house at which he has lived. He is not robust, but of wiry type, and has enjoyed good health all his life.

About the 1st of June, 1908, he began to "feel out of sorts," had some loss of appetite and feeling of discomfort in region of liver, but no attacks of sharp pain. He attributed his indisposition to a torpid liver induced by sedentary habits. Was slightly jaundiced. Did not get weighed, but thinks he lost about 15 pounds in weight during the two weeks in which he felt indisposed. He began to feel well again about the 1st of August and since then has been in his usual health.

Laboratory examinations: Feces examined July 19. One of the Endo plates showed three suspicious colonies; fished and planted in broth; 24-hour culture in broth of one of these colonies agglutinated with antityphoid serum in dilution of 1:400; failed to ferment in lactose broth, and plated back on Endo in pure culture gave colonies characteristic of *B. typhosus*. The other two fished colonies proved not to be typhoid.

Examinations of feces obtained on July 29 and on October 8 were negative for *B. typhosus*.

On July 29 blood was examined. Negative for *Plasmodium malariæ*, some eosiniphilia observed. Feces examined for animal parasites, but result negative.

Blood gave negative result to Widal test on July 29.

2. C. E. Male; white; age 14 years; school boy. Born in Washington. Has lived at present home, 301 Massachusetts avenue NE. for past five years. Eleven in family—father, mother, and nine children. No one in family had typhoid fever except boy's mother, whose attack occurred forty-two years ago.

No one in the family has been ill in any way within the past four years. C. E. had a slight cold (nasal) about the middle of August. The cold lasted for only a few days and did not interfere with his activities.

Laboratory examinations: Feces on September 3 gave positive result for *B. typhosus*.

Examination of feces obtained September 15 and on October 24 both negative for *B. typhosus*.

On September 3, at the time the specimen of feces giving positive result was delivered for examination, 4 control specimens were examined. One of these, containing 5 drops of 24-hour broth culture of typhoid bacillus, added to the feces just before examination, was not detected by the examination.

3. J. K. Male; white; age 9 years; school boy. Born in Baltimore, Md., where he lived until about 6 years old. Since then has lived in Washington, at 933 Twenty-fifth street NW. Eight in family—father, mother, and six children. None ever had typhoid fever. No sickness in family within past six months. Rooms occupied by family to rear and on floor above a small grocery store owned and run by boy's father and mother.

Laboratory examinations: Urine, on September 21, gave positive result for *B. typhosus*. Examinations of urine obtained September 25, October 8, and October 24, all were negative for *B. typhosus*. Examinations of feces on September 25, October 8, and October 24 were negative for *B. typhosus*.

As the plates made from the specimens of urine examined on September 21 showed, beside the typhoid colonies, a large number of colonies like those of *B. coli* and other bacteria it is probable that the urine was caught in a soiled vessel before being placed in the clean specimen bottle.

The possibility of the typhoid bacilli found in this specimen of urine having come from the excreta of some other members of the family was considered; but repeated examinations (altogether 32) of specimens of feces and urine from other members of the family were negative for the typhoid bacillus.

The organisms isolated from the specimens of excreta of C. E. and of J. K. were carried through the various culture media. Their cultural characteristics and their serum reactions were those of the typhoid bacillus.

In work of this kind the possibility of experimental error must be conceded, particularly when 30 or more specimens of feces are examined in one day. However, the likelihood of such error occurring by the misplacing or mislabeling of plates should have been reduced to practically nothing by the exercise of the utmost care. Considering each of the three positive results with a view to possibility of error, we have the following:

1. Specimen of feces of C. H. J.: On the day that this specimen was examined only two other specimens were examined, so there was no need for hurry in doing the work. No control specimens containing typhoid bacilli were presented for examination that day; so the possibility of a control plate having been mistaken for one of the C. H. J. plates can be eliminated. The only apparent reasons for doubting this positive finding seems to be that the organism was not tested on all the culture media which may be used to identify the typhoid organism and only one serum was used to determine its agglutinability. However, the typical appearance of the colonies on Endo's medium, the failure of the organism to ferment lactose broth to gas formation, its typical growth in this medium, and its

agglutination in high dilution with antityphoid serum make it appear probable that this organism was the typhoid bacillus.

Later in the season one of us (Lumsden) isolated from human feces an organism, culturally the colon bacillus, which would agglutinate quite readily in dilutions of 1 to 300 with this same (rabbit's) antityphoid serum. A number of other strains of *B. coli* were then tried with this serum, but all failed to agglutinate in dilutions as low as 1 to 20. There seemed to be some specific relation between the organism and this particular rabbit's serum dependent upon some agglutinin other than typhoid in the serum, because two serums obtained from other animals—one rabbit and one horse—both failed to agglutinate the organism.

The positive finding in the case of C. H. J. has some possible clinical support in the fact that during the five or six weeks previous he had been indisposed—had symptoms suggesting the possibility of a typhoid cholecystitis.

2. Specimen of feces from C. E.: On the day this specimen was examined 41 other collected specimens of feces and 4 control specimens of feces containing typhoid culture were examined, all on the same table. With such a large number of plates to handle there was a possibility, but we do not say a probability, of error.

One of the 4 control specimens containing 5 drops of typhoid culture freshly added examined that day was reported negative. Identity of organism isolated established by full cultural study and by agglutination in high dilution with two antityphoid serums obtained from different animals.

3. Specimen of urine from J. K.: The day this specimen was examined, 19 collected specimens of feces, one other collected specimen of urine, and 4 control specimens of feces containing typhoid culture were examined.

The Endo plates from this urine presented a large number and quite a variety of colonies, giving the plates more the appearance of having been made from feces than from urine. This probably was due to the urine having been collected in a vessel actually soiled with fecal matter. Therefore the typhoid bacilli isolated from this specimen may have come originally from the feces of this boy or from that of some other member of the family. All the control specimens examined that day were reported positive.

Identity of the organism isolated was established by complete cultural study and by its high agglutinability with two different antityphoid serums. Examinations of three specimens of urine and of three specimens of feces subsequently obtained from this boy were all negative for *B. typhosus*. Repeated examinations of the feces and urine of other members of the family were negative for *B. typhosus*.

The second specimen of urine from J. K. was obtained four days after the first. No treatment had been given the boy in the meanwhile, and yet the typhoid organism, neither at the second nor at subsequent examinations, was found.

If these three positive results are accepted, several interesting considerations present themselves:

(*a*) These three persons are chronic typhoid bacillus carriers. The fact that the bacillus was not found in any of the specimens after the first for each case may have been due to all three of these "carriers" having been in periods, known to occur with bacillus carriers, when the excreta are apparently free from the organism.

It seems to us, however, that it is improbable that all three of the persons were chronic carriers, in view of the fact that all specimens after the first ones gave negative results.

(*b*) That these three persons were temporary bacillus carriers and perhaps harbored the bacilli for but a few days or weeks. That they did not have the disease may have been due to the absence of other factors concerned in the etiology of typhoid infection. Still, if they are viewed as temporary carriers, which in face of the evidence seems more reasonable than the view that they are chronic carriers, we must admit that, if there are in the District of Columbia about 900 persons during the typhoid season who without having typhoid fever clinically do discharge typhoid bacilli, if only for a few days, they must be considered as important factors in the spread of infection.

(*c*) The finding of typhoid bacilli in the feces of two or three healthy persons per 1,000 of population in a community may have diminished significance in the epidemiology of typhoid fever. If these organisms do not cause disease of the hosts in whose intestinal canals they are found, it is evident that other factors are necessary in the etiology of typhoid fever.

This is following the view suggested by Pettenkofer, that the causation of typhoid fever may be represented by XYZ In this combination the typhoid bacillus is X, and unless Y and Z are also operative, disease does not result.

In this connection it is interesting to consider the possibility of typhoid bacilli being harbored as harmless parasites in the intestinal canal, gall bladder, hepatic ducts, or elsewhere in the human body for months or even years and then, by having added the other (YZ) elements of the etiologic complex, produce the disease.

The possibility in some cases of a long period of time elapsing between the time of taking the bacilli into the body and the onset of the disease must be considered, even if the typhoid bacillus is looked upon as the sole cause of the disease, specific susceptibility to this organism being the only other factor concerned. Accepting this hypothesis, it is conceivable that typhoid bacilli, like tubercle

bacilli, may be stored in the body but their growth inhibited in some way until the specific resistance of the persons becomes lowered, and allows the organisms to multiply and produce the disease.

That prolonged periods of storing (incubation) of the organisms are the rule is not borne out by the numerous observations that pronounced outbreaks of typhoid fever in a given community cease quite abruptly within about a month after the known source of infection causing the outbreak is removed. The possibility of exceptional and individual cases being accounted for by such long periods of storage of the infecting organisms, however, must be recognized. This is a possible explanation of the case occurring at the Government Hospital for the Insane. (See p. 35.)

NEWCOMERS.

Data relative to the newcomers in the residences at which cases occured are given in the following table. By "newcomers" is meant persons who moved into the house within three months before the onset of the case.

Number of cases at private residences at which there were one or more newcomers.	33
At hotels, rooming houses, hospitals, and other public institutions	34
Not determined	1
No newcomers	474
Total	542

Of the 33 cases occurring at private residences at which there were newcomers, there were 12 cases associated with newcomers who were said never to have had typhoid fever, 6 with newcomers whose typhoid history could not be learned, 1 with a newcomer who had had typhoid fever within one year, 1 with a newcomer who had had typhoid between 4 and 5 years previous, 8 with newcomers who had had typhoid from 6 to 10 years previous, 3 with newcomers who had had typhoid from 13 to 20 years previous, and 2 with newcomers who had had typhoid from 40 to 45 years previous.

Specimens of feces and urine from 10 newcomers suspected to be possible bacillus carriers were examined bacteriologically, but all were negative for the typhoid bacillus.

SERVANTS.

Seventy-two, or about 13 per cent, of the 542 cases occurred at homes at which servants were employed. Of the 523 cases investigated in 1907, 71, or about 13 per cent, and of the 306 cases investigated in regard to servants in 1906, 69, or about 22 per cent, occurred at homes at which servants were employed.

The decided decrease for the last two years in the proportion of cases among persons of the servant-employing or wealthier class is interesting. Of the 72 cases occurring in 1908 at homes at which

servants were employed, 6 were at homes where white servants were employed and resided, 2 were at a home where two white servants were employed, one of whom resided at the house and the other went to his own home at night; one was at a house where two white and one colored servants were employed, one white servant residing at the house and the other white and the colored servant going to their own homes at night.

Nineteen were at homes where colored servants were employed and resided, 41 at homes where colored servants were employed but who went to their own homes at night, 3 at homes at each of which two colored servants were employed, one servant residing at the place and one going home at night.

Of the 86 servants employed at the homes of these 72 cases, the typhoid fever histories were as follows:

White resident servants who had had typhoid fever		0
White resident servants who had not had typhoid fever		8
White nonresident servants who had had typhoid fever		0
White nonresident servants who had not had typhoid fever		1
Colored resident servants who had had typhoid fever—		
Four years prior	1	
Five years prior	1	
Ten years prior	1	
Sixteen years prior	1	
		4
Colored resident servants who had not had typhoid fever		23
Colored resident servants' typhoid history not obtained		1
Colored nonresident servants who had had typhoid fever—		
Six years prior	2	
Ten years prior	1	
Forty-five years prior	1	
		4
Colored nonresident servants who had not had typhoid fever		37
Colored nonresident servants' typhoid history not obtained		8
Total		86
Cases at homes at which servants were employed		72
Cases at hotels, hospitals, institutions, etc		31

Thirty-one cases occurred at hospitals, hotels, or other public institutions at which there was a shifting servant population, so that accurate data regarding servants could not be obtained.

As in the 1907 period, there was no instance in which a history was obtained of the existence of typhoid fever cases at the homes of the servants during the three or four weeks prior to onset of the cases, nor was there any instance in which the information obtained indicated that the infection was conveyed by the servants to the members of the employers' households.

It appears from the data that servants play but little rôle in spreading the disease in Washington.

TWO OR MORE CASES IN SAME HOUSE.

There were 35 instances in which two or more cases developed in the same house and in these 35 houses there were 81 cases all told. The largest number of cases occurring in one house was 4, and there were 2 such instances.

Of these 81 cases, 39 were secondary to previous cases in the same households and were attributed to infection by direct contact, 17 were attributed to milk infection, and 25 to undetermined factors.

AVENUES OF INFECTION BY PERSONAL CONTACT.

Of the cases investigated in 1908 there were 17 which developed among persons living in buildings in which grocery stores were located, 1 in a building in which there was a bakery, and 1 in a building in which there was a saloon. The patients were cared for at these dwellings during a whole or a part of their illness, and in some instances a member of the family or other person who attended the patient was closely associated with the business. Furthermore, in some instances the precautions taken in the disposal of the stools, urine, etc., of the patient were decidedly inefficient.

The extent and freedom of association between persons of all walks of life in a large urban community makes it easy to understand how the infection from a given case of typhoid fever may be conveyed by devious and multiple ways to a number of persons widely separated socially and geographically. Many cases are no doubt due to direct or indirect personal contact which can not be traced.

Our studies of typhoid fever in Washington in 1908 have substantiated the view we held in 1907 that typhoid fever here is in large part a contact disease and that it should be treated as a quarantinable disease. It is infinitely easier to destroy the infection in the excreta of the patient than in the various foods and beverages, on the hands of persons, bodies of insects, etc., by which it may be disseminated after it leaves the bedside.

PROPHYLAXIS.

Of the total 665 cases investigated, 324, or 48.7 per cent, were treated at hospitals. One case a few days after onset of illness was taken for treatment to a place outside of the District of Columbia.

The time of admission to hospital of the cases considered as having contracted the infection in the District of Columbia was as follows:

	Cases.
Within 5 days after onset of illness	143
Within 10 days after onset of illness	74
Within 15 days after onset of illness	18
Within 20 days after onset of illness	3
Within 25 days after onset of illness	1
Total	239

Of the 103 cases considered as undoubtedly having contracted the infection outside of the District of Columbia, 73 were treated at hospital; and of the 20 cases considered as almost certainly having contracted the infection outside of the District of Columbia, 12 were treated at hospital. Of these 85 imported cases treated at hospital, 56 were brought from places out of the District of Columbia and sent directly to hospital, and 29 were sent to hospital after being treated at private residences in the District of Columbia, as follows:

At private residence:	Cases.
One day	3
Two days	7
Three days	4
Four days	2
Five days	3
Six days	4
Seven days	2
Eight days	2
Eighteen days	1
Nineteen days	1
Total	29

In 1908, as in 1907 and in 1906, the good proportion of cases cared for at hospitals was one of the most encouraging features met with in our investigation of the typhoid fever situation in Washington. The facilities for preventing the spread of infection from typhoid fever patients are much better at hospitals than they are in the vast majority of instances at private homes, and we believe in the light of the present knowledge of the subject that it would be entirely justifiable to make provision for and require all typhoid fever patients at whose homes the necessary prophylactic measures are not or can not be carried out to be sent to hospital for treatment.

Of the 340 cases cared for at private residences, 98 during a part or the whole of their illness were attended by professional nurses. The treatment of stools and urine with disinfectants was considered efficient for 152 cases, inefficient for 146, of doubtful efficiency for 38, and method not ascertained for 4.

Of the 146 patients whose stools and urine were inefficiently treated, no attempt was made at disinfection for 46; the treatment was inefficient on account of the small quantity of disinfectant used for 70; on account of the shortness of time of exposure of excreta to disinfectant for 30.

For 38 of the patients the treatment of stools and urine was considered of doubtful efficiency because the agent used for accomplishing the disinfection was of doubtful value. In some instances a little powdered air-slaked lime was sprinkled over the stools and disinfection expected. In more frequent instances some patented and widely advertised preparation was relied upon as a disinfectant, although such preparations are readily proved by laboratory experiments to

have practically no germicidal effect. We found that in some instances these patented and aesthetic preparations were recommended for use by the physician, or even by the professional nurse, in attendance on the patient.

Of other measures to prevent the spread of the infection, such as treatment of clothing, bedding, dishes, hands of attendants, etc., in contact with the patient, the precautions carried out were considered efficient for 90 cases, fairly efficient for 83, inefficient for 163, and not determined for 4.

The following table shows the prophylactic measures carried out for the patients treated at private residences during the periods of 1908, 1907, and 1906:

	Number of cases.		
	1908.	1907.	1906.
Treatment of stools and urine:			
Efficient	152	128	145
Inefficient	146	180	286
Of doubtful efficiency	38	48	51
Method not determined	4	3	10
Total	340	359	492
Other measures to prevent spread of infection:			
Efficient	90	79	.....
Fairly efficient	83	96	212
Inefficient	163	181	270
Not determined	4	3	10
Total	340	359	492

The findings, as shown by this table, make it evident that there is yet in the District of Columbia much that can and should be done to prevent the spread of infection from the patient's bedside, which is the fountain head of typhoid fever infection.

SUMMARY AND CONCLUSIONS.

Summary for 1908, with corresponding figures for 1907 and 1906.

	1908 (May 1 to November 1).		1907 (May 1 to November 1).		1906 (June 1 to November 1).	
	Cases.	Percentages.	Cases.	Percentages.	Cases.	Percentages.
Infection contracted out of District of Columbia	144	21.80	174	25.97	129	14.89
Infection attributed to milk	52	7.82	48	7.17	85	9.81
Infection attributed to contact	114	17.14	102	15.22	54	6.23
Accounted for	310	46.76	324	48.46	268	30.93
Unaccounted for	355	53.24	346	51.64	598	69.07
Total	665	100.00	670	100.00	866	100.00

Or, considering only the 542 cases, of which 438 undoubtedly and 104 probably contracted the infection in the District of Columbia, we have—

	1908 (May 1 to November 1).		1907 (May 1 to November 1).		1906 (June 1 to November 1).	
	Cases.	Percentages.	Cases.	Percentages.	Cases.	Percentages.
Infection attributed to milk	52	9.59	48	9.18	85	11.30
Infection attributed to contact	114	21.03	102	19.50	54	7.30
Accounted for	166	30.62	150	28.68	139	18.60
Unaccounted for	376	69.38	373	71.32	608	81.40
Total	542	100.00	523	100.00	747	100.00

Prevalence.—In 1907 and 1908 the typhoid fever rate in the District of Columbia was lower than that of any other year of which there is record. The reduced rate for both these years as compared with that of 1906 was accounted for by the reduction in the number of cases occurring in the summer periods.

Diagnosis.—Practically every case of clinical typhoid fever in Washington is reported to the health officer. Some cases not typhoid fever are reported as such. The official records, therefore, show more typhoid fever than exists in a clinical sense.

There should be official confirmation of the diagnosis of all cases of typhoid fever and of all cases suspected to be typhoid fever. This would greatly aid in the suppression of the disease.

Race.—During the warm season the disease prevails proportionately among white and colored. In the cold weather, however, the disease prevails proportionately more heavily among the whites.

Sex.—The disease is somewhat more prevalent among males, especially among those between the ages of 15 and 34 years.

Age.—The disease in 1908 again was especially prevalent among children, suggesting that milk and contact are responsible for more cases than can be definitely traced to these factors.

Endemicity.—The majority of cases occur among persons who have not been absent from Washington within the thirty days previous to onset of illness, and moreover among those who have lived in Washington all their lives, showing that Washington is a true "endemic" center.

Seasonal prevalence.—In 1906 and 1908 the typhoid season and the summer season were both early. In 1907 there were a cool spring and late summer, also a belated typhoid curve, showing strikingly the correlation between the beginning of summer weather and the increased prevalence of the disease.

In 1907 and 1908 the high typhoid fever rate continued until the cessation of warm weather; in 1906, however, there was a very marked decline in the typhoid fever rate eight weeks before the marked decline in the warm weather, indicating that in this year some factor or factors independent of warm weather *per se* operated to a relatively greater extent than in 1907 and 1908.

Geographical distribution.—During the three years there has been a general and fairly uniform distribution of cases throughout the city.

Sanitary conditions.—The majority of the cases occur among persons who live in houses of good or fairly good sanitary condition. In 1907 and 1908 the proportion of cases among persons living under the best sanitary conditions was much smaller than in 1906.

Sewerage system.—There is little difference in the prevalence of the disease in the sewered and in the nonsewered districts. There has been no especial grouping of cases, nor an excessive prevalence of the disease in the sections of the city in which a number of privies remain.

Flies.—In a well-sewered city, as Washington now is, flies would not be expected to play much of a part; and the lack of correlation between the seasonal curve of fly abundance and that of typhoid fever seems to corroborate this view.

Servants.—The majority of the cases occur in households without servants. There is no evidence to support the supposition that day servants frequently convey infection to the households of their employers.

Imported cases.—With the decreased prevalence of the disease in 1907 and 1908 the relative percentages of imported cases have increased, but the actual number has remained practically the same during the three years.

Contact.—Our studies again show that "contact" is one of the major factors in the spread of the disease.

Bacillus carriers.—The results of the examination of the excreta of about a thousand apparently healthy persons indicate that the typhoid bacillus is more commonly distributed among persons than the actual number of clinically recognized cases of the disease suggests.

Milk.—About 10 per cent of the cases in 1908, as in 1906 and 1907, were definitely attributed to infected milk. In the 1908 outbreak the milk was infected by a bacillus carrier. Our studies for the three years indicate that if all the market milk of Washington were pasteurized under official supervision the amount of typhoid fever here would be materially reduced.

Shellfish.—It is now evident that oysters and other shellfish do not play much part.

Water.—According to the accepted bacteriologic standards, the filtered Potomac river water during the typhoid seasons of 1907 and 1908 was of good sanitary quality and it does not seem probable that such water could have been directly responsible for much, if any, of the infection. There is not yet sufficient evidence, however, for a positive conclusion to be drawn as to just what part the Potomac river water has played in the causation of the disease in previous years.

Washington's "excessive" typhoid fever rate.—Considering the climatic and general sanitary conditions of Washington, the typhoid fever rate is still comparatively high for a city with no water-borne infection.

Prophylaxis.—The results of three years of study show that the disinfection of excreta of patients is frequently inefficient or neglected, and that there is a need of legal control of typhoid fever patients and typhoid bacillus carriers.

We are convinced that a vigorous campaign against typhoid fever as a "contagious" disease, and the adoption of measures that would prevent the spread of the infection in milk, would eliminate the greater part of typhoid fever from the District of Columbia.

LIST OF HYGIENIC LABORATORY BULLETINS OF THE PUBLIC HEALTH AND MARINE-HOSPITAL 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 formaldehyd 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 etheral 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.—Miscellaneous zoological papers. 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 echidninus*). 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.—Milk and its relation to the public health (revised edition). By various authors.

No. 50.—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. 51.—Further studies upon the phenomenon of anaphylaxis. By M. J. Rosenau and John F. Anderson.

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

In citing these bulletins, beginning with No. 8, bibliographers and authors are requested to adopt the following abbreviations: Bull. No. ——, Hyg. Lab., U. S. Pub. Health & Mar.-Hosp. Serv., Wash., pp. ——.

MAILING LIST.

The Service will enter into exchange of publications with medical and scientific organizations, societies, laboratories, journals, and authors. ALL APPLICATIONS FOR THESE PUBLICATIONS SHOULD BE ADDRESSED TO THE "SURGEON-GENERAL, U. S. PUBLIC HEALTH AND MARINE-HOSPITAL SERVICE, WASHINGTON, D. C.," EXCEPT THOSE MARKED (*).

The editions of the publications marked (*) available for distribution by the Surgeon-General of the Public Health and Marine-Hospital Service have been exhausted. Copies 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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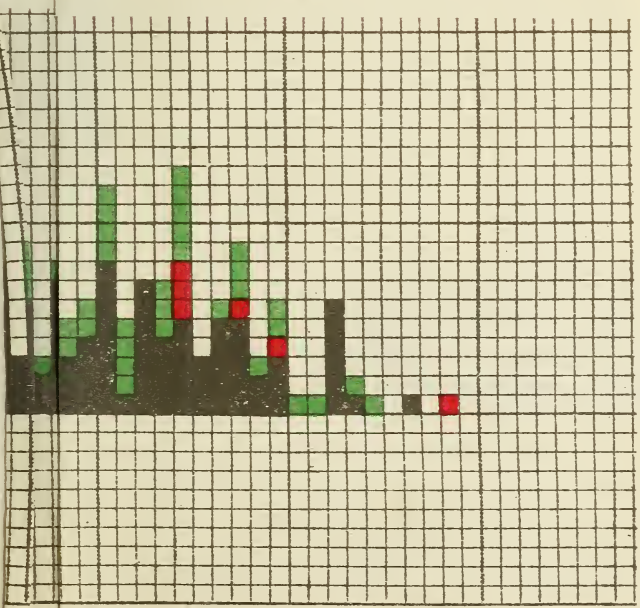

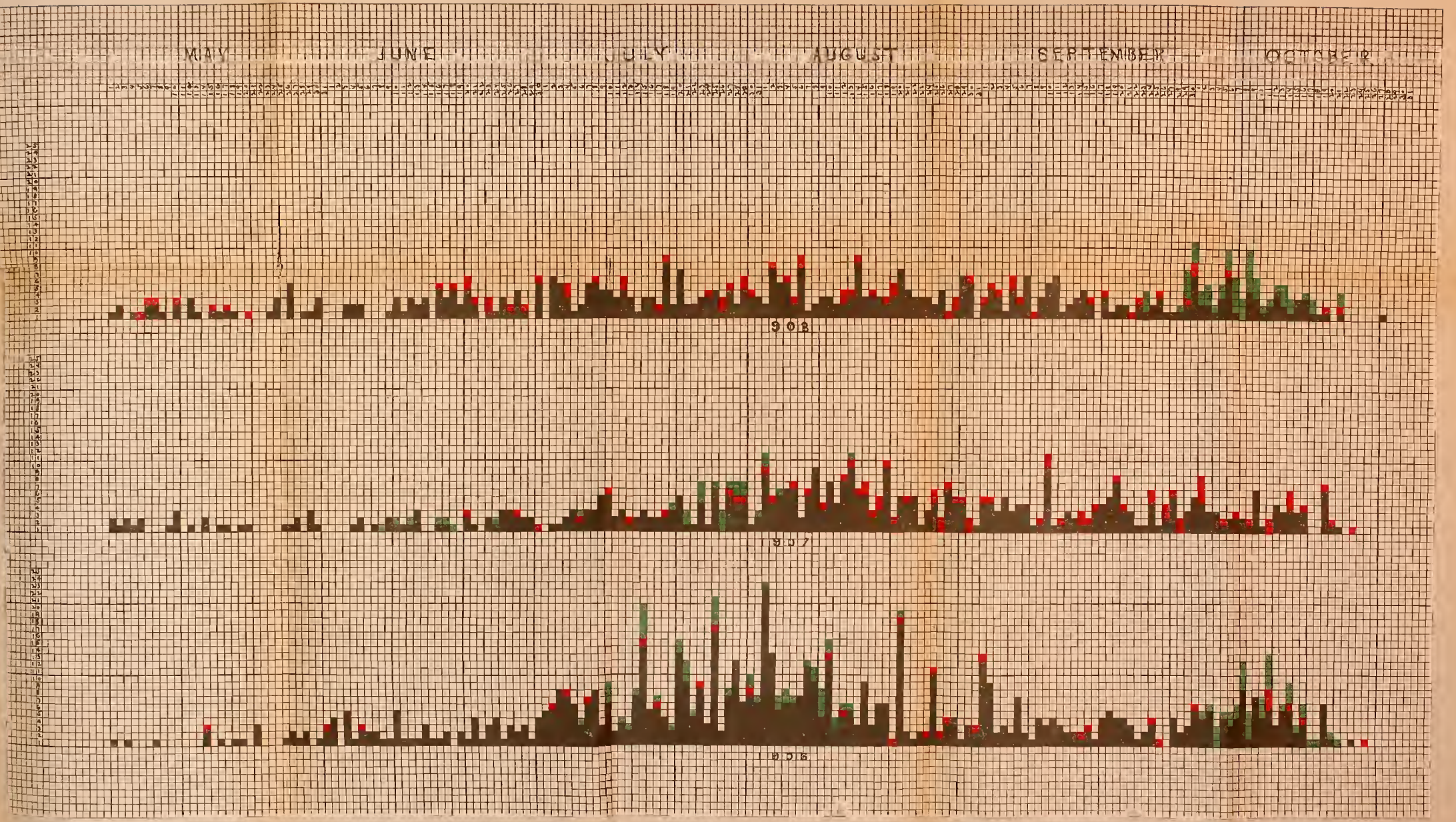

Chart No. 2.—SHOWING CASES OF TYPHOID FEVER IN THE DISTRICT OF COLUMBIA, BY DATE OF DEFINITE ONSET AND ACCORDING TO ATTRIBUTED CAUSATION.

BULLETIN 52, HYGIENIC LABORATORY, P. H. & M. H. S.

■ = Cases due to infection in milk.
■ = Cases due to direct contact with previous cases in the febrile stage of the disease.
■ = Cases not definitely accounted for.

OCT. 21
1100
1050
1000
950
900
850
800
750
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650
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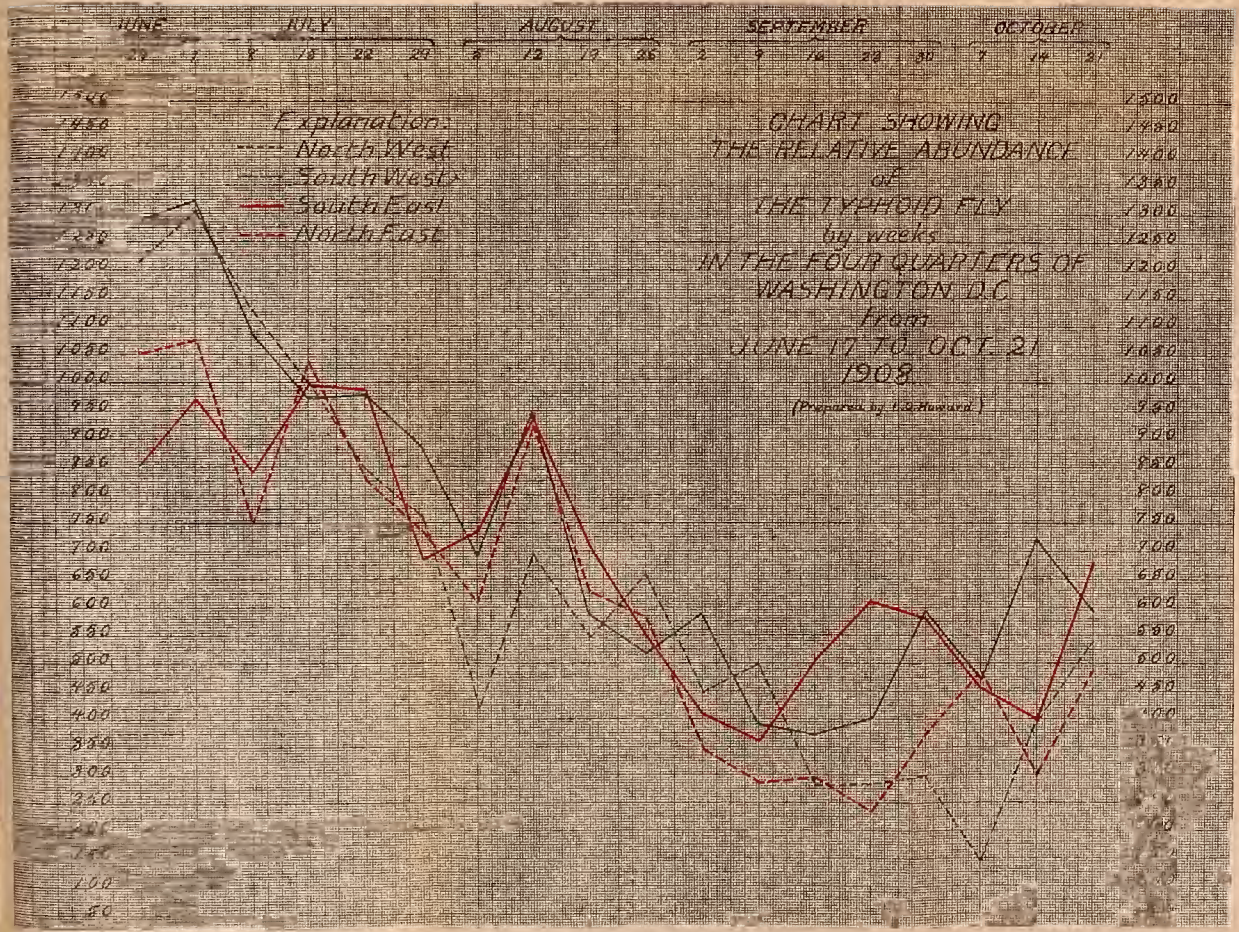
500
450
400
350
300
250
200
150
100
50

CHART No. 5.


JUNE
JULY
AUGUST
SEPTEMBER
OCTOBER
Explanation:
North West
South West
South East
North East
CHART SHOWING
THE RELATIVE ABUNDANCE
of
THE TYPHOID FLY
by weeks
IN THE FOUR QUARTERS OF
WASHINGTON D.C.
from
JUNE 17 TO OCT. 21
1908.
(Prepared by W.D. Howard)

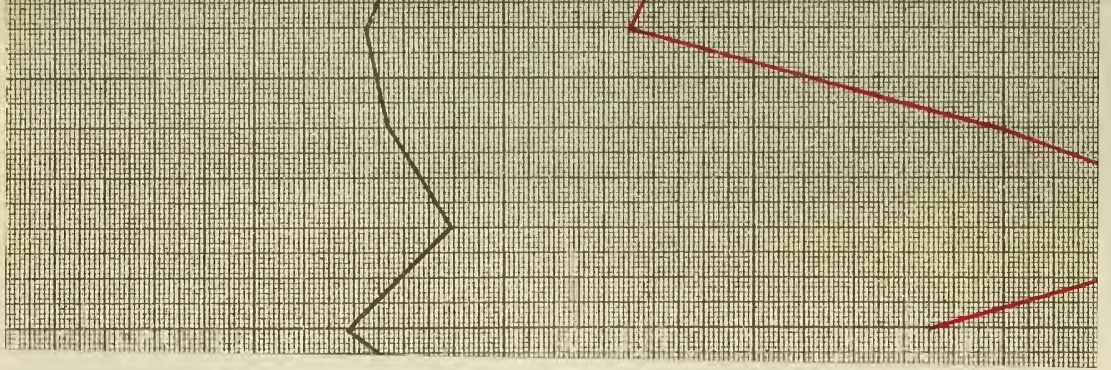
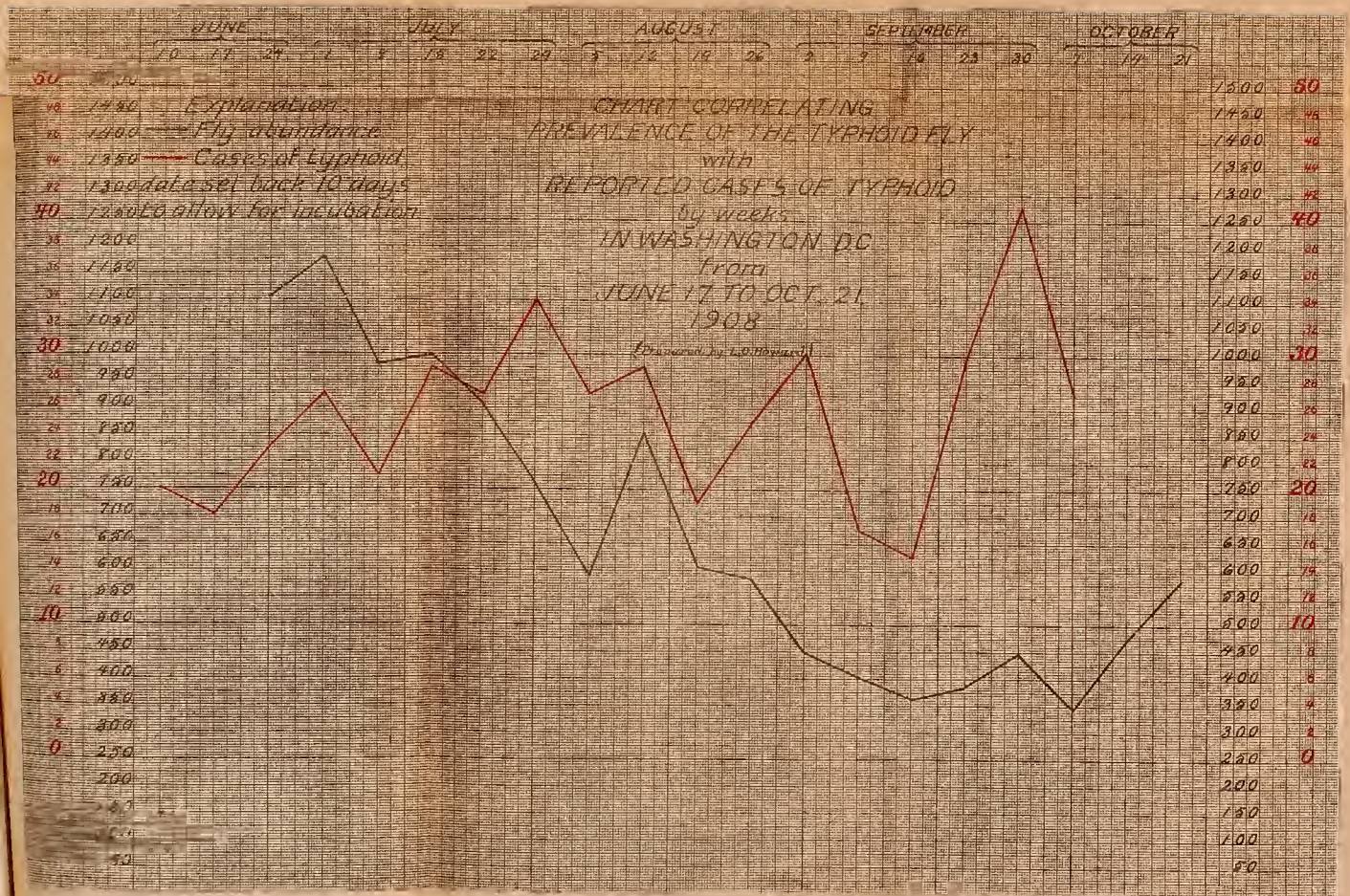


CHART No. 6.


JUNE
JULY
AUGUST
SEPTEMBER
OCTOBER
17 24 1 8 15 22 29 5 12 19 26 2 9 16 23 30 7 14 21
Explanation
Fly abundance
Cases of Typhoid
date set back 10 days
to allow for incubation
CHART CORRELATING
PREVALENCE OF THE TYPHOID FLY
with
REPORTED CASES OF TYPHOID
by weeks
IN WASHINGTON, D.C.
from
JUNE 17 TO OCT. 21
1908
Prepared by L.O. Howard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TREASURY DEPARTMENT
Public Health and Marine-Hospital Service of the United States

HYGIENIC LABORATORY.—BULLETIN No. 53

SEPTEMBER, 1909

THE INFLUENCE OF CERTAIN DRUGS UPON THE TOXICITY OF ACETANILIDE AND ANTIPYRINE

By

WORTH HALE

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THE INFLUENCE OF CERTAIN DRUGS UPON THE TOXICITY OF ACETANILIDE AND ANTIPYRINE.[a b]

By Worth Hale,

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Summary.—The results of the experiments which are recorded in this bulletin indicate that the deleterious effect of acetanilide upon the heart is very imperfectly antagonized by caffeine. They show that so far as the contractile power of the heart is concerned the antagonism is very weak or even not present at all, and in some cases that the two drugs seem to combine to depress the heart to a greater degree than acetanilide does when given alone. The heart rate, on the other hand, is not slowed after a mixture of acetanilide and caffeine are given, as is the case when acetanilide is given alone, and the decreased heart rate following the exhibition of the former alone tends to become normal upon the subsequent injection of caffeine. Caffeine is further shown to increase the toxicity of acetanilide mixtures when given to the intact animal, and in certain experiments this is not only a summation effect but even some synergistic action is to be observed.

Sodium bicarbonate, quite in contrast, appears to markedly lessen the poisonous effects of acetanilide upon the heart, which is shown to be less depressed than when the alkali is not given. The lessened toxicity also appears in the experiments upon the intact animal, in which case acetanilide when given alone proved to be far more toxic than in mixtures with the alkalies.

The combinations of the alkaloids of the morphine group also increase the toxic effects of acetanilide, while mixtures containing salicylic acid and the bromides seem not to alter its poisonous effects in any way.

[a] Submitted for publication April 29, 1909.

[b] The experiments recorded in this bulletin were suggested by some preliminary experiments carried out by Doctor Hunt, in February, 1908, his results indicating that caffeine increased the toxicity of both acetanilide and antipyrine very materially.

These experiments indicate, therefore, that caffeine increases the danger of acetanilide mixtures, as do also the opium alkaloids. On the other hand, in these experiments upon the lower animals sodium bicarbonate appears to be a fairly good antagonist and would possibly be of use in acetanilide poisoning in man.

In Part II it is shown that caffeine is not materially antagonistic to the circulatory depression following antipyrine, but that it prevents the slowing in the heart rate. In the experiments upon the intact animal the mixtures of the above drugs were invariably more poisonous than antipyrine alone. In contrast, and as in the acetanilide experiments, sodium bicarbonate was somewhat antagonistic to the heart effect of antipyrine, but when given to the intact animal it did not seem to lessen the toxicity of the antipyrine in any degree.

HISTORICAL.

The year 1884 has become notable in the history of therapeutics on account of the introduction of antipyrine, the first of a large series of drugs as agents for the reduction of excessive temperature. This was followed very shortly (in 1886) by the second member of the antipyretic group, acetanilide, and in quick succession a large number of similar bodies appeared, all of which possessed in a general way the same action. The powerful influence of these drugs upon high temperature made them all extremely popular, and especially so since up to this time the other drugs used in the relief of fever were not only uncertain in their action but were very much less powerful.

Although having been preceded by antipyrine by two years, acetanilide quickly outranked it in popularity, and it has never been supplanted to any great extent by any of the large number of antipyretics appearing later. Many cases of poisoning appeared almost from the first, due not only to the enormous doses that were prescribed, but also to the inherent poisonous properties of these drugs. This, however, did not seem to detract from its popularity, which seems to have been based partly upon the idea that it was less poisonous than antipyrine,[a] and certainly less poisonous than other drugs of the series excepting acetphenetidin and partly upon the fact that it was somewhat more efficient,[b] at least in the doses used, and thought to be necessary. The chief reason for its greater popularity, however, seems to have been neither its comparative smaller degree of toxicity nor its greater efficiency, but its comparative cheapness.[c]

The immediate popularity of this group of compounds arose from their decided antipyretic action, but it was quickly observed that they were also very efficacious in relieving various more or less

[a] Barr, Pharm. Jour. and Tr., Lond., 1887, XVIII, 170.
[b] Faust, Deutsch. med. Wchnschr., 1887, XIII, 575.
[c] Hinzelmann, Munch. med. Wchnschr., 1887, XXXIV, 36.

obscure pains, generally neuralgic in character, as facial neuralgia, hemicrania,[a] and the lancinating pains of tabes dorsalis. At the present time enormously large amounts are used for the relief of symptoms of this sort, and comparatively very little as a means of reducing fever.

Drugs to relieve pain have always been especially popular with the general public, who prescribe for themselves all sorts of preparations with absolutely no idea of their poisonous properties. Hence the legitimate use of the antipyretics quickly was made subservient to an indiscriminate use, especially in the treatment of headache, so that Siefert,[b] as early as 1888, pointed out the great danger of allowing the apothecaries to dispense these preparations directly to the general public. Despite this early recognition of the danger of their promiscuous use in this class of disorders their use has become almost universal, and at the present time they are dispensed directly to the laity over the counters of every drug store and at almost every soda fountain with no warning as to their danger and with meager directions as to dosage.

The early history of this popular use of the antipyretics, especially of acetanilide, is closely connected with their exploitation in proprietary remedies. Appearing as one of the first, if not the first of these, were the notorious "Antikamnia" preparations. The promoters of these products claimed to have discovered a new and wonderful member of the antipyretic series which was far more efficacious than those in common use and without their deleterious effects. These claims were such that chemists both in this country and abroad became interested and a large number of analyses were made. These showed that instead of a new and harmless remedy Antikamnia was really a mixture which sometimes contained one thing, sometimes another, but always the already well-known aniline compound, acetanilide. Among the first analyses was that of Hall,[c] who found 77.5 per cent acetanilide and 19.3 per cent sodium bicarbonate. In the same year Goldman[d] reported acetanilide 70 per cent, sodium carbonate 20 per cent, and caffeine 10 per cent.

There seems to be no literature bearing directly upon the exhibition of caffeine and the alkaline carbonates with the antipyretics and it will probably never be known just why caffeine was introduced into the general type acetanilide prescription. Two reasons may be suggested, however. It had been known for a very long time that caf-

[a] Chomjäkow u. Ljwow, Wratsch., 1885, VI, 887. White, N. Y. Med. Rec., 1886, XXX, 293. Ungar, Centralbl. f. klin. Med., 1886, VII, 777. Secretan, Revue med. de la Suisse rom., 1887, VII, 29.

[b] Siefert, Munch. med. Wchnschr., 1888, XXXV, 850, 867.

[c] Hall, Druggists Circular, 1891, XXXV, 99.

[d] Goldman, Pharm. Ztg., 1891, XXXVI, 255.

feine in itself was useful in certain forms of headache, and it appeared in several formulæ combined with the bromides a number of years before the introduction of antipyrine and acetanilide. The natural inference, therefore, is that it was a direct transfer of caffeine from the old to the new type of headache remedies. The other explanation of its presence is to be found in the literature relating to the treatment of cases of acetanilide poisoning. Lepine [a] seems to have first suggested caffeine as an antidote, having reported that the cyanosis of acetanilide poisoning disappeared after large doses of this drug. In 1889 Mahnert [b] suggested that the excitants be used, and in treating three cases made use of ether injections, wine, and powdered caffeine, the latter being especially recommended by him. Hartge [c] treated a case of poisoning with coffee and brandy and later with camphor and ether injections. Falk [d] used caffeine, but thought that alcohol was distinctly contraindicated, owing to the increased solubility of acetanilide in this menstruum and therefore its more rapid absorption. Such a course of treatment for acetanilide poisoning might easily have suggested the addition of one of the above drugs to acetanilide mixtures with the idea that poisoning would be prevented. Whether caffeine was introduced into them as an active agent in the cure of headache or merely to give an additional safety to a drug with known poisonous properties it is of course impossible to say, and both factors may have played some part. At any rate caffeine thus introduced has been almost invariably a constituent of all prescriptions or proprietary formulæ containing acetanilide.

The generally prevailing idea at the present time is that caffeine is added to prevent the deleterious effects of the coal-tar drugs upon the heart,[e] although this does not seem to have been the original reason for its administration. No direct observations concerning its antidotal value seem to have been made, but on purely theoretical grounds it would apparently be useful as a stimulant to the respiratory center, which becomes markedly embarassed from the formation of methæmoglobin and to a lesser degree to the heart. It does not seem probable that it would have any special influence upon the cyanosis unless indirectly through increased respiratory activity.

The combination of alkali carbonates with acetanilide also became popular about 1890, but the reason for their presence in acetanilide

[a] Lepine, Rev. de med. Par., 1887, VII, 531.

[b] Mahnert, Memorabilien, Heilbr., 1889, XXXIV, 321.

[c] Hartge, St. Petersb. med. Wchnschr., 1890, VII, 69.

[d] Falk, Therap. Monatsh., 1890, IV, 257.

[e] McFarline, Canad. Pharm. Jour., Toronto, 1906, XXXIX, 360, says in speaking of headache powders: "It will be noted that in most cases the depressant effect upon the heart is sought to be counteracted by the addition of caffeine, bicarbonate of soda, or other drugs of like character."

mixtures is largely a matter of conjecture. Herczel[a] in 1887 carried out some experiments upon dogs and was able to show that the exhibition of acetanilide definitely decreased the alkalinity of the blood. Although all methods for determining the alkalinity of the blood are unreliable this fact would afford an experimental basis for their presence, but the small amount present in the usual type of acetanilide mixture would probably be insufficient to make the blood materially less acid.

In this connection it is important to remember that acetanilide causes the formation of methæmaglobin in the blood and that this may be hastened by a decrease in the alkalinity of the blood has been pointed out by Kobert[b] who states that the alkalies prevent the breaking up of the blood cells and the formation of methæmaglobin. The exhibition of alkalies, according to Kobert, also serves to aid the regeneration of the oxyhæmaglobin, an alkali-methæmaglobin being formed as an intermediary product which changes the blood from a chocolate brown to a red and from this oxyhæmaglobin is formed.

Another reason for the combination of alkalies with acetanilide has to do with increasing the solubility of the drug, the idea formerly prevailing that acetanilide was made more soluble and hence more easily absorbed when thus prescribed. In 1891 Hall[c] gave as a reason for the greater activity that had been claimed for antikamnia its finely divided state and the presence of sodium bicarbonate, which he said made it more soluble. The idea of greater solubility when given with alkalies seems to have been held as late as 1906 for Ritter,[d] in commenting on the introduction of a compound acetanilide powder into the eighth decennial revision of the U. S. Pharmacopœia, wrote that it mattered very little whether the alkaline salt be a carbonate of ammonium or sodium or a bicarbonate, as it was only added to increase the solubility of the acetanilide. Puckner[e] could find no experimental basis for this, however, and was able to show in direct contradiction that no increased solubility occurred in alkaline solutions. Acids, on the other hand, especially strong solutions, increased the solubility to some extent, and he concluded therefore that acetanilide is probably even less soluble when taken with an alkalie because of the partial neutralization of the acid gastric contents. A further reason for the presence of alkalies in mixtures with the antipyretics, more particularly antipyrine, is found in the occasional gastric irritation that results from their administration,

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[a] Herczel, Wien. med. Wchnschr., 1887 XXXVII, 1022.
[b] Kobert, Lehrbuch der Intoxikationen, 1902, 73–74.
[c] Hall, Druggist Circular, 1891, XXXV, 99.
[d] Ritter, Jour. Am. Med. Ass., 1906, XLVII, 683.
[e] Puckner, Ibid., 1206.

both sodium bicarbonate and seltzer water having been suggested as a means of lessening these disagreeable symptoms.[a] Looked at from a purely pharmaceutical standpoint the presence of carbonates in such mixtures, when dispensed in tablet form, would aid in the disintegration of the tablet because of the chemical action of the acids of the gastric juice.

No branch in the manufacture of proprietary medicine has offered such inducements for the introduction of special formulæ or special nomenclature as has that dealing with the preparation of headache remedies. And almost invariably this has meant acetanilide mixtures. The universal use of such drugs in the relief of such a common symptom has led to multiplication and remultiplication of the different preparations until they are numbered by the hundreds. These have in a general way followed the general type of formula as illustrated by antikamnia, containing acetanilide as a basis and occasionally antipyrine or acetphenetidin, although the cheapness of the former drug made it by far the most popular with the manufacturers. The other ingredients of these mixtures have usually included caffeine and an alkaline carbonate and less often the salicylates, the bromides, morphine, and codeine.

The fact that many of these preparations were advertised and sold to the physician on the one hand and directly to a drug-addicted public on the other, that they and similar proprietaries were often frauduently advertised as panaceas of unusual and wonderful virtue, and finally that their composition was shown to be notoriously variable, was sufficient to arouse a sentiment against all such preparations. In line with this, in the last revision of the United States Pharmacopœia, certain formulæ were introduced, the purpose of which was to give the physician official preparations to take the place of the many similar ones which he had previously been prescribing. This accounts for a number of formulæ which are now pharmacopœial, and especially for Pulvis Acetanilide Compositus. Although there may be some reasons for criticising this step, it certainly was desirable that the physician should be able to order an acetanilide mixture the composition of which was known and which contained a definite and constant proportion of the several ingredients.

It is the purpose of this investigation to determine through experiments upon animals to what extent the presence of such a combination of drugs is justifiable upon the basis of a lessened or altered toxicity of the contained acetanilide; also to determine to what extent the toxicity of other coal-tar combinations is altered by the addition of the various other drugs most frequently found with them in the various formulæ.

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[a] Am. Jour. Pharmacy, 1888, XVIII, 180.

DETERMINATION OF HEART ACTION.

The popular belief in the value of caffeine in preventing the poisonous heart effects of the coal-tar products suggested the experimental determination of any modifications in the action upon this organ which might occur when it was exhibited in combination with acetanilide. Experiments were carried out upon both warm and cold blooded animals, using the myocardiograph method to record the changes in the dog's heart, the perfusion method in estimating the changes in the frog's heart.

Action upon the frog's heart.—The perfusion method was adopted as being most suitable for determining the changes occurring in the frog's heart after acetanilide and combinations of this drug with caffeine citrate or an alkaline carbonate. This method was believed to be especially suitable for this purpose on account of the relative insolubility of acetanilide in an aqueous solvent, since comparatively small amounts are sufficient to produce profound changes in the isolated heart. It is also of advantage because by its use secondary and extraneous effects are excluded, any action being limited to the intrinsic nerves of the heart or to the cardiac muscle substance.

In all cases frogs of the same variety (Rana pipiens) captured at the same time and kept under the same conditions were used. As far as possible those of the same weight were chosen and this was always done when comparisons between the relative effects produced on several frogs were to be noted. In most instances each heart was made its own control, so that these precautions were usually unnecessary. The frogs were pithed (both brain and cord) and the heart exposed in the usual manner. After removal of the pericardial sac the right branch of the aortic arch was dissected out and ligated, and a cannula inserted into the left branch as far from the heart as possible. By gentle traction upward the heart may be separated from the œsophagus and other tissues, best accomplished by running a blunt dissecting needle between these structures and the sinus venosus. If the animal is small it is probably easier to insert the inflow cannula into the posterior vena cava by turning the heart over with the base downward (frogs weighing 10 grams have been successfully employed using this procedure). If the animal is large, 25 to 50 grams, the venous cannula is usually inserted with the heart lying in the normal position.

Two Mariotte bottles of about 150 c. c. capacity were used to hold the perfusing fluid. They were mounted on a stand and into the upper opening of each bottle a glass tube reaching to within one-half c. m. of the bottom was inserted through a tightly fitting stopper in order to allow ingress of air and thus preserve a constant pressure no matter what the level of the fluids in the bottles might be. The outlets of the two bottles were connected through a Y tube held

immovably by a clamp. Ringer's solution, (sodium chloride 0.7, potassium chloride 0.03, and calcium chloride 0.026 per cent), was used as a perfusing fluid. The venous cannula was connected with the outlet of the Y tube, while the aortic cannula was supported in such a manner that the perfused fluid would flow back over the heart, thus keeping it moist and in good condition. The heart with certain exceptions as noted below was started by perfusing it with Ringer's solution at a temperature of 20 to 22° and the rate and output per five-minute periods were recorded. When a normal had been ascertained acetanilide in varying per cent in Ringer's perfusing fluid was substituted, and after the effect of this solution had been determined this was replaced by acetanilide of the same strength, but to which had been added either caffeine citrate or sodium bicarbonate and the output and rate again recorded as before.

In estimating the effect of acetanilide alone and in combination with other drugs, it was first planned to use a sufficient concentration to poison the heart to such an extent that it would stop beating after twenty or thirty minutes. The drug in large doses being of itself a depressant of the heart, it was thought that the rate and output would both grow gradually less and less, and that finally the heart would stop beating. Such a course of progressive poisoning, however, does not seem to follow. After perfusing with strong solutions (one-half to one-fifth per cent) the heart is found to cease beating almost instantly; slightly weaker solutions (one-sixth to one-tenth per cent) would stop the heart in some cases either at once or after a few minutes, or again would not stop it although perfused through it for an hour or more. Protocols illustrating this action of the drug are given in Table I.

Table I.—*Perfusion of the isolated frog's heart with Ringer's solution and acetanilide.*

Protocol 20, October 12, 1908.			Protocol 25, October 13, 1908.		
Time.	Rate.	Output per 5 minutes.	Time.	Rate.	Output per 5 minutes.
		C. c.			*C. c.*
9.50	26	.....	2.00	28	.....
9.55	26	21	2.05	29	16
10.00	25	25	Acetanilide, ½ per cent.		
Acetanilide, ½ per cent.			2.10	12	5
10.02	13	.....	2.14	0	.....
10.05	13	9			
10.10	10	7			
10.15	10	7			
10.20	9	7			
10.30	11	8			
10.40	12	9			
10.50	12	10			
11.10	14	.....			

In other respects the effect on the rate is very regular. Almost immediately after the introduction of the drug the rate decreases to

about half, although in some cases the slowing is more progressive and drops by degrees to a certain point or in certain cases rapidly to zero. The rate in both auricle and ventricle usually remains the same until late in the poisoning, when the auricles are observed to be less affected than the ventricle and continue to beat for some time after the latter has ceased to contract.

Another rather peculiar feature of the poisoning is that after the heart has been exposed to the action of the drug for some time the heart muscle seems to gain some tolerance for the poison, for although never regaining its normal rhythm, the toxic effect appears to become less and often the rate and output are secondarily augmented. The same thing is shown in other instances by the stoppage of the heart following the first introduction of the drug. After a short time it is allowed to regain its original rhythm by perfusing it with Ringer's solution, whereupon a second introduction of the drug, although slowing the rate, shows no tendency to check it entirely. Cahn and Hepp [a] in perfusing the hearts of frogs (species not recorded) reported some slowing but no change in the energy, but their work may be questioned because of an insufficient range in the per cent of acetanilide used. Weil [b] noted a primary increase in both rate and energy, but this was followed by a secondary decrease in both. Lepine [c] reported that acetanilide increased the energy but decreased the heart rate. My own observation was that the energy was sometimes considerably augmented, but this effect was always of very short duration. Less often there appeared also a momentary increase in heart rate, but in the case of all my experiments this was quickly followed by a very marked slowing.

The next problem was to determine what strength of caffeine citrate could be used to the greatest advantage in perfusing the heart of Rana pipiens. It is well known that rigor is produced in the skeletal muscles and the heart of frogs, although the latter is not so readily affected, and small doses cause definite stimulation, both rate and output being increased. It is obvious, of course, that an amount of the drug should be used too small to produce rigor and yet sufficiently large to cause stimulation. By experiment this was found to be between one-fifteen hundredth per cent and one-five thousandth per cent. Below one-fifteen hundredth per cent the heart rate was most often slowed, and even at this dilution the output was very materially lessened. In stronger solutions both rate and output rapidly fell to zero. Examples of the effects produced are shown in Table II.

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[a] Cahn and Hepp, Berl. klin. Wchnschr., 1887, XXIV, 27.

[b] Weil, Thesis, Paris. De l'Acetanilide, 1887, 47 pp.

[c] Lepine, Revue de med., Par., 1887, VII, 310.

TABLE II.—*Perfusion of the isolated frog's heart with caffeine citrate in Ringer's solution.*

Protocol 13, October 7, 1908.			Protocol 14, October 8, 1908.		
Time.	Rate.	Output per 5 minutes.	Time.	Rate.	Output per 5 minutes.
		C. c.			*C. c.*
11.05	24	.....	10.10	27	.....
11.10	24	18	10.15	27	19
Caffeine citrate $\frac{1}{500}$ per cent.			10.20	28	21
11.11	26	.....	10.25	27	20
11.15	20	10	10.30	27	20
11.18	0	.....	Caffeine citrate $\frac{1}{2000}$ per cent.		
11.20	.....	4	10.32	29	.....
			10.35	30	22
			10.40	27	23
			10.45	28	21
			11.05	28	.....

Having thus determined the manner in which the frog's heart reacts when perfused with definite percentages of caffeine or acetanilide, a third series of experiments were carried out to determine to what extent and in what manner the action of acetanilide in solutions of various strengths could be modified by the addition of such amounts of caffeine as had been shown to possess a definite stimulant action. Two methods of determining these effects were employed, one based upon the differences in the time necessary to stop the heart, the other upon changes in rate and output. It had been shown by a number of experiments, using one-seventh of one per cent solutions of acetanilide alone, that the heart was stopped by the poisonous action of the drug at the following intervals: 37, 17, 9, and 30 minutes, or on the average 23.1 minutes after the perfusion of the drug was begun. Accordingly one-seventh per cent acetanilide solutions to which one two-thousandth per cent caffeine citrate had been added were perfused at the same temperature through another series using frogs of approximately the same weight. The intervals between the introduction of the combined drugs and the stoppage of the heart were as follows: 25, 3, 15, and 8 minutes, or on the average 12.7 minutes after the drug was started. In both series marked irregularities will be noted. This is probably in great part due to the irregularity in the course of acetanilide poisoning and because of the great differences in the same series it becomes impossible to draw absolute conclusions. The results as they stand, however, indicate considerably more toxicity to the heart from the drugs when exhibited in combination than from acetanilide alone.

The second method of experimenting consisted in poisoning the heart with smaller quantities of acetanilide with the idea of keeping it beating for some time and then noting the changes in rate and output upon the substitution of the acetanilide caffeine combination. These experiments are also somewhat disappointing because of their

failure to show striking modifications in action. It seems possible, however, in going carefully over the results obtained in this manner to point out certain definite changes. In five experiments there was clearly a lessened toxicity as a result of using the drugs in combination. In fourteen experiments the results were exactly the opposite. The rate was lessened in five and in the others there was no change, but in all cases there was a decrease in the output over that produced by acetanilide alone. Two protocols illustrating this increased toxicity are given in Table III.

TABLE III.—*Perfusion of the isolated frog's heart with acetanilide, and with acetanilide and caffeine citrate in combination.*

Protocol 17, October 10, 1908.			Protocol 40, October 23, 1908.		
Time.	Rate.	Output per 5 minutes.	Time.	Rate.	Output per 5 minutes.
		C. c.			*C. c.*
9.50	29	.....	9.20	28	.....
9.55	32	20	9.25	36	23
10.00	32	25	9.30	35	22
10.05	33	27	Acetanilide $\frac{1}{8}$ per cent + caffeine citrate $\frac{1}{30000}$ per cent.		
Acetanilide $\frac{1}{10}$ per cent.			9.31	0	.....
10.07	19	.....	9.32	Ringer's solution.	
10.10	17	25	9.35	26	10
10.15	15	23	9.40	33	19
Acetanilide $\frac{1}{10}$ per cent + $\frac{1}{2000}$ per cent caffeine citrate.			Acetanilide $\frac{1}{8}$ per cent.		
10.17	13	.....	9.42	19	.....
10.20	14	26	9.45	13	8
10.25	11	27	Acetanilide $\frac{1}{8}$ per cent + caffeine citrate $\frac{1}{30000}$ per cent.		
10.30	11	25	9.46	0	.....
Acetanilide $\frac{1}{10}$ per cent.					
10.32	13	.....			

It must be admitted that the changes are not very definite, but as in the results obtained by the other method there is some indication of increased toxicity from the combination. The results at any rate are confirmatory of each other. It may be very clearly stated too that there is no lessened toxicity when using the drugs in the above strengths and this again substantiates the results shown by the first series of experiments.

In another series of experiments, carried out to determine changes in rate and output, smaller amounts of the drugs were used. In these such small amounts of the drugs were perfused that the perfusion was begun with solutions of acetanilide in Ringer's solution. The second perfusion bottle in addition to the acetanilide contained either caffeine citrate or sodium bicarbonate. After a series of readings using acetanilide had been taken, the combined drugs were perfused and the resulting changes in heart rate and output were noted. Acetanilide was used in one-fifteenth per cent strength and caffeine citrate in one three-thousandth per cent, and with few exceptions the

results in this series indicated that caffeine if sufficiently dilute would antagonize, to some extent at least, the poisonous effects of acetanilide. There were some experiments which still showed greater toxicity from the combination of drugs than from acetanilide alone, but these were only occasional. However, they were sufficiently frequent to illustrate very well how incomplete the antagonism between these drugs really is.

Protocols showing the results of this series of experiments are given in the following table:

TABLE IV.—*Effect upon the isolated frog's heart of an acetanilide Ringer's solution and a similar solution to which caffeine citrate had been added.*

Protocol 53.			Protocol 50.		
Time.	Rate.	Output per 5 minutes,	Time.	Rate.	Output per 5 minutes,
		C. c.			C. c.
Acetanilide $\frac{1}{15}$ per cent.			Acetanilide $\frac{1}{15}$ per cent.		
9.35	22	----------	11.00	20	----------
9.40	20	54	11.10	15	21
9.45	20	52	11.15	17	20
9.50	21	50	11.20	17	20
9.55	22	53	11.25	16	19
10.00	22	56	Acetanilide $\frac{1}{15}$ per cent+caffeine citrate $\frac{1}{3000}$ per cent.		
Acetanilide $\frac{1}{15}$ per cent+caffeine citrate $\frac{1}{3000}$ per cent.			11.27	16	----------
10.02	27	----------	11.30	16	18
10.05	26	58	11.35	14	17
10.10	27	56	11.40	14	15
10.15	27	57	11.45	14	14
10.20	25	54	Acetanilide $\frac{1}{15}$ per cent.		
Acetanilide $\frac{1}{15}$ per cent.			11.47	13	----------
10.22	23	----------	11.50	12	13
10.25	23	58	12.00	12	10
10.35	22	55			

To determine to what extent the presence of alkaline carbonates in the perfusion fluid would modify or prevent the toxic effects of acetanilide was then made the subject of a series of experiments. In this work no effort was made to determine the length of time necessary to produce stoppage of the heart, but as in the later caffeine-acetanilide experiments the comparison was made by noting the changes in rate and output. Acetanilide was used in only one-eighth per cent solution, but the amount of sodium bicarbonate and ammonium carbonate was varied from one three-hundredth to one-twentieth per cent. As far as could be determined there seemed to be no difference in the antagonistic value whether the base were sodium or ammonium. In all cases there proved to be a considerable degree of antagonism, but this was not at all sufficient to abolish or even prevent quite marked slowing and weakening from the toxic action of the acetanilide. The degree to which sodium bicarbonate is antidotal to acetanilide when perfused through the frog's heart is shown by the protocols given in Table V.

TABLE V.—*Perfusion of the isolated frog's heart with acetanilide and with acetanilide and sodium bicarbonate in Ringer's solution.*

Protocol 35, October 15, 1908.			Protocol 38, October 16, 1908.		
Time.	Rate.	Output per 5 minutes.	Time.	Rate.	Output per 5 minutes.
		C. c.			
3.20....	34	.....	1.55.....	42	
3.25....	46	27	2.00.....	38	
3.30....	40	30	2.05.....	33	
Acetanilide $\frac{1}{8}$ per cent+ $\frac{1}{100}$ $NaHCO_3$.			Acetanilide $\frac{1}{8}$ per cent+$\frac{1}{200}$ per cent $NaHCO_3$.		
3.32....	32	.....			
3.35....	18	19	2.07.....	21	Not recorded.
3.40....	17	15	2.10.....	22	
3.45....	17	12	2.20.....	22	
3.50....	17	13	2.28.....	21	
Acetanilide $\frac{1}{8}$ per cent.			Acetanilide $\frac{1}{8}$ per cent.		
3.52....	14	.....			
3.55....	10	11	2.30.....	16	
4.00....	7	3	2.35.....	14	
4.03....	0	.....			

Action on the dog's heart.—The effect of acetanilide upon the mammalian heart is considered such an important factor in the poisoning in man that the results of the control experiments done in determining the nature of the effect of acetanilide caffeine combinations will be described in detail. Evans,[a] in describing the effect of acetanilide upon the circulation, states that it caused a rise of pressure and a slight acceleration in the heart rate when given to rabbits in doses of 15 to 75 milligrams. Lepine[b] reported in experiments on dogs that there was an increased heart rate, increased energy, and greater tension in the arteries, but that this was followed by slowing and lessened tension. Hare[c] also worked with dogs and found a slight fall in pressure, and Osler[d] in clinical observations noted a decrease of the pulse of from 20 to 30 beats per minute. Weil[e] seems to have summed up the action of the drug in his experiments by stating that the first effect was an increase of rate and energy, but that this was followed by a decrease in both. It is generally concluded from experimental evidence, therefore, that small doses increase the heart action, but that larger amounts cause depression. No experiments seem to have been made in which the heart action of acetanilide was recorded by a myocardiograph. Accordingly it seems worth while to again report upon the action of this drug upon the circulatory organs as a means of emphasizing its dangers, especially as myocardiograph tracings demonstrate this action so clearly.

It is generally recognized that the mammalian heart reacts to drugs and poisons in much the same manner as does the heart of cold-

[a] Evans, Therap. Gazette, 1887, XI, 237.
[b] Lepine, Rev. de Med., Par., 1887, VII, 310.
[c] Hare, Therap. Gazette, 1887, XI, 382.
[d] Osler, Ibid., 165.
[e] Weil, Paris Thesis, 1887, De la Acetanilide.

blooded animals. This reaction is entirely qualitative and, aside from differences due to variation in the action of the vagi, is always very uniform. In estimating the effects of a drug upon the human heart, however, it is preferable to experiment upon warm-blooded animals, and to choose those in which the vagus action is well developed. In this way the experimental data may be said to represent not only the qualitative but also quite closely the quantitative effects in man. Therefore, to check the results obtained by perfusing the frog's heart with acetanilide and with mixtures of acetanilide and other drugs, a series of experiments were carried out, using dogs as experimental animals. These animals were anæsthetized by giving hypodermic injections of morphine sulphate, 0.010 gram per kilogram body weight, and this was followed in the course of a half hour to an hour by chloretone, 0.180 gram per kilogram dissolved in a small amount of alcohol (1 gram chloretone to 2 c. c. 95 per cent alcohol), which, after dilution with a small amount of water, was introduced into the stomach by means of a stomach tube. Following the appearance of complete anæsthesia a tracheal cannula was introduced to provide for artificial respiration, the air being properly warmed by passing it through a coil submerged in hot water. The heart was then exposed by a median incision reaching to the diaphragm, the pericardial sac removed, and the ventricle attached to a modified form of the Roy-Adami myocardiograph. Blood pressure tracings from the carotid were also taken, using the ordinary mercury manometer to record the changes in pressure produced by the drug. Cannulæ were placed in both the right and left saphenous veins—one for the injection of the acetanilide, the other for the injection of the caffeine citrate or sodium bicarbonate, which was used to determine if there were any antagonism between these drugs and acetanilide.

Acetanilide is so slightly soluble in physiological salt solution that it was necessary to inject it as an emulsion. This was formed with mucilage of acacia, the amount used being as small as possible, and then diluted with salt solution. To insure uniformity of dosage the emulsion was thoroughly shaken before each injection, since the suspension of the acetanilide was only temporary.

Injections of small amounts (0.200 to 0.600 gram) of acetanilide was usually followed by a momentary increase in the strength of the heart, the systolic phase being more complete and the relaxation only slightly lessened. This effect lasted for a few seconds only and was succeeded by a rapid and marked decrease in efficiency, the contractions growing quickly less complete, while the relaxations were scarcely affected at all. In some instances the amplitude (efficiency (?)) was slightly greater after recovery from or as a late effect of the drug. As in the case of the perfusion experiments it

was also noted that following the primary injection the later injections caused much less weakening of the heart. For example, a preliminary dose of 1 gram lessened the amplitude 70 millimeters, a

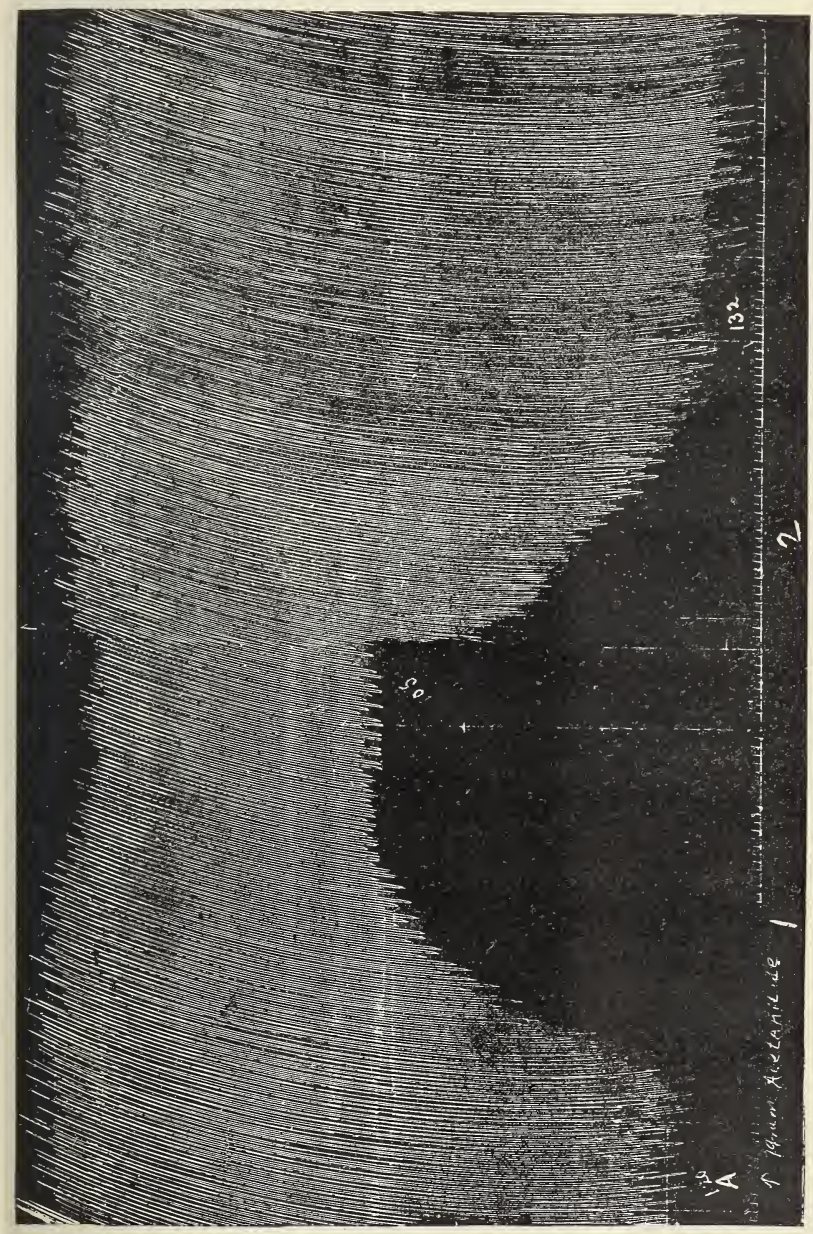

FIG. 1.—Tracing of the ventricular contractions under acetanilide. The lever moves downward during systole. "A" marks the point of injection. The rhythm of the heart is somewhat slower (120 to 105) after the drug and the lever does not descend so far, indicating a less complete systole. The diastole is also slightly less as is shown by the failure of the lever to reach so far upward. A late effect is the increased systole, indicated by the nearer approach of the lever to the base line. Between 1 and 2, one minute.

second injection only 38, a third injection of 1.5 grams 36, and a fourth also of 1.5 grams only 8. Figure No. 1 is given to illustrate

the deleterious effect on the dog's heart produced by a 1-gram dose of acetanilide.

The gradual decrease in the action of acetanilide makes the determination of possible antagonistic effects of caffeine and sodium bicarbonate somewhat difficult. As has been noted, the effect of

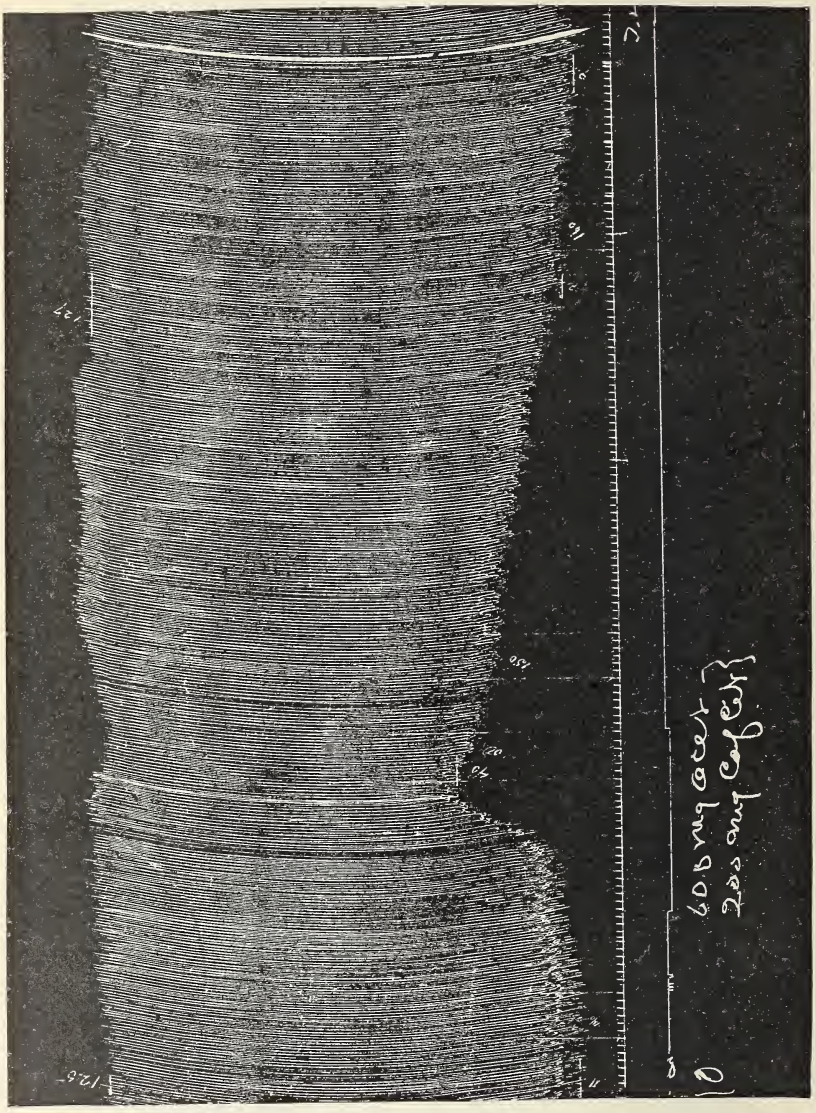

FIG. 2.—Tracing of the ventricular contractions under the acetanilide and caffeine citrate. A signal marker was used to indicate the time of injection. The failure of the heart to draw the lever downwards indicates a lessened systole. The diastole is little influenced as the upper line scarcely is changed by the drugs.

acetanilide is to lessen the contractile power of the heart, and caffeine should prevent this symptom if it acted as an antagonist. A mixture of the two drugs was injected, but in no case was the decrease in the systolic phase prevented—whether it may have been less pronounced is of course difficult to determine since, as has been

noted, the effect of the poison appears to become less at subsequent injections. This is well illustrated by the tracings given in figures 2 and 3.

Figure No. 2 shows the effect after 0.600 gram acetanilide and 0.200 gram caffeine citrate had been injected into the saphenous

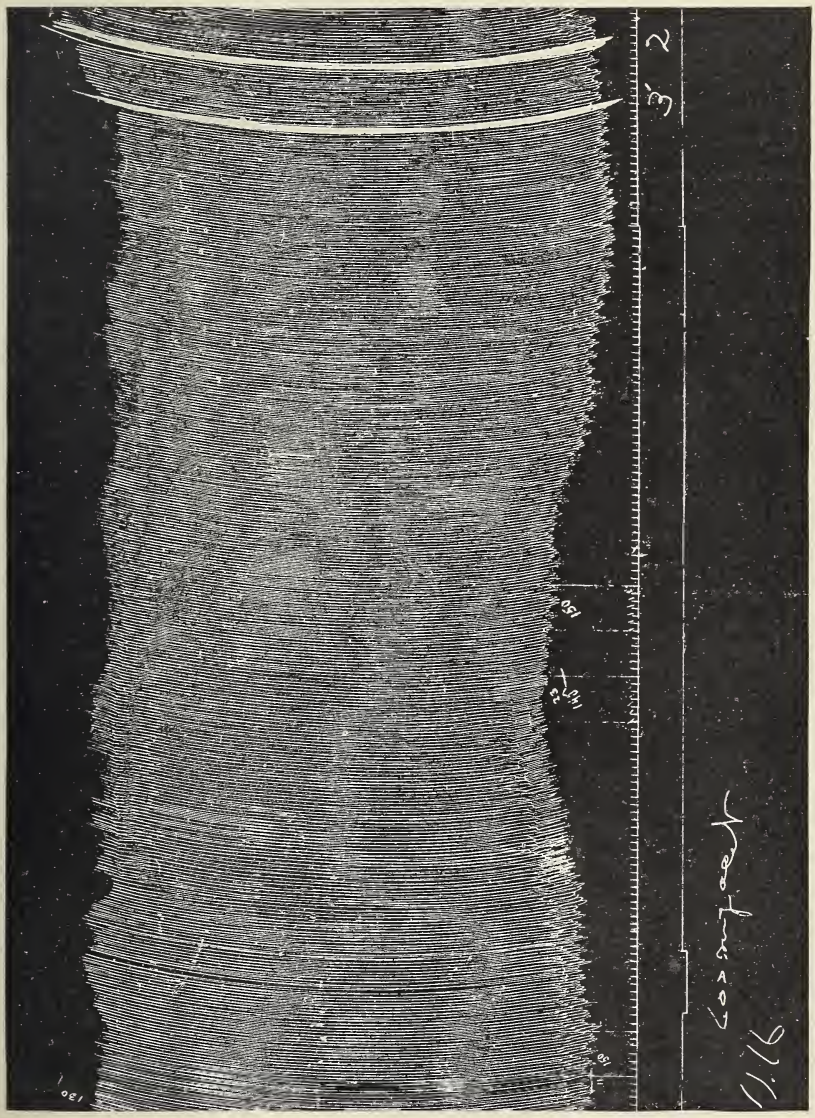

Fig. 3.—Tracing of the ventricular contractions under acetanilide, 0.600 gram dose. The lever moves downwards during systole. Compare with Fig. 2 and note the lessened contraction, although this tracing followed that in Fig. 2 after an interval of six minutes.

vein; figure No. 3, the effect after an injection of 0.600 gram acetanilide, this tracing immediately following that given in figure 2. It may also be inquired whether the caffeine is not responsible for the greater weakening shown in figure 2; but this is not likely, judging from other experiments where the reverse order of injection was used.

When the caffeine acetanilide mixture followed the plain acetanilide the effect of the combined drugs was less instead of greater as in the above case. Usually the heart does not become accustomed to the poison quite so quickly as in this instance, animals differing very widely in this respect.

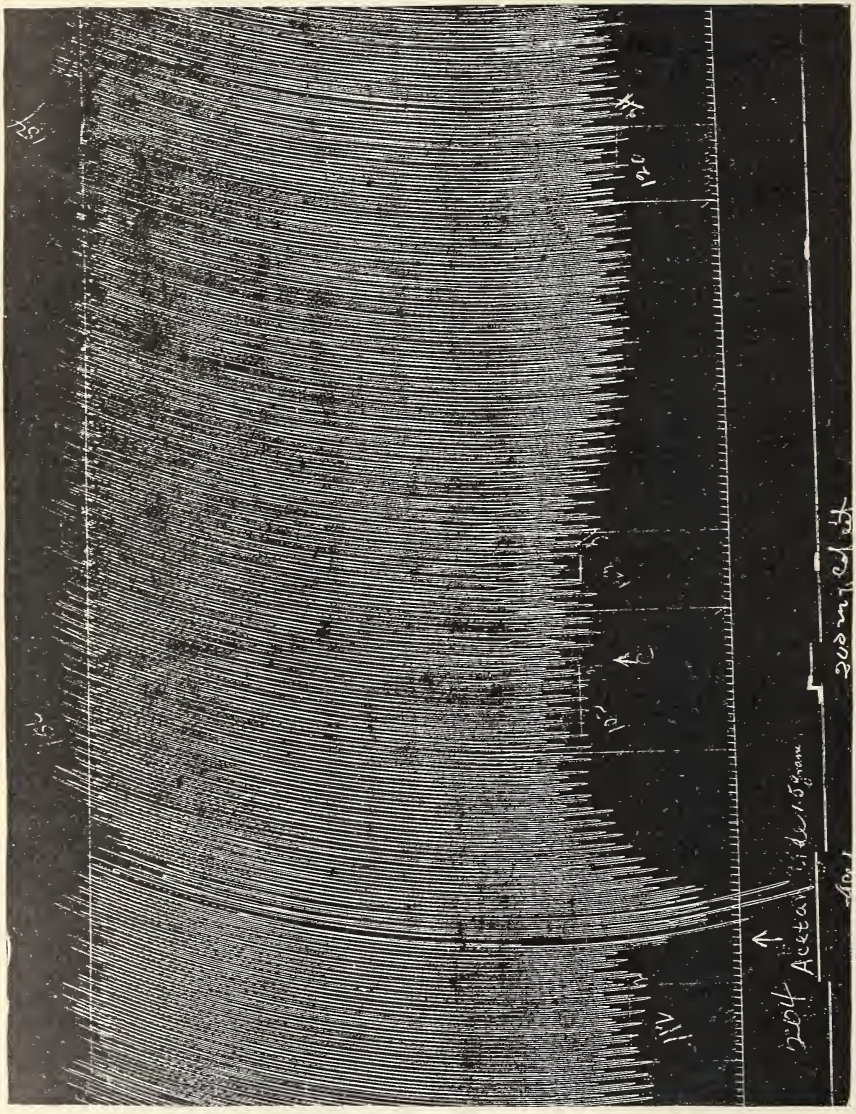

FIG. 4.—Tracing of the ventricular contractions under acetanilide and under caffeine citrate. The lever moves downward in systole. Note the momentary increase in the systole after the injection of acetanilide and the very slight lessened contraction after the injection of caffeine at "C."

Further experiments were carried out in which caffeine citrate was injected as soon as the acetanilide effect became pronounced. In some instances this procedure showed a possible slight increased contraction coincident with the caffeine injection, but almost equally

often such an injection of caffeine was followed by a decrease in the completeness of systole. In other cases there was a momentary slight increase in strength followed by a short decrease immediately following the caffeine injection. This is illustrated by figure 4.

No increase in heart rate was observed following the injection of acetanilide in the doses used in these experiments. On the other hand, there was generally a decrease of from 10 to 20 beats per minute, the slowing evidently being due to a direct action on the heart muscle, since paralysis of the vagi by atropine did not prevent it. As regards the rate, caffeine proved to be completely antagonistic, when injected at the same time usually preventing any slowing and when injected subsequent to an injection of acetanilide causing the heart rate to return at once to the normal or in some cases to a rate more rapid than normal.

There is a marked fall in blood pressure immediately following the injection of acetanilide, which is probably due in a great measure to the lessened efficiency of the heart. The injection of caffeine, although restoring the rate to normal, has hardly any effect upon the blood-pressure curve after injections of acetanilide. In general, there is a slight upward tendency, but in certain instances caffeine seemed to check the return to normal, agreeing in this respect to the occasional tendency of caffeine to lessen the completeness of the heart's contraction. Figure 5 illustrates the lack of antagonism between caffeine and acetanilide upon the blood pressure.

Sodium bicarbonate was used to antagonize the action of acetanilide only after the heart had become seriously poisoned. The immediate effect of large doses (2 grams) was to slightly increase the depression as marked by the systolic phase. This was quickly followed, however, by a rather marked and prolonged increase in the contractile power of the ventricle, as is shown by the nearer approach of the writing lever to the base line

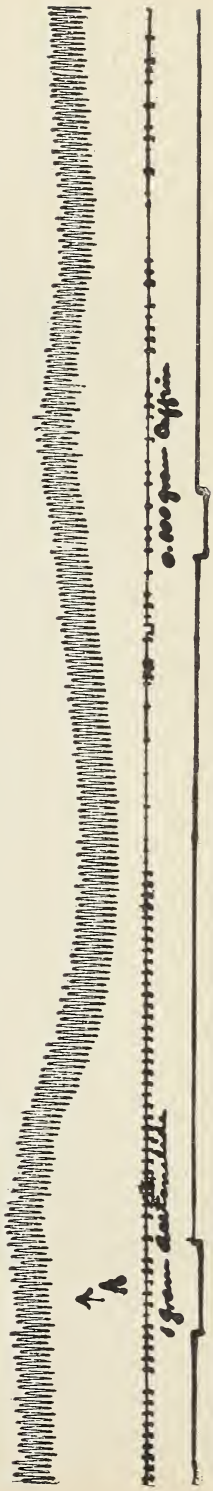

FIG. 5.—Blood pressure tracing taken from the carotid of dog. Note especially the secondary fall immediately following the injection of 0.075 gram caffeine citrate. 0.500 gram acetanilide injected at A.

in Figure 6. Note also the great dilation of the heart as a late effect of acetanilide poisoning, both diastole and systole being much less complete as compared with the normal, a portion of which is also included in the cut.

DETERMINATION OF GENERAL TOXIC ACTION.

In view of the fact that the cases of acetanilide poisoning are due to an involvement of all the vital organs of the body and not on

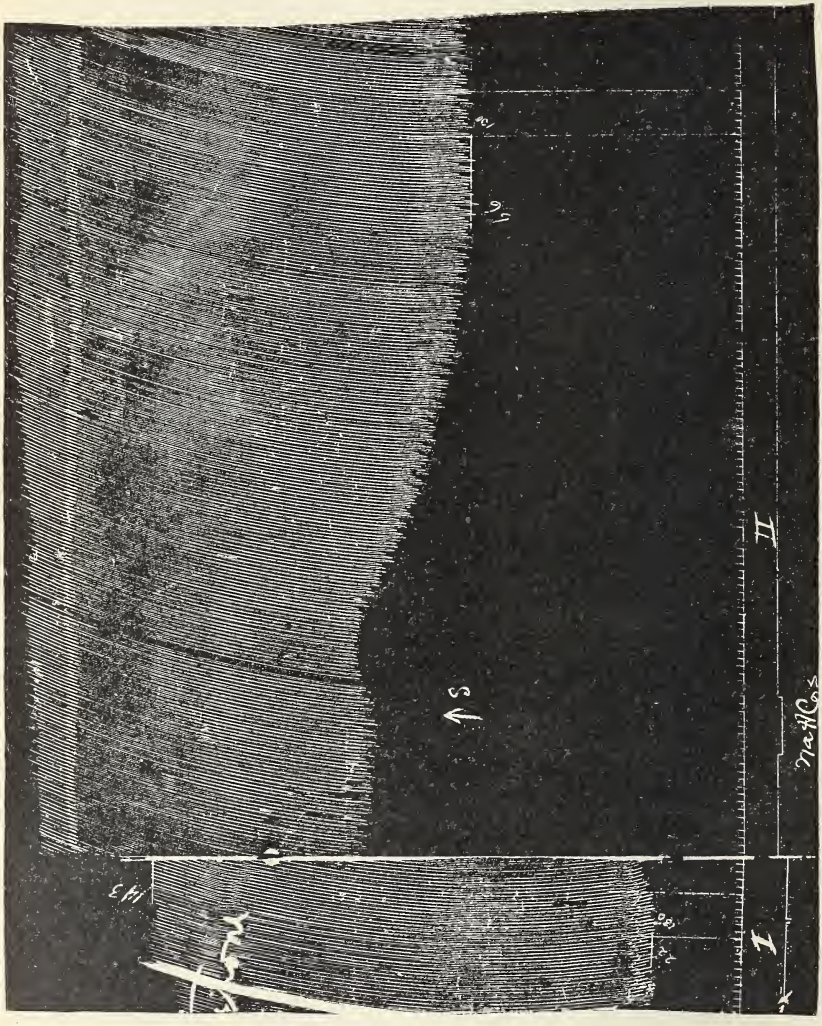

FIG. 6.—Tracing of the ventricular contractions of the normal heart and of the heart after the injection of acetanilide. Sodium bicarbonate was injected at S. Note the increased systole after the introduction of the carbonate. 1, normal heart; 2, the same heart one hour and a half later poisoned with acetanilide.

account of its heart action alone, a series of experiments was carried out to determine what modification of the general toxicity could be secured by administering it to intact animals with the drugs commonly found in acetanilide mixtures. In these experiments the drugs were administered to white mice by hypodermic injection and to mice and guinea pigs by the stomach.

In the first series of experiments acetanilide and an acetanilide-caffeine mixture were given to white mice by hypodermic injection. The mice were obtained from the same lot for each series of experiments and after being weighed they were placed in separate jars and the dose calculated in terms of grams of body weight. Acetanilide is so slightly soluble in water that it was found necessary to dissolve it in dilute alcohol (55 per cent) in order to make the hypodermic method available. This unfortunately introduces an additional factor, and to lessen the amount of alcohol injected as far as possible a supersaturated solution (at ordinary temperature) was formed, each cubic centimeter of the solution representing 200 milligrams of the drug. It was necessary to heat the solution to about 40° before the injections were made, and to prevent precipitation when drawn up in the syringe the latter was also kept in a warm place and the injection under the loose skin of the back made as quickly as possible. The minimum lethal dose of acetanilide when given in this way was found to be 0.0013 gram per gram of body weight. In control mice somewhat more than double the amount of alcohol was just sufficient to cause death, showing that the poisonous action of acetanilide was the principal toxic agent, although its effects were possibly modified to some extent by the solvent. The following table (Table VIII) gives the result of a determinative series of experiments:

TABLE VIII.—*Determination of the minimum lethal dose of acetanilide for white mice, hypodermic injection. Dose given represents grams of drug per gram body weight.*

[−=survived; +=death.]

	Weight in grams.	Dose per gram body weight.	Result.	Hours till death.
Series I	15.12	0.0010	−	.....
	16.10	.0012	−	.....
	14.41	.0014	+	23
Series II	24.18	.0011	−	.....
	19.24	.0012	+	26
	23.66	.0013	+	25
Series III	17.45	.0012	−	.....
	18.96	.0012	−	.....
	14.32	.0013	+	30
	15.92	.0013	+	14

As a means of control the following experiments were carried out to determine the fatal dose of 55 per cent alcohol:

TABLE IX.—*Determination of minimum lethal dose of 55 per cent alcohol for white mice. Dose calculated in terms of grams of acetanilide for sake of comparison.*

[−=survived; +=death.]

	Weight in grams.	Dose per gram body weight.	Result.	Hours till death.
Series I	30.93	0.0014	−	.....
	25.72	.0016	−	.....
Series II	26.60	.0020	−	12
	26.06	.0024	−	.....
	26.65	.0028	+	3
Series III	20.60	.0020	−	.....
	21.96	.0024	−	.....
	19.82	.0028	+	6

The minimum lethal dose for caffeine citrate was determined in the same manner, excepting that the drug was dissolved in physiological salt solution. The dose just sufficient to cause death was found to be 0.0007 gram.

The results of this series of experiments are found in Table X.

TABLE X.—*Determination of the minimum lethal dose of caffeine citrate for white mice, hypodermic injection.*

[−=survived; +=death.]

	Weight in grams.	Dose per gram body weight.	Result.	Minutes till death.
Series I	13.72	0.0005	−	.....
	14.39	.0008	+	127
	13.28	.0010	+	70
Series II	12.51	.0006	−	.....
	16.92	.0007	+	25
	12.52	.0008	−	.....
Series III	14.46	.0006	−	.....
	14.09	.00075	+	17
	12.82	.00085	+	5(

Having thus determined the minimum fatal dose for acetanilide and caffeine citrate, both drugs were mixed in varying proportions, dissolved in 55 per cent alcohol, and injected in the manner as in the previous experiments. A series of mice were injected with mixtures containing 0.0012 of acetanilide and 0.0001 and 0.0002 gram of caffeine per gram of body weight, but this dosage proved to be invariably fatal, thus proving the absence of any antidotal properties for caffeine in the above amounts. A further series of experiments was then carried out, using smaller amounts of acetanilide to determine whether the effect might not represent a summation of the toxic action of the two drugs. In estimating the doses to be given the fatal doses of each drug, as already determined, were added

together, it being believed that if there were a decrease in toxicity the half of this sum or any proportion representing half this sum would surely not cause death. The sum of the minimum fatal doses being 0.0020, the initial doses were estimated as about one-half of this amount, or 0.0010. But when given in proportions the sum of which equaled 0.0010 the mixture also proved fatal, showing not only no antagonism but a summation effect even. Further experiments were then carried out in which smaller amounts than half the sums of the minimum lethal doses were injected. Likewise these doses, even when slightly less than half the amount of the minimum lethal dose, always proved fatal, thus indicating more emphatically not only a summation effect in the toxic action of the two drugs but even some synergistic action. The time after the injections were made until death of the animal was also shorter, and this served as further proof of an increased toxicity. Some of the results obtained in these experiments are given in Table XI.

TABLE XI.—*Determination of the minimum lethal dose for mice of mixtures of acetanilide and caffeine citrate, hypodermic injection.*

[−=survived; +=death; A=acetanilide, and C=caffeine citrate.]

Weight.	Dose per gram body weight.	Result.	Hours till death.
17.51	A 0.0006 C .0001	−	.....
14.15	A .0006 C .0002	−	.....
16.01	A .0007 C .0001	−	.....
12.77	A .0007 C .0002	+	7.30
12.96	A .0007 C .0002	+	9.00
14.62	A .0008 C .0001	+	4.50
12.55	A .0008 C .0001	+	2.30
14.34	A .0009 C .0001	+	3.37
20.83	A .0009 C .0001	+	3.12
16.73	A .0007 C .0003	+	9.52
15.04	A .0007 C .0004	+	4.40
15.73	A .0009 C .0002	+	4.49
14.56	A .0009 C .0002	+	2.20

FEEDING EXPERIMENTS.

The hypodermic method of administering the antipyretics is rarely, if ever, used, at least in recent years, in the therapeutic application of these drugs. In order, therefore, to carry out experiments more closely simulating the ordinary methods of giving them, a series of feeding experiments was planned, using white mice and guinea pigs as experimental animals. In all cases the animals for

the same series were taken from the same lot and kept under the same conditions tc lessen the chances for the individual variation in results.

The mice were weighed, placed in separate jars, and fed according to the method of Ehrlich upon cakes made up with cracker meal to which the drug or mixture of drugs to be tested had been added. Each cake represented 4 grams of the meal and constituted the daily ration for each mouse. Because the whole cake was not always eaten entirely up, the daily amount of drug ingested necessarily varied to some extent, being somewhat less than the amount computed as the daily dose. In some cases, too, the death of the animal was probably only in part due to the toxic action of the drugs, in part to a dislike for the medicated cakes, and consequent partial starvation. To lessen the chances for error from this cause and from individual variations in the susceptibility of the mice, etc., a number of different combinations of the drugs were fed and several series of mice (in different series mice of different lots but always of the same lot for the same series) were used in testing the various combinations.

Control experiments were always carried out for each series. In the first series unmedicated cakes were used as well as cakes containing the drugs which are commonly found in the ordinary type of acetanilide mixtures. In the later experiments the control with plain cakes was omitted as unnecessary, since the controls using the drugs exhibited in the above mixtures usually lived as long or approximately as long as the mice fed upon plain cakes, the controls with acetanilide being an exception. In certain cases three different drugs were mixed together and fed, but usually the simpler combination of acetanilide with only one other drug was used. Caffeine and sodium bicarbonate, the two drugs most commonly exhibited with acetanilide, were fed to a greater number of mice than the drugs appearing less frequently. The controls of caffeine were fed 0.040 gram, an amount from two to four times that used in the mixtures of this drug with acetanilide, although even in the above amount it appeared practically nontoxic, the controls living about the same time as the mice receiving plain cakes.

Toxicity of acetanilide-caffeine mixtures.—In the experiments recorded below white mice were fed on unmedicated cakes, on cakes containing acetanilide, caffeine, and a mixture of acetanilide and caffeine.

The three tables which follow show the results of feeding experiments carried out as controls to other experiments in which a mixture of caffeine citrate and acetanilide was used:

SERIES I.—*Controls.*

[Plain cakes: Feeding mice unmedicated cakes (4 grams cracker dust to 4 parts water, dried at 50–60° C.).]

Date.	Weight of mice in grams.			
	1.	2.	3.	4.
1908.				
December 3	20.38	18.04	18.40	20.94
December 7	19.76	16.95	17.76	18.64
December 11	19.75	16.38	17.78	18.29
December 18	18.50	16.02	17.40	18.53
December 23	18.14	15.52	17.24	15.12
December 27	16.65	14.55	15.87	14.00
December 30	16.28	14.01	15.47	13.16
1909.				
January 4	15.02	13.33	14.97	14.47
January 7	.....	13.60	14.37	13.59
January 11	.....	11.97	14.49	12.41
January 15	.....	.....	14.07	12.23
January 19	.....	.....	.....	11.45
January 23	.....	.....	.....	11.40

No. 1, dead January 7, 35 days; No. 2, dead January 12, 40 days; No. 3, dead January 18, 46 days; No. 4, dead January 25, 53 days.

[Caffeine: Feeding mice 0.040 gram caffeine citrate per cake.]

Date.	Weight of mice in grams.			
	1.	2.	3.	4.
1908.				
December 3	14.78	18.48	17.50	22.88
December 7	13.12	17.32	15.06	21.52
December 11	12.62	17.24	14.73	19.81
December 18	11.45	16.71	13.70	20.05
December 23	12.18	17.15	14.21	20.47
December 27	10.90	15.87	13.30	20.82
December 30	11.68	14.51	13.76	20.26
1909.				
January 4	11.08	14.33	13.00	19.91
January 7	.....	14.51	12.69	15.60
January 11	.....	13.47	12.31	15.31
January 15	.....	11.99	11.99	15.20
January 19	.....	.....	11.92	15.02
January 23	.....	.....	.....	14.96

No. 1, dead January 7, 35 days; No. 2, dead January 15, 43 days; No. 3, dead January 16, 44 days; No. 4, dead January 23, 51 days.

[Acetanilide: Feeding mice 0.050 gram acetanilide per cake.]

Date.	Weight of mice in grams.			
	1.	2.	3.	4.
1908.				
December 3	20.13	17.38	20.36	16.11
December 5	17.10	13.47	16.65	14.20
December 7	.....	11.57	17.15	12.39
December 9	.....	.....	17.96	11.62
December 11	.....	.....	.....	11.16
December 15	.....	.....	.....	9.91
December 19	.....	.....	.....	10.24
December 21	.....	.....	.....	9.30
December 23	.....	.....	.....	8.79

No. 1, dead December 6, 3 days; No. 2, dead December 8, 5 days; No. 3, dead December 10, 7 days; No. 4, dead December 23, 20 days.

Having determined the period which mice fed on the simple drugs, a series of mice were fed upon a mixture of these drugs with the following results:

SERIES I.—*Acetanilide-caffeine.*

[Feeding mice acetanilide 0.050 gram+caffeine citrate 0.020 gram per cake.]

Date.	Weight of mice in grams.			
	1.	2.	3.	4.
1908.				
December 3	14.71	15.42	14.16	14.20
December 5	11.70	12.64	11.97	11.77
December 7	.....	11.75	12.50	12.06
December 9	.....	.....	.....	11.51

No. 1, dead December 7, 4 days; No. 2, dead December 8, 5 days; No. 3, dead December 8, 5 days; No. 4, dead December 10, 7 days.

A somewhat decreased toxicity was observed when smaller amounts of caffeine were given. The results are tabulated below:

[Feeding mice acetanilide 0.050 gram+caffeine citrate 0.010 gram per cake.]

Date.	Weight of mice in grams.			
	1.	2.	3.	4.
1908.				
December 3	16.29	16.53	17.41	14.42
December 5	13.39	13.71	14.08	12.25
December 7	.....	13.09	13.08	11.02
December 9	.....	12.79	11.00	9.73

No. 1, dead December 7, 4 days; No. 2, dead December 10, 7 days; No. 3, dead December 10, 7 days; No. 4, dead December 11, 8 days.

In the following tables are given the results of a later series of experiments. Control mice fed caffeine citrate 0.040 gram lived a somewhat shorter time than in the previous series, namely, 13, 34, 35, and 40 days. The table showing this control may be omitted, since the relative small degree of toxicity of this drug in this dose is apparent in the above figures. The protocols of control mice fed on simple acetanilide cakes and mice fed on a mixture of acetanilide and caffeine are to be found in the following tables:

SERIES II.—*Control: Acetanilide.*

[Feeding mice acetanilide 0.050 gram per cake.]

Date.	Weight of mice in grams.			
	1.	2.	3.	4.
1909.				
February 11	18.34	20.78	18.92	20.00
February 13	15.43	17.80	15.59	17.29
February 15	13.67	15.80	14.06	14.44
February 17	14.15	14.23	13.26	12.65
February 20	.....	.....	.....	10.80

No. 1, dead February 18, 7 days; No. 2, dead February 18, 7 days; No. 3, dead February 19, 8 days; No. 4, dead February 21, 10 days.

Acetanilide-caffeine

[Feeding mice acetanilide 0.050 gram+caffeine citrate 0.020 gram per cake.]

Date.	Weight of mice in grams.			
	1.	2.	3.	4.
1909.				
February 11	20.68	16.68	22.10	26.26
February 13		14.00	17.97	22.78
February 15		13.11	18.25	20.52
February 17				

No. 1, dead February 13, 2 days; No. 2, dead February 16, 5 days; No. 3, dead February 16, 5 days; No. 4, dead February 16, 5 days.

A still later series using acetanilide and caffeine mixtures showed the following results. The caffeine control mice lived 8, 34, 41, and 65 days.

SERIES III.—*Control: Acetanilide.*

[Feeding mice acetanilide 0.050 gram per cake.]

Date.	Weight of mice in grams.			
	1.	2.	3.	4.
1909.				
March 6	15.20	15.28	18.25	15.08
March 8	13.99	15.12	16.61	13.23
March 11	11.96	13.69	14.43	12.61
March 13			13.32	11.94

No. 1, dead March 12, 6 days; No. 2, dead March 12, 6 days; No. 3, dead March 14, 8 days; No. 4, dead March 14, 8 days.

Acetanilide-caffeine.

[Feeding mice acetanilide 0.050 gram+caffeine citrate 0.020 gram per cake.]

Date.	Weight of mice in grams.			
	1.	2.	3.	4.
1909.				
March 6	15.95	13.44	15.21	15.88
March 8	14.65	11.84	14.00	13.45
March 11				12.19

No. 1, dead March 9, 3 days; No. 2, dead March 10, 4 days; No. 3, dead March 11, 5 days; No. 4, dead March 13, 7 days.

Another lot fed smaller amounts of caffeine gave results as follows:

[Feeding mice acetanilide 0.050 gram+caffeine citrate 0.010 gram per cake.]

Date.	Weight of mice in grams.			
	1.	2.	3.	4.
1909.				
March 6	15.43	16.39	14.99	16.02
March 8	14.78	14.21	13.39	14.82
March 11		13.63	12.42	14.10
March 13				13.85

No. 1, dead March 10, 4 days; No. 2, dead March 11, 5 days; No. 3, dead March 12, 6 days; No. 4, dead March 15, 9 days.

A summary of the results given in the above tables is given here for the sake of comparison.

SUMMARY.

Series.	Control.			Acetanilide mixtures.	
	Plain cakes.	Caffeine, 0.040.	Acetanilide, 0.050.	Acetanilide 0.050, caffeine 0.020.	Acetanilide 0.050, caffeine 0.010.
	Days.	*Days.*	*Days.*	*Days.*	*Days.*
I	35	35	3	4	4
	40	43	5	5	7
	46	44	7	5	7
	53	51	20	7	8
Average	43.50	43.25	8.75	5.25	6.50
II	.....	13	7	2	.....
	.....	34	7	5	.....
	.....	35	8	5	.....
	.....	40	10	5	.....
Average	.....	30.50	8.00	4.25	.....
III	.....	.....	6	3	4
	.....	.....	6	4	5
	.....	.....	8	5	6
	.....	.....	8	7	9
Average	.....	.....	7.00	4.75	6.00
General average.	43.50	36.87	7.91	4.75	6.25

NOTE.—The figures in this table refer to the number of days from the beginning of the experiment until the death of the animal.

As will be noted, the figures in the above summary show that mice fed on acetanilide lived almost two times as long as those fed on a mixture of acetanilide and caffeine. This indicates that instead of any antagonism, caffeine markedly increases the toxicity of acetanilide when given to white mice with their food, although caffeine itself is scarcely toxic at all even when given in doses from two to four times greater.

Unfortunately, in these and in all the other experiments the mice lost weight quite rapidly, so that starvation may be argued as a partial reason for the animal's death. This seems to be an insufficient reason, however, as there is no relation between the loss in weight and the death of the animal, some decreasing in weight as much as 50 per cent before death, others decreasing only 10 to 20 per cent. A more important argument is furnished by certain experiments in which a record was kept of the number of cakes each mouse ate. Excepting in those cases where the animal died within the first two or three days, there was approximately the same average amount of cake eaten per day, whether the cakes contained acetanilide alone or a mixture of acetanilide and caffeine. The figures covering this point are as follows: Average amount of cake eaten per day—acetanilide, 0.51 per cent; of mixture containing 0.020 gram caffeine citrate, 0.62 per cent; of mixture containing 0.010 gram caffeine citrate, 0.69 per cent. In other words, the animal eating the most cake per day, grouping the mixtures containing caffeine, died in the

shortest length of time, and if starvation were a factor it is evident that they should have lived the greater period of time.

Again, it may be argued that death naturally resulted more quickly from the toxic action of the greater amount of acetanilide consumed by those mice which ate the most cake. But the figures do not bear out this assumption. In the first place, of the two lots receiving caffeine that receiving the smaller amount lived the longer, and yet this lot consumed more cake, and therefore acetanilide in the ratio of 62 to 69. In the second place, the length of time until death of the acetanilide control mice and the acetanilide mixture (0.020 gram caffeine) mice are not in even an approximate ratio to the amount of cake eaten: Ratio of cake eaten, 100 to 119; ratio of days until death, 100 to 166. The conclusion seems unavoidable, therefore, that caffeine adds to the toxicity of acetanilide.

TOXICITY OF ACETANILIDE AND SODIUM BICARBONATE MIXTURES.

Other experiments were carried out in which mice were fed cakes containing sodium bicarbonate mixed with acetanilide. In one series, also, caffeine citrate was added to this mixture, giving a compound acetanilide powder quite closely imitating the United States pharmacopœial preparation of this name. Control mice were given sodium bicarbonate 0.020 gram per cake, which amount showed no toxic properties. Other controls fed on sodium bicarbonate 0.020 gram plus caffeine citrate 0.020 gram appeared relatively nontoxic, the animals living for 25, 27, 35, and 36 days. The other controls for this series of experiments have already been given, this work being carried out at the same time and as a part of the series already tabulated. Accordingly they will not be recorded again, the only data to be repeated appearing in the summary of the following experiments:

SERIES I.—*Acetanilide—caffeine—sodium bicarbonate.*

[Feeding mice acetanilide 0.050 gram + caffeine citrate 0.020 gram + sodium bicarbonate 0.020 gram per cake.]

Date.	Weight of mice in grams.			
	No. 1.	No. 2.	No. 3.	No. 4.
	Days.	*Days.*	*Days.*	*Days.*
December 3	16.92	21.26	18.15	15.84
December 5	13.13	17.90	15.15	15.50
December 7	12.39	14.58	12.89	.....
December 9	11.53	.....	.....	.....

No. 1, dead December 16, 7 days; No. 2, dead December 9, 6 days; No. 3, dead December 9, 6 days; No. 4, dead December 6, 3 days.

The following table gives the results of giving smaller amounts of caffeine.

[Feeding mice acetanilide 0.050 gram + caffeine citrate 0.010 gram + sodium bicarbonate 0.020.]

Date.	Weight of mice in grams.			
	No. 1.	No. 2.	No. 3.	No. 4.
	Days.	*Days.*	*Days.*	*Days.*
December 3	16.39	16.16	14.92	18.40
December 5	14.06	13.47	12.06	.....
December 7	13.48	12.44	11.86	.....
December 9	12.95	13.80	.....	.....
December 13	13.65	.....	.....	.....
December 15	12.54	.....	.....	.....
December 17	13.57	.....	.....	.....

No. 1, dead December 17, 14 days; No. 2, dead December 11, 8 days; No. 3, dead December 9, 6 days; No. 4, dead December 5, 2 days.

In a later series of experiments sodium bicarbonate was mixed with acetanilide, the caffeine being omitted. The results of experiments of this sort are tabulated below:

SERIES II AND IV.—*Acetanilide and sodium bicarbonate.*

[Feeding mice acetanilide 0.050 gram + sodium bicarbonate 0.020 gram per cake.]

Date.	Weight of mice in grams.			
	No. 1.	No. 2.	No. 3.	No. 4.
1909.	*Days.*	*Days.*	*Days.*	*Days.*
February 11	21.50	21.61	22.68	18.52
February 13	18.50	18.23	20.10	16.02
February 15	16.95	16.10	16.84	14.50
February 17	15.90	14.71	15.42	13.46
February 20	14.50	13.80	.....	.....
March 15	15.82	17.58	17.30	19.18
March 18	13.66	16.51	16.00	16.18
March 20	12.03	14.10	13.73	13.82
March 22	10.73	12.15	12.50	13.43
March 24	10.70	11.44	11.50	.....
March 26	10.41	10.55	.....	.....

No. 1, dead February 21, 10 days, and March 28, 13 days; No. 2, dead February 21, 10 days, and March 27, 12 days; No. 3, dead February 20, 9 days, and March 25, 10 days; No. 4, dead February 19, 8 days, and March 24, 9 days.

As a control for Series IV mice were fed on acetanilide 0.050 per cake and lived for 7, 7, 9, and 13 days, respectively.

To determine whether a larger amount of the carbonates would still further lessen the toxicity, a series of mice were fed cakes each containing 0.040 gram sodium bicarbonate. Again this mixture showed the antidotal action of an alkali carbonate, and while this is somewhat greater with the increase of alkali the figures do not indicate any special advantage. The averages are given in the following table:

Series.	Acetanilide 0.050.	Acetanilide 0.050, $NaHCO_3$ 0.020.	Acetanilide 0.050, $NaHCO_3$ 0.040.
	Days.	*Days.*	*Days.*
II+IV	8.50	10.10 Advantage 1.60	.....
III	7.00	.....	8.75 Advantage 1.75

The following summaries are arranged in order to bring the results of these experiments together for the sake of comparison:

SUMMARY.

Series.	Control.		Acetanilide mixture.
	Caffeine 0.020, $NaHCO_3$ 0.020.	Acetanilide 0.050.	Acetanilide 0.050, $NaHCO_3$ 0.020.
	Days.	*Days.*	*Days.*
II	25	7	8
	27	7	9
	35	8	10
	36	10	10
IV	.....	7	9
	.....	7	10
	.....	9	12
	.....	13	13
Average	30.75	8.50	10.10

By comparing the results as given in the above summary it will be noted that in the case of mice sodium bicarbonate is antagonistic to acetanilide to a certain degree in the general average of the two series, as 8.50 is to 10.10. This same antagonism is also shown in the experiments in which caffeine was also a constituent of the mixture, a summary of which is given below:

SUMMARY.

Series.	Control.			Acetanilide mixture.	
	Acetanilide 0.050.	Acetanilide 0.050, caffeine 0.020.	Acetanilide 0.050, caffeine 0.010.	Acetanilide 0.050, caffeine 0.020, $NaHCO_3$ 0.020.	Acetanilide 0.050, caffeine 0.010, $NaHCO_3$ 0.020.
	Days.	*Days.*	*Days.*	*Days.*	*Days.*
I	3	4	4	3	2
	5	5	7	6	6
	7	5	7	6	8
	20	7	8	7	14
Average	8.75	5.25	6.50	5.50	7.50

These results do not show quite so marked antagonism between acetanilide and sodium bicarbonate as is shown by the results given in the preceding summary. Their agreement serves, nevertheless, as very valuable contributory evidence of the lessened toxicity of a mixture of acetanilide containing sodium bicarbonate. It will be observed, however, that despite some antidotal action this is still insufficient to make acetanilide mixtures containing caffeine as non-toxic as those which omit it entirely. This is illustrated by the following data, the averages of series No. I, II, and III:

	Acetanilide 0.05.	Acetanilide 0.05, caffeine 0.02.	Acetanilide 0.05, caffeine 0.02, $NaHCO_3$ 0.02.	Acetanilide 0.05, $NaHCO_3$ 0.02.
General average	7.91	4.75	5.50	10.10

This may be represented graphically as follows:
The degree of toxicity being represented by the length of the bars.

TOXICITY OF ACETANILIDE AND THE OPIUM ALKALOIDS.

Experiments were carried out upon white mice to determine the toxicity of mixtures composed of acetanilide and codeine and acetanilide and morphine. Control mice were fed morphine sulphate 0.002 gram per cake lived 20, 27, 36, and 45 days. Controls of codeine-fed mice lived 10, 29, 61, and 65 days. As in the previous experiments where the results of the same series have been recorded, the acetanilide controls will not be given again except as data to complete the summary of these experiments.

SERIES II.—*Acetanilide-codeine.*

[Feeding mice acetanilide 0.050, codeine 0.010 gram per cake.]

Date.	Weight of mice in grams.			
	1.	2.	3.	4.
1909.				
February 11	22.85	15.20	17.08	15.88
February 13	20.34	12.86	14.47	13.25
February 15	18.74	10.98	14.38	11.31
February 17	16.38	9.59	12.16	9.30
February 20	16.18	9.36	11.74	8.92
February 24	14.69			

No. 1, dead February 26, 15 days; No. 2, dead February 23, 12 days; No. 3, dead February 21, 10 days; No. 4, dead February 21, 10 days.

[Feeding mice acetanilide 0.050, codeine 0.005 gram per cake.]

Date.	Weight of mice in grams.			
	1.	2.	3.	4.
1909.				
February 11	15.48	19.34	17.06	14.48
February 13	14.55	16.63	15.42	13.62
February 15	12.02	13.88	13.10	12.34
February 17	11.19	11.93	12.05	
February 20	10.38	10.48		

No. 1, dead February 21, 10 days; No. 2, dead February 21, 10 days; No. 3, dead February 20, 9 days; No. 4, dead February 16, 5 days.

A summary of the results of this series is given in the following table to make the comparison of the toxicity easier.

SUMMARY.

Series.	Control.		Acetanilide mixtures.	
	Codeine, 0.010.	Acetanilide, 0.050.	Acetanilide, 0.050, codeine, 0.005.	Acetanilide, 0.050, codeine, 0.010.
	Days.	*Days.*	*Days.*	*Days.*
II	10	7	5	10
	29	7	9	10
	61	8	10	12
	65	10	10	15
Average	41.25	8.00	8.50	11.75

The data shown in this summary indicates that codeine is antidotal to the general toxic effects of acetanilide. This result was rather unexpected, but the fact that the mice receiving the larger dose of codeine in combination with acetanilide lived the longer served to confirm these findings. The number of mice fed upon mixtures of this sort was too small however to give results from which to draw absolute conclusions and accordingly a further series of mice were fed, using heroine and morphine in addition to codeine. The results of these experiments are given in the following tables.

Control[a] acetanilide.

[Feeding mice acetanilide 0.050 gram per cake.]

Date.	Weight of mice in grams.			
	1.	2.	3.	4.
1909.				
April 17	17.17	19.39	15.19	19.30
April 19	14.20	16.63	12.82	16.83
April 21	13.83	15.04	12.55	15.05
April 23	12.16	13.89	11.03	14.55
April 26	11.27	12.44	10.56	.....

[a] The control mice receiving 0.010 gram codeine, 0.002 gram morphine sulphate, and 0.00075 gram heroine hydrochloride are still alive at this date (April 29, 1909).

No. 1, dead April 28, 11 days; No. 2, dead April 27, 10 days; No. 3, dead April 27, 10 days; No. 4, dead April 24, 7 days.

In the following table the protocols of mice fed upon acetanilide and codeine are given:

SERIES V.—*Acetanilide-codeine.*

[Feeding mice acetanilide 0.050 gram plus codeine 0.010 gram per cake.]

Date.	Weight of mice in grams.			
	1.	2.	3.	4.
1909.				
April 17	17.73	20.67	18.57	22.31
April 19	14.75	16.63	15.71	17.85
April 21	13.45	15.43	14.08	16.76
April 23	12.62	14.24	12.28	.....
April 26	10.86	.....	.....	.....

No. 1, dead April 27, 10 days; No. 2, dead April 24, 7 days; No. 3, dead April 23, 6 days; No. 4, dead April 23, 6 days.

The following table gives the results of feeding mice acetanilide 0.050 plus morphine sulphate 0.002 gram per cake:

Acetanilide-morphine.

Date.	Weight of mice in grams.			
	1.	2.	3.	4.
1909				
April 17	15.23	15.09	14.26	16.48
April 19	15.73	14.92	11.23	13.07
April 21	15.29	13.27	9.78	12.30
April 23	14.78	12.12	.....	.....

No. 1, dead April 24, 7 days; No. 2, dead April 24, 7 days; No. 3, dead April 23, 6 days; No. 4, dead April 22, 5 days.

Another series of mice were fed cakes containing a smaller amount of morphine, with the following results:

Acetanilide-morphine.

[Feeding mice acetanilide 0.050 gram plus 0.001 gram morphine sulphate per cake.]

Date.	Weight of mice in grams.			
	1.	2.	3.	4.
1909.				
April 17	14.53	14.89	13.43	15.52
April 19	12.22	12.60	12.43	12.91
April 21	12.22	12.30	11.44	11.08
April 23	12.16	11.26	10.16	.....

No. 1, dead April 25, 8 days; No. 2, dead April 25, 8 days; No. 3, dead April 24, 7 days; No. 4, dead April 23, 6 days.

A series of mice were also fed acetanilide 0.050 gram and heroine 0.00075 gram per cake with the following results:

Acetanilide-heroine.

Date.	Weight of mice in grams.			
	No. 1.	No. 2.	No. 3.	No. 4.
1909.				
April 17	19.10	15.88	17.10	14.32
April 19	16.75	13.69	14.37	12.53
April 21	15.56	11.42	16.23	.....
April 23	14.01	.....	.....	.....

No. 1, dead April 24, 7 days; No. 2, dead April 23, 6 days; No. 3, dead April 22, 5 days; No. 4, dead April 21, 4 days.

A second series of mice fed upon codeine, acetanilide, and heroine and morphine gave results the general averages of which are as follows: Acetanilide 0.050 gram and codeine 0.010 gram, lived six days; acetanilide 0.050 gram and morphine 0.002 gram, three days; acetani-

lide 0.050 gram and heroine 0.00075 gram, 7 days. These results are brought together in the following summary for the sake of comparison:

Series.	Control.	Acetanilide mixture.			
	Acetanilide, 0.050.	Acetanilide 0.050, morphine 0.002.	Acetanilide 0.050, morphine 0.001.	Acetanilide 0.050, codeine 0.010.	Acetanilide 0.050, heroine 0.00075.
V	7	5	6	6	4
	10	6	7	6	5
	10	7	8	7	6
	11	7	8	10	7
VI	6	3	.....	6	6
	9	8	.....	5	8
	11	10	.....	8	8
	11	.....	.....	.....	.....
Average..	9.37	6.57	7.25	6.85	6.28

As will be noted, the figures given in this summary do not confirm those of Series III, but show exactly the opposite effect, the toxicity of acetanilide being increased by codeine, heroine, and morphine. The contributory evidence of morphine and heroine is of special value when it is remembered the similarity in the effects produced by the drugs of the opium series and serves to substantiate the findings with codeine. The much larger number of mice and the uniformity in the results of both Series V and VI make the results of Series III probably incorrect. The longer life of acetanilide-codeine mice in the earlier series is probably to be explained by some external variation in the condition of the mice which escaped the notice of the experimenter.[a]

The results of these experiments indicate, therefore, that the toxicity of acetanilide is considerably increased when combined with the above opium alkaloids.

TOXICITY OF ACETANILIDE AND SALICYLIC ACID.

In a series of experiments mice were fed on cakes each containing 0.050 gram acetanilide and 0.020 gram salicylic acid. The control mice fed on acetanilide alone, 0.050 gram per cake, lived 7, 7, 8, and 10 days, or an average of 8 days. The mice receiving the two drugs mixed in the above proportions lived 6, 7, 9, and 10 days, an average of 8 days,[b] or, in other words, lived the same length of time as the acetanilide controls. Another series fed on slightly larger amounts of salicylic acid (0.030 gram with acetanilide 0.050 gram per cake)

[a] Mice are especially sensitive to changes in temperature, and the unusual results of this series may be explained upon these grounds. During the course of the experiments the mice were moved into a room where part were much closer to a steam radiator than others, and it is very likely that those kept in the warmest position lived the longer.

[b] The control mice fed on 0.030 gram salicylic acid lived for 50, 62, and 66 days.

lived 6, 6, 7, 10 days, an average of 7.25 days. The control mice fed on acetanilide alone, 0.050 gram per cake, lived 6, 6, 8, and 8 days, an average of 7 days. These results show that salicylic acid neither increases nor decreases the toxic effect of acetanilide.

TOXICITY OF ACETANILIDE AND SODIUM BROMIDE.

Mice fed upon a mixture of acetanilide 0.050 and sodium bromide 0.030 gram per cake lived approximately as long as the control, an average of 8 days, the controls an average of 8.25 days. This indicates no alteration in toxicity.

The method of feeding mice upon cakes gives the results from long continued use of a drug, but the amount taken is entirely dependent upon the appetite of the animal. Therefore, to confirm the above results, to make more certain the exact amount of drug given, and to test the action of acetanilide mixtures when given in amounts sufficiently large to produce acute poisoning, the following experiments were carried out upon guinea pigs. These animals were all of about the same weight, obtained from the same lot, and were kept under the same conditions. The dose was estimated per gram of body weight, each dose being weighed separately and made into pills of suitable size with mucilage of acacia and arrow-root starch. These were dried until a slight crust was formed on the outside and then fed in such a manner that none of the drug was lost during administration. The protocols in the following table show the toxicity of acetanilide as determined by this method of administration:

[Acetanilide control: Determination of the minimum lethal dose of acetanilide for guinea pigs. −=survived; +=death.]

Weight in grams.	Dose per gram weight in grams.	Result.	Hours till death.
515	0.0008	−	.....
490	.0010	−	.....
500	.0010	−	.....
450	.0010	−	.....
500	.0010	+	14
465	.0012	−	.....
445	.0012	−	.....
422	.0014	+	10½
485	.0014	+	12
406	.0016	+	9½
465	.0016	+	32
402	.0018	+	12½

Control experiments with caffeine citrate gave the following results:

[Caffeine control: Determination of the minimum lethal dose of caffeine citrate for guinea pigs. −= survived; += death.]

Weight in grams.	Dose per gram weight in grams.	Result.	Hours till death.
530	0.0003	−	.....
480	.0004	−	.....
495	.0004	−	.....
405	.0004	+	16
435	.00045	−	.....
415	.0005	−	.....
410	.0005	−	.....
565	.0005	+	15
405	.00055	+	15
415	.0006	+	7

In like manner a series of guinea pigs were given pills containing acetanilide and caffeine citrate. The results of these experiments are tabulated below:

Acetanilide-caffeine mixture.—Determination of the minimum lethal dose of a mixture of acetanilide and caffeine citrate.

[−= survived; += death; A=acetanilide; C=caffeine citrate.]

Weight in grams.	Dose per gram weight in grams.	Result.	Hours till death.
555	A 0.0006 C .0002	−	.....
545	A .0006 C .0003	−	.....
580	A .0008 C .0002	−	.....
520	A .0008 C .0003	−	.....
600	A .0010 C .0002	+	21
380	A .0010 C .0002	+	13
460	A .0010 C .0003	+	17
350	A .0012 C .0002	+	20
425	A .0012 C .0003	+	112
385	A .0014 C .0002	+	23½
450	A .0014 C .0003	+	19½

The minimum lethal dose of acetanilide for guinea pigs appears by these experiments to be approximately 0.0013 gram and that of caffeine citrate about 0.00055. The mixture of caffeine (caffeine about one-third of the least fatal dose) and acetanilide shows an increased toxicity, a dose of 0.0010 gram acetanilide in a mixture with caffeine being sufficient to invariably cause death instead of 0.0013 gram per gram of body weight, as is the case when given

alone. These results serve to confirm the results of the feeding experiments upon mice and show very clearly the absence of any antagonistic action.

TOXICITY OF ACETANILIDE AND SODIUM BICARBONATE.

Experiments using equal parts of acetanilide and sodium bicarbonate were carried out in the same manner as the above experiments. The animals used in this series had been given acetanilide a week previously and had apparently recovered. In the controls for this series a smaller fatal dose showed that they were more susceptible, however, than in the earlier experiments, death invariably resulting from 0.0011 gram per gram of body weight. The pigs receiving the mixture containing sodium bicarbonate were somewhat more resistant, with one exception, the least fatal dose appearing to be approximately 0.0014 gram per gram of body weight, thus indicating that the carbonates are antagonistic to acetanilide to a certain extent. These experiments are tabulated below.

ACETANILIDE-SODIUM BICARBONATE.

[Determination of the lethal dose of a mixture of acetanilide and sodium bicarbonate. − = survived; + = death; A = acetanilide; S = sodium bicarbonate.]

Weight in grams.	Dose per gram weight in grams.	Result.	Hours till death.
365	A 0.0010 S .0010	+	36
380	A .0012 S .0012	−	.....
375	A .0014 S .0014	−	.....
340	A .0014 S .0014	+	13½
485	A .0015 S .0015	+	50
315	A .0016 S .0016	+	7¾

The lessened toxicity of this mixture was further confirmed by comparing the duration of life under the same dosage either of the simple remedy or the mixture. The time guinea pigs lived after increasing doses of acetanilide alone was 20, 4¾, 21, 9⅓, 4½, 13, and 26⅓ hours. Pigs receiving corresponding doses of the mixture lived 36, 13½, 50, 7¾, 13½, 15, and 18 hours. The sum of the hours of duration of life after dosage therefore is 99½ for the simple remedy; 153¾ hours for the mixture. Rather little emphasis is intended for this point, however, because of the great irregularity in the time the animals lived, as will be noted in the above figures, which are arranged in order of the increase in amount of drug given.

PART II.

THE TOXICITY OF ANTIPYRINE MIXTURES.

Although it is now generally recognized that antipyrine is less toxic than acetanilide[a] it has never been so popular as an ingredient of headache mixtures. It is occasionally dispensed with other drugs, however (as in bromopyrine for example), and accordingly a series of experiments were made to determine whether these altered its toxicity.

ACTION UPON THE FROG'S HEART.

The method used to determine the effect of antipyrine and mixtures of antipyrine upon the frog's heart was the same as that described for acetanilide, page 13. The species of frog and the various precautions used to obtain uniform results were also the same.

Although affecting the frog's heart when perfused through it in much the same way as acetanilide, antipyrine is much less toxic to it. This ratio is about 1 to 6 or 7. A 1 per cent solution of antipyrine usually stopped the heart at once; one-half per cent solutions decreased the rate quite markedly, but this effect was far more pronounced immediately after the introduction of the drug. Later the heart apparently became accustomed to the poisonous effects and little or no further slowing resulted, while in some cases actual increase in rate was observed after the primary slowing, a phenomenon which was also present after acetanilide, but less frequently. Solutions of 0.8 per cent were generally found to be strong enough to cause marked changes and only occasionally stop the heart within a short time. This latter reaction appeared so erratically, however, that no conclusions could be drawn from the results obtained. In one case the heart might continue to beat for a half hour or more after the primary slowing with absolutely no further changes in rate. In other instances in experiments carried out in exactly the same manner, the heart would stop as soon as the drug reached it, a variability in action which seems to be somewhat more marked with this drug than with acetanilide. The changes in output were also similarly irregular, so that any conclusions were necessarily indefinite.

The addition of caffeine citrate to an antipyrine solution caused no material change in the course of the poisoning, especially in regard to the efficiency of the heart. The primary slowing induced by 0.8 per cent solutions of antipyrine was not prevented, the experiments indicating on the other hand that the slowing was greater from the combined action of the two drugs than from antipyrine alone. This represents the general result from a large number of experiments, but exceptions occasionally occurred.

[a] According to Cushny, Pharmacology and Therapeutics, 1906, p. 371, antipyrine is more toxic than phenacetine and less toxic than acetanilide.

The amount of fluid perfused is so irregular that no conclusions can be drawn from changes in this factor. Experiments illustrative of these changes are given in table I.

TABLE I.—*Perfusion of the isolated frog's heart with antipyrine and with antipyrine and caffeine citrate in Ringer's solution.*

Protocol 14.			Protocol 17.		
Time.	Rate.	Output per 5 minutes.	Time.	Rate.	Output per 5 minutes.
		C. c.			*C. c.*
10. 45	39	30	9. 25	20	35
10. 50	38	32	9. 30	18	34
Antipyrine 0.8 per cent + caffeine $\frac{1}{2000}$ per cent.			9. 35	19	34
10. 52	19	.....	Antipyrine 0.8 per cent + caffeine $\frac{1}{2000}$ per cent.		
10. 55	15	17	9. 40	17	25
11. 00	16	13	9. 45	18	24
11. 10	20	5	9. 50	13	10
11. 30	18	6	Antipyrine 0.8 per cent.		
11. 40	19	9	9. 55	17	5
Antipyrine 0.8 per cent.			10. 00	17	4
11. 42	21	.....	10. 10	18	5
11. 45	21	10	10. 53	Still beating.	
11. 50	20	9			
11. 55	19	10			

As had been the result in the case of acetanilide it was hoped that more dilute solutions of the drugs might give more definite results. Accordingly in a series of experiments the perfusions were begun with antipyrine in 0.5 per cent solution, and after sufficient readings had been recorded to establish a normal a caffeine-antipyrine solution was turned on. It was shown by these experiments that caffeine quite consistently lessened the toxic effect of antipyrine to a slight extent, although occasional exceptions occurred. The protocols in Table II illustrate the results obtained.

TABLE II.—*Perfusion of the isolated frog's heart with antipyrine and antipyrine-caffeine dissolved in Ringer's solution.*

Protocol 26.			Protocol 29.		
Time.	Rate.	Output per 5 minutes.	Time.	Rate.	Output per 5 minutes.
		C. c.			*C. c.*
Antipyrine 0.5 per cent.			Antipyrine 0.5 per cent.		
9. 55	25	53	3. 30	24	49
10. 00	22	51	3. 35	23	46
10. 05	22	46	3. 40	19	46
10. 10	22	45	3. 45	19	46
10. 15	20	42	Antipyrine 0.5 per cent + caffeine $\frac{1}{3000}$ per cent.		
Antipyrine 0.5 per cent + caffeine $\frac{1}{3000}$ per cent.			3. 47	23	.....
10. 17	22	.....	3. 50	24	56
10. 20	23	45	3. 55	19	52
10. 25	22	44			
10. 30	22	44			
10. 35	20	44			
10. 40	19	41			

The use of sodium bicarbonate[a] has been suggested to relieve the gastric irritation which sometimes follows the use of antipyrine. Accordingly a series of perfusion experiments were carried out, using sodium bicarbonate in conjunction with antipyrine. The effect was to lessen but not abolish the poisonous action of antipyrine, both rate and output being increased to a considerable degree, although never to normal. The following protocols illustrate this antagonism:

TABLE III.—*Perfusion of the isolated frog's heart with antipyrine and with antipyrine and sodium bicarbonate in Ringer's solution.*

Protocol 81.			Protocol 85.		
Time.	Rate.	Output per 5 minutes.	Time.	Rate.	Output per 5 minutes.
		C. c.			*C. c.*
10.35	35	Antipyrine 0.8 per cent.	3.20	32	Antipyrine ½ per cent.
10.40	24	20	3.50	23	26
10.45	24	13	3.53	21	27
10.50	24	8	4.00	21	27
Antipyrine 0.8 per cent + $NaHCO_3$ ⅕₀ per cent.			Antipyrine ½ per cent + $NaHCO_3$ ⅕₀ per cent.		
10.52	28	.....	4.02	24	.....
10.53	32	.....	4.05	22	32
10.55	25	14	4.10	19	30
11.00	28	17	4.15	20	32
11.15	27	16	4.20	21	30
			Antipyrine ½ per cent.		
			4.22	17	.....
			4.25	16	21
			4.29	0	.....

ACTION ON THE MAMMALIAN HEART.

The changes in the dog's heart induced by antipyrine injections were also determined, using the same methods as for acetanilide, except that the perfect solubility of antipyrine made its injection as an emulsion with acacia unnecessary. As in the perfusion experiments upon the frog's heart, antipyrine was found to be very much less poisonous to the dog's heart than acetanilide. Small doses (0.5 to 1 gram) were practically devoid of any depressant action, and a dose of 0.500 gram was actually stimulant, increasing the amplitude 15 millimeters through an increase in the completeness of the systole. Doses of 1 gram also increased the amount of contraction, but the diastole was less complete, so that the result was a slight decrease in the amplitude. The injections of still larger amounts produced a very definite decrease in the heart's action, but the change was not at all comparable to the depression following acetanilide in the same dose. Figure 7 is given to show the stimulant action of 1 gram antipyrine (compare this with the depressant action of 1 gram acetanilide. (Fig. 1, p. 21.)

[a] Am. J. Pharm., 1888, XVIII, 180.

Antipyrine in 500 milligram doses has only a slight depressant action on the heart rate, but with the injection of larger amounts the slowing became quite pronounced. The blood pressure is also markedly affected, being lowered to such a degree that neither the slowing

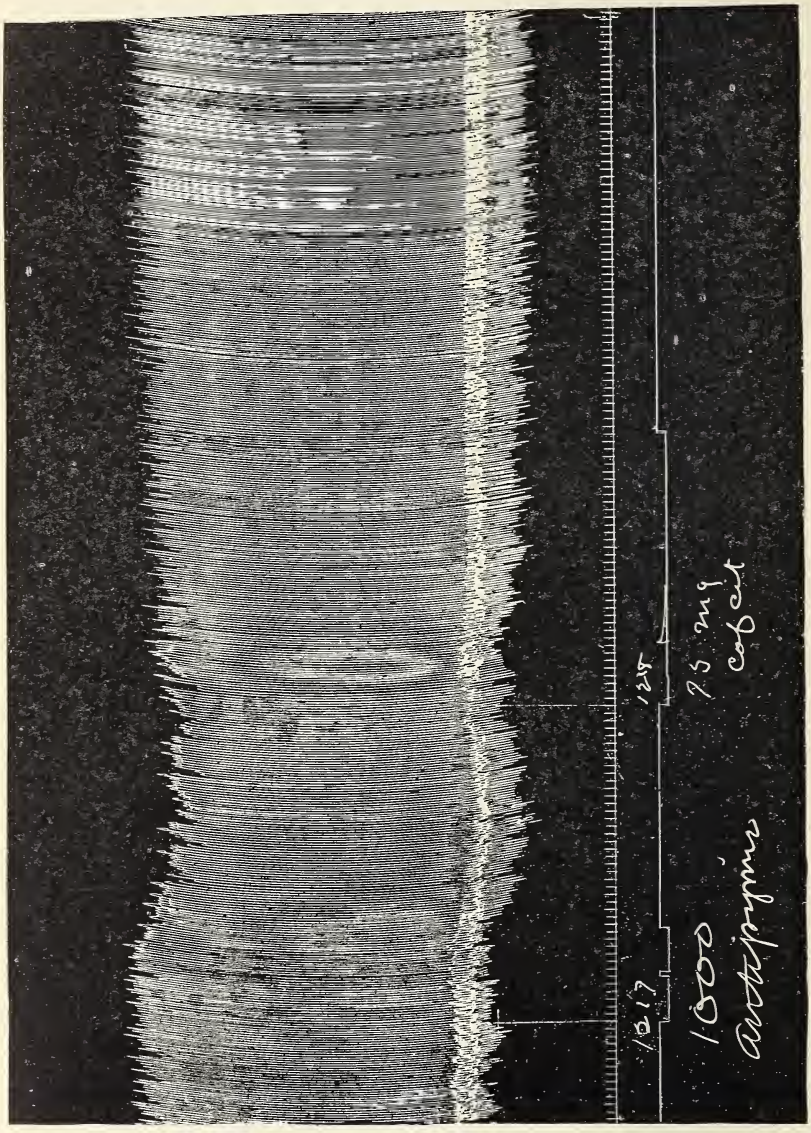

FIG. 7.—Tracing of ventricular contractions under antipyrine and caffeine citrate. The contraction of the heart pulls the lever downward. This tracing is given to show the difference between the heart effect of antipyrine and acetanilide, Fig. 1, page 21.

nor the changes in the efficiency of the heart afford a sufficient reason for its depression. For instance, with a lessened amplitude of 3 millimeters and a decrease in rate of only 10 beats per minute, the pressure fall after an injection of a 1 gram dose amounted to 58

millimeters mercury, or a fall of 42 per cent (fig. 8). The obvious conclusion, therefore, is that antipyrine either depresses the vasomotor center or dilates the peripheral vessels from a local action, and both factors may play some part in this marked fall in pressure. The decrease in the heart rate is not prevented by paralysis of the vagi, and would be due, therefore, to a direct depression of the heart muscle.

Caffeine was injected coincident with and also subsequent to the injection of antipyrine, but appeared to be devoid of any antagonistic action upon either the depressant effect of antipyrine on the strength of the heart or upon the blood pressure. As a matter of fact, when caffeine was injected at the time when the heart was most depressed or just beginning to recover from the antipyrine, caffeine appeared to delay the recovery or to cause a secondary weakening in both the heart strength and the blood pressure. Some antagonism was shown upon the heart rate which was restored to normal by the caffeine injections.

GENERAL TOXIC ACTION.

The general toxic effect of antipyrine and mixtures containing antipyrine when given hypodermically to mice was the subject of a further series of experiments. The easy solubility of antipyrine in water obviated the introduction of the additional factor, alcohol, into the problem, as in the case of acetanilide. The drug was given by the method already described and the minimum lethal dose determined. This was found to be 0.0010 gram, which amount caused death in about half an hour. By comparison, it will be noted that this is not only a smaller dose than was required in the case of acetanilide, but also that the time the animal survived was much shorter. The reason for this apparently greater toxicity (upon the frog's heart about seven times less toxic) when compared with acetanilide is probably dependent upon the relative solubility of the two drugs in the fluids of the tissues, and hence upon the rate of their absorption. Table IV gives protocols showing the determination of the minimum lethal dose for this drug.

TABLE IV.—*Minimum lethal dose of antipyrine for white mice, hypodermic injection.*

[−=survived; +=death.]

Weight.	Dose.	Result.	Minutes till death.
14.33	0.0008	−	.....
20.00	.0008	−	.....
19.70	.0009	−	.....
17.17	.0010	+	30
19.92	.0010	+	80
15.47	.0011	+	25
18.89	.0012	+	35

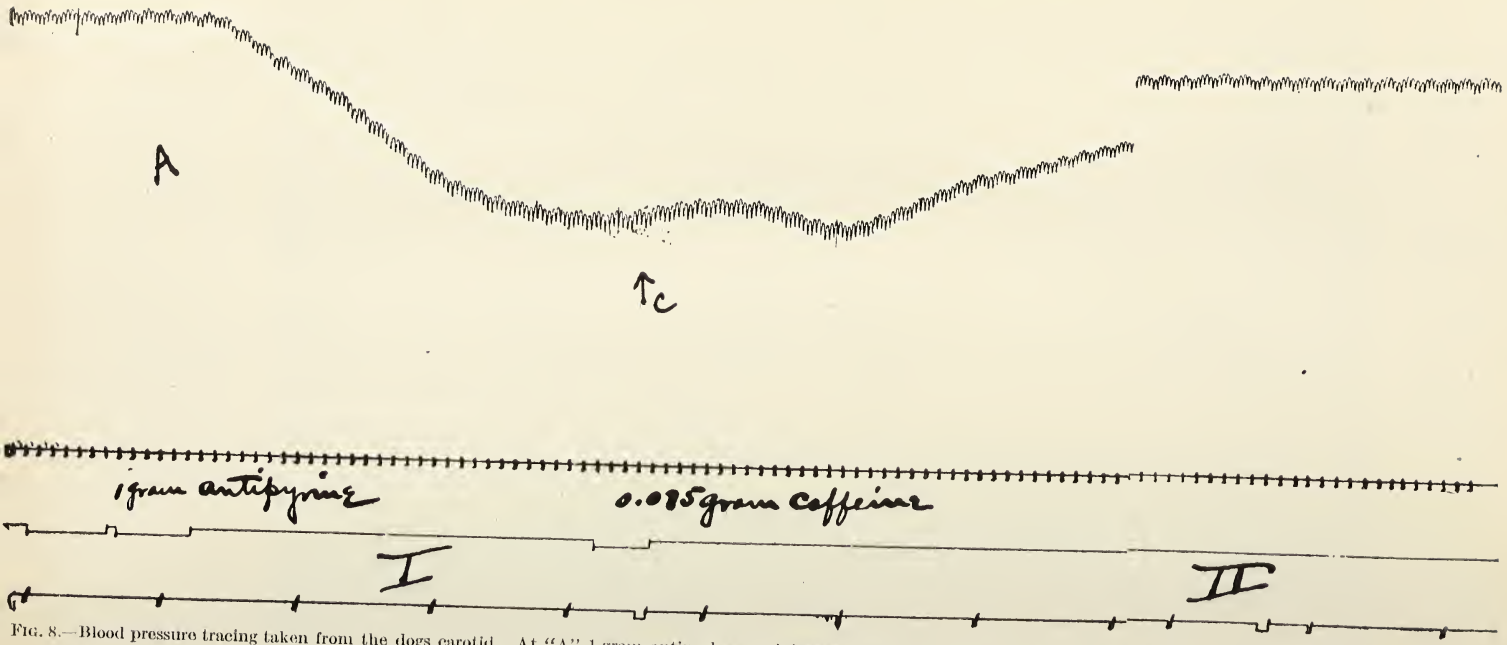

FIG. 8.—Blood pressure tracing taken from the dogs carotid. At "A" 1 gram antipyrine was injected causing a fall in pressure of 58 mm. mercury. The injection of caffeine at "C" indicates a very slight depressant action. II indicates the recovery from the drugs; taken after an interval of 3 minutes.

The mice being of the same lot as in the determination of the minimum lethal dose for caffeine, this was not redetermined in these experiments.

The experiments with acetanilide and caffeine having shown no antagonistic action, the experiments using a mixture of antipyrine and caffeine were begun with relatively small doses. Half the sums of the minimum lethal dose for each drug were used as a basis for estimating the probable amount of the mixture necessary to cause death. Antipyrine and caffeine were given in varying proportions, except that the relative amount of antipyrine was always the greater. The result of the whole series was to show that the two drugs were not antagonistic, death resulting invariably from half the sums of the doses of the two drugs, and when exhibited in smaller amounts failing to kill. This indicates mere summation, but no synergistic action, as in the case of acetanilide-caffeine mixtures. Protocols taken from this series of experiments are given in Table V.

TABLE V.—*Determination of the minimum lethal dose of a mixture of antipyrine and caffeine citrate for white mice, hypodermic injection.*

[−=survival; +=death; A=antipyrine; C=caffeine.]

Weight.	Dose per gram body weight.	Result.	Hours till death.
13.49...	A 0.0007 C .0001	−	.....
15.91...	A .0007 C .0001	−	.....
15.14...	A .0007 C .0002	+	2.50
13.28...	A .0008 C .0001	+	2.35
13.17...	A .0008 C .0001	+	16.10
16.53...	A .0006 C .0003	−	.....
14.82...	A .0006 C .0003	+	1.35
15.92...	A .0006 C .0003	+	2.47
13.20...	A .0006 C .0003	+	1.30

FEEDING EXPERIMENTS.

Guinea pigs were given a mixture composed of antipyrine and caffeine citrate to determine whether any modification in the toxicity of the former resulted. The method used was practically the same as that used in the experiments on acetanilide, the only modification being in the method of administration. Since both drugs are easily soluble in water, they were given in solution by means of a stomach tube (a small sized, semielastic urethral catheter). No control experiments were carried out with caffeine citrate, the control experiments of the acetanilide series serving this purpose, as the animals in both

cases belonged to the same lot, and the experiments were carried out at the same time. Protocols of the control experiments using antipyrine and of the experiments using a mixture of antipyrine and caffeine citrate are given in the tables which follow.

Control—antipyrine.

[Determination of the minimum lethal dose of antipyrine for guinea pigs. — Survived; + death.]

Weight in grams.	Dose per gram.	Result.	Hours till death.
465	0.0011	—	.....
415	.0012	—	.....
345	.0012	—	.....
395	.0013	—	.....
380	.0014	+	7.30
365	.0014	+	10.45
435	.0015	—	.....
435	.0015	+	11.45
445	.0016	+	12.45
470	.0016	+	3.00

A series of guinea pigs belonging to the same lot were given a mixture of antipyrine and caffeine in the determination of the least fatal dose, with results as follows:

[Antipyrine and caffeine mixture: Determination of the least fatal dose of a mixture of antipyrine and caffeine citrate for guinea pigs. —survived; +death.]

Weight in grams.	Dose per gram.	Result.	Hours till death.
375	A 0.0008 C .0002	—	.....
395	A .0008 C .0003	—	.....
340	A .0008 C .0003	—	.....
490	A .0010 C .0002	+	110
400	A .0010 C .0003	—	.....
400	A .0012 C .0002	+	12
490	A .0012 C .0002	+	8
475	A .0012 C .0003	+	7
400	A .0012 C .0003	+	7
495	A .0014 C .0002	+	4

Estimating the minimum lethal dose of antipyrine for guinea pigs as 0.0014 gram per gram body weight, the experiments with a mixture of antipyrine and caffeine indicate an increased toxicity to the simple drug, the amount of antipyrine producing death in the mixture being 0.0012 gram per gram body weight, or an increased toxicity in the inverse ratio of 12 to 14.

TOXICITY OF ANTIPYRINE AND SODIUM BICARBONATE.

Guinea pigs were given doses of equal parts of antipyrine and sodium bicarbonate to see whether any antagonism, as was apparent in the case of acetanilide, could be developed. The control experiments using antipyrine alone are tabulated on the preceding page. No controls using sodium bicarbonate were thought necessary on account of its nonpoisonous character. The results of the experiments using the mixture of antipyrine and sodium bicarbonate are tabulated below.

[Antipyrine and sodium bicarbonate: Determination of the minimum lethal dose for guinea pigs of a mixture of antipyrine and sodium bicarbonate. — Survived; + death; A, antipyrine; S, sodium bicarbonate.]

Weight in grams.	Dose per gram.	Result.	Hours till death.
315	A 0.0012 S .0012	—	.....
365	A .0012 S .0012	—	.....
485	A .0014 S .0014	+	18
420	A .0014 S .0014	+	15
445	A .0016 S .0016	+	9
355	A .0016 S .0016	+	8

These protocols indicate that a mixture containing sodium bicarbonate is of approximately the same toxicity as antipyrine given alone, the minimum lethal dose being 0.0014 gram per gram body weight in each case. A lessened toxicity is suggested by the longer period of life after equivalent doses of the mixture, but at best this is so slight as to be almost negligible.

SALIPYRIN.

Salipyrin, a chemical combination of antipyrine and salicylic acid, was made the subject of a further series of experiments in order to compare its toxicity with a simple mixture of antipyrine and salicylic acid when given in the same proportions as occurs in the chemical compound. Special interest in this comparison was stimulated by the abstracts and reprints sent out by the American firm selling this product, since these invariably point out its nontoxic character. These may be abstracted as follows:

Lohman[a] reported that it was more active than its components and was free from the secondary action so often observed after anti-

[a] Lohman, Deutsch. med. Ztg., 1903, XXIV, 1142.

pyrine. Lubowski[a] stated that there was complete absence of secondary effects such as are common when its components are used. According to Buettner[b] the dangerous heart effect of antipyrine is avoided by its administration as salipyrin. Muhlbauer[c] reported no bad effects subsequent to a dose of 10 grams of the drug.

In entire disagreement to these reports a large number of others[d] have appeared reporting the deleterious effects from the use of salipyrin even in small doses. The symptoms generally present were various skin eruptions, burning in the region of the stomach, profuse sweating, dilated pupils, great air hunger, marked heart distress, and fear of impending death. In Dumstrey's cases these symptoms appeared after 1 gram of the drug had been taken.

As will be recognized, these are the ordinary symptoms associated with antipyrine or with salicylic acid poisoning, and they certainly do not bear out the statements found in the advertising literature as to the absence of toxic and secondary symptoms. From the comparatively large number of cases of poisoning it would seem probable also that the salipyrin was fully as toxic as a simple mixture of its components. Theoretically also this would seem probable, since this compound is broken up in the body into its constituents,[e] and it would therefore produce an effect in the body similar to that of the two drugs from which it is compounded.

In proof of this point the following experiments were carried out to determine the relative toxic values for animals of salipyrin and of a mixture composed of antipyrine and salicylic acid in the same proportions as occur in the chemical compound. In the first series of experiments the drugs were injected beneath the skin of the back of white mice. Mice belonging to the same lot and kept under the same conditions were weighed and the dose given was estimated upon the basis of grams of body weight. On account of the insolubility of salipyrin and salicylic acid in water it was found necessary to dissolve the drugs in 50 per cent alcohol in such amounts that 1 c. c. of the solution represented 100 milligrams of salipyrin in one case and 57.7 milligrams antipyrine and 42.3 milligrams of salicylic acid, the proportionate amount of these drugs entering into the compound, in the other. The amount of alcohol injected in this way is so small that the chief symptom from it was merely some unsteadiness in the animal's

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[a] Lubowski, Allg. med. Centr.-Ztg., 1903, LXXII, 682.

[b] Buettner, Cor. Bl. f. schweiz. Aerzte, 1900, XXX.

[c] Muhlbauer, Wien. med. Wochenschr., 1897, XLVII, 196.

[d] Schmey, Therap. Monatshefte, 1897, XI, 175. Dumstrey, Deutsche med. Wochenschr., 1903, XXIX, 461. Dittmer, Med. Woche, 1903, IV, 579. Scharfe, Therap. Monatshefte, 1903, XVII, 163. Ritter, Berl. klin. Wochenschr., 1908, XLV, 338.

[e] Cushny, Pharmacology and Therapeutics, 1906, p. 380.

movements. After about twenty minutes this was followed by lessened movements, the animal sitting quietly and acting as if cold. The fur was roughened and occasionally slight convulsions appeared. No difference in the symptoms of poisoning could be determined in the two series of experiments. The toxicities of the mixture and of the compound, salipyrin, were also approximately the same as is shown by the protocols given in the following table:

SERIES I.

[Effect of salipyrin and of an antipyrine-salicylic acid mixture upon white mice, hypodermic injection.]

Dose per gram body weight, in grams.	Salipyrin.	A. and S. mixture.
	Hours till death.	Hours till death.
0.0010	0	0
.0011	0	3.39
.0012	2.33	2.47
.0014	1.25	1.03

SERIES II.

Dose per gram body weight, in grams.	Salipyrin.	A. and S. mixture.
	Hours till death.	Hours till death.
0.0013	2.12	1.44
.0013	2.21	2.27
.0013	3.02	2.50

Feeding experiments were also carried out to determine the toxic effect of salipyrin and of the antipyrine-salicylic acid mixture when given in a manner more closely simulating therapeutic administration. Guinea pigs of about the same weight and belonging to the same lot were used in the first series. The dose was estimated per gram body weight, each dose being weighed separately and made up into pills of suitable size and then fed in such a manner that none of the drug was lost in their administration. The results of the first series, using 0.0016 milligram per gram body weight, indicated that salipyrin was most toxic, two pigs receiving salipyrin dying after ten and twelve hours, respectively. Two days later a second dose was administered in the same manner to the surviving animals, using, however, 0.0018 milligram of the combination or of the mixture per gram body weight. In this series the toxicities appeared to be approximately the same,

as judged by the time the animals survived the introduction of the drug. The results are as follows:

SERIES I.

[Protocols of experiments to determine relative toxicity of salipyrin and of a mixture of antipyrine and salicylic acid when given to guinea pigs by the stomach. —=survived; +=death.]

[Salipyrin.]

Weight.	Dose.	Result.	Hours till death.
620	0.0016	+	12
480	.0016	+	9
470	.0016	—	.....
450	.0016	—	.....
430	.0016	—	.....
470	.0016	—	.....

[Antipyrine 57.76+salicylic acid 42.3 per cent.]

530	0.0016	—	.....
470	.0016	—	.....
610	.0016	—	.....
490	.0016	—	.....

SERIES II.

[Salipyrin.]

Weight.	Dose.	Result.	Hours. till death.
530	0.0018	+	3.30
405	.0018	+	5
410	.0018	+	6
420	.0018	+	14

[A. and S. mixture.]

455	0.0018	+	2.30
405	.0018	+	3.30
505	.0018	+	5
430	.0018	+	26

Feeding experiments were carried out on white mice, using the method already described for acetanilide, page 29. Each cake contained 0.100 gram salipyrin as a control; the cakes made up with the simple mixture each contained 0.0577 gram antipyrine and 0.0423 gram salicylic acid. In other words, the control cakes contained just the same proportionate amount of the two drugs as did those containing the mixture.

The feeding of these cakes to mice obtained from the same lot was followed by a slow but equal decrease in weight, and the duration of life in the case of the mice receiving the mixture and those receiving the same

amount of drugs chemically combined was approximately the same. The protocols taken from these experiments are as follows:

Control, salipyrin.

[Feeding mice salipyrin 0.100 gram per cake. − = survived; + = death.]

Date.	Weight of mice, in grams.			
	No. 1.	No. 2.	No. 3.	No. 4.
1909.				
February 13	19.84	18.36	18.08	17.71
February 17	19.19	17.16	16.70	17.05
February 20	20.12	18.50	18.47	16.76
February 24	19.30	17.74	18.08	16.10
February 27	18.89	17.30	16.99	14.65
March 1	17.40	16.34	15.45	14.30
March 3	15.99	15.19	15.23	13.30
March 6	13.95	14.85	14.76	.....
March 9	15.62	15.57	.....	.....
March 11	14.02	14.14	.....	.....

No. 1, dead March 2, 27 days; No. 2, dead March 11, 26 days; No. 3, dead March 7, 22 days; No. 4, dead March 6, 21 days.

In like manner a series of mice were fed upon a mixture of antipyrine and salicylic acid given in the same proportions in which they combine and form salipyrin. The results of these experiments are given below.

Antipyrine-salicylic acid mixture.

[Feeding mice antipyrine, 0.0577 gram plus salicylic acid 0.0423 gram per cake.]

Date.	Weight of mice, in grams.			
	No. 1.	No. 2.	No. 3.	No. 4.
1909.				
February 13	22.51	19.75	16.15	20.04
February 17	21.19	18.92	15.71	19.70
February 20	22.09	18.46	15.81	18.30
February 24	21.54	16.86	14.50	18.38
February 27	20.34	16.01	14.40	16.85
March 3	18.36	14.28	13.39	16.30
March 6	16.57	13.46	12.62	.....
March 9	16.08	13.92	.....	.....

No. 1, dead March 10, 25 days; No. 2, dead March 10, 25 days; No. 3, dead March 7, 22 days; No. 4, dead March 5, 20 days.

The average duration of life for mice fed salipyrin was twenty-four days; for mice receiving the mixture, twenty-three days—a difference that is quite negligible in experiments of this sort, on account of the small toxic action of the drugs used.

In conclusion, it may be said that the results of the whole series of experiments indicate no change in the toxicity of a mixture of antipyrine and salicylic acid when formed into a definite compound by chemical means.

LIST OF HYGIENIC LABORATORY BULLETINS OF THE PUBLIC HEALTH AND MARINE-HOSPITAL 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 formaldehyd 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.

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*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.

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*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.

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*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.

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4. A reexamination of the original specimen of *Tænia saginata abietina* (Weinland, 1858), by Ch. Wardell Stiles and Joseph Goldberger.

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

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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 echidninus*). By W. W. Miller.

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No. 49.—Digests 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, L. 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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Public Health and Marine-Hospital Service of the United States

HYGIENIC LABORATORY.—BULLETIN No. 54.

JULY, 1909

THE FIXING POWER OF ALKALOIDS ON VOLATILE ACIDS AND ITS APPLICATION TO THE ESTIMATION OF ALKALOIDS WITH THE AID OF PHENOLPHTHALEIN OR BY THE VOLHARD METHOD

By

ELIAS ELVOVE

WASHINGTON

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THE FIXING POWER OF ALKALOIDS ON VOLATILE ACIDS AND ITS APPLICATION TO THE ESTIMATION OF ALKALOIDS WITH THE AID OF PHENOLPHTHALEIN OR BY THE VOLHARD METHOD.[a]

By Elias Elvove.

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The volumetric estimation of the vegetable alkaloids has been a subject of much study, and numerous methods have been proposed which, with more or less success, have been practiced by various authors. The desirability of perfecting a suitable volumetric method for the quantitative determination of this class of substances grows out not only of the general advantages of rapidity and ease of manipulation which volumetric methods in general possess over gravimetric processes, but also because in this case the most commonly practiced gravimetric processes are open to serious errors which render them quite unreliable. Thus Kebler[b] expresses this fact in connection with the estimation of the vegetable alkaloids in the following words:

> From the hundreds of assays made by the author, he feels justified in stating that all of the gravimetric processes yield products containing considerable non-alkaloidal matter, and hopes that the day is not far distant when all gravimetric results will at least be supplemented by volumetric methods, if not displaced by them.

Most of the volumetric methods given in the literature for the estimation of this class of substances naturally fall into two classes:

(1) Those which are based upon the precipitation of the alkaloid from solution and measuring the amount of reagent required to effect complete precipitation.

(2) Those which are based upon the alkaline nature of most alkaloids toward certain indicators and measuring the amount of acid required for complete neutralization.

As the most important of the methods belonging to class (1) we may mention the various modifications of the old and well known method of estimating alkaloids by means of potassium mercuric iodide

[a] Manuscript submitted for publication July 9, 1909.

[b] Jour. Amer. Chem. Soc., **17**, 822–831 (1895).

(Mayer's reagent), as well as those methods which employ various modifications of Wagner's reagent (a solution of iodine in potassium iodide) as the precipitant, chief among which we may place Kippenberger's[a] process. To class (2) we may assign all the acidimetric or alkalimetric processes, including Gordin's[b] method.

When we compare the merits of the methods of class (1) with those of the acidimetric or alkalimetric processes included under (2), we find them to be largely in favor of the latter. For not only do the former lack the advantage of being based upon a more or less general characteristic property of the alkaloids, namely the alkaline nature of most alkaloids toward certain indicators, as is the case with the latter, but they also possess serious inherent defects. Thus Linde[c] points out among other things, that the precipitate formed with Mayer's reagent has no definite composition, that an allowance has to be made for its solubility, and that the end point of the reaction is affected by the concentration of the alkaloid in the solution titrated: while according to Scholtz[d] the method of Kippenberger is so untrustworthy as to be entirely inapplicable.

The acidimetric or alkalimetric methods are thus left to us as the only ones wherein we may look for a satisfactory volumetric method for the estimation of the vegetable alkaloids. With more or less success such methods have been worked out for most of the more common alkaloids through the employment of a number of various indicators. Thus, according to Kippenberger,[e] satisfactory results may be obtained, with lacmoid as indicator, in titrations of atropine, morphine, veratrine, codeine, cocaine, nicotine, coniine, papaverine, or narcotine: with iodeosin as indicator, in titrations of thebaine, codeine, emetine, or coniine; with cochineal as indicator, in titrations of coniine, morphine, thebaine, emetine, brucine, or pelletierine; with azolitmin as indicator, in titrations of aconitine, strychnine, or quinine; while in the U. S. Pharmacopœia (1905) hæmatoxylin or iodeosin are recommended for use in various alkaloidal assays.

A closer examination of the literature, however, shows that there exists considerable difference of opinion, and even contradiction, as to the suitability of the various indicators which have been proposed for use in the estimation of the vegetable alkaloids. Thus Kippenberger's statement cited above, in which he recommends azolitmin in titrations of quinine, is contradicted by Rammstedt,[f] who denies the applicability of azolitmin as indicator in quinine titrations: the

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[a] Zeit. anal. Chem., **35**, 10–27 and 422–471 (1896).
[b] Ber., **32**, 2871–2876 (1899).
[c] Arch. Pharm., **237**, 172–185 (1899).
[d] Arch. Pharm., **237**, 71–80 (1899).
[e] Zeit. anal. Chem., **39**, 201–229 (1900).
[f] Apoth. Zeit., **22**, 1117 (1907).

German Pharmacopœia[a] recommends hæmatoxylin as indicator in the titration of quinine, while according to Hille[b] there is an error when quinine is titrated with hæmatoxylin as indicator which is in many cases equivalent to a whole cubic centimeter of N/10 alkali. Likewise, Messner[c] has tested the various indicators recommended for the purpose of titrating the cinchona alkaloids and found most of them, with the exception of lacmoid, to be unsuitable. Similarly, the adoption of hæmatoxylin as indicator in certain of the alkaloidal assays given in the U. S. Pharmacopœia (1905) is condemned by Lyons[d] and also by Francis,[e] who prefer cochineal as the indicator. Finally, we may perhaps best illustrate the imperfect condition of the acidimetric processes for estimating alkaloids by the use of various indicators, each more or less suitable to particular alkaloids, by the circumstance that the revisers of the U. S. Pharmacopœia (1905), at present official, apparently deemed it better to include no quantitative method whatever for the determination of the actual amount of alkaloid in specimens of such an important alkaloid as quinine,[f] for example, rather than adopt an acidimetric estimation by means of any of the several indicators proposed. And the reason for such course is also readily seen; for having apparently selected iodeosin and hæmatoxylin as the most suitable indicators in the alkaloidal titrations, the former could not be used in the case of quinine, since it is entirely unsuitable as an indicator in quinine titrations, as shown by Kippenberger,[g] while hæmatoxylin, although better suitable than iodeosin, is still incapable of yielding satisfactory results, as pointed out by Hille.[h]

Another method for the estimation of alkaloids, in which the indicator employed is phenolphthalein, is the method proposed by Gordin.[h] This author found that the periodides or mercuriodides of alkaloids, precipitated by Wagner's and by Mayer's reagent respectively, always carry down with them an amount of hydriodic acid which appeared to be proportional to the amount of alkaloid in the solution. Gordin therefore sought to apply this property of alkaloids to their volumetric estimation with the aid of phenolphthalein as indicator. His method as applied to morphine, for example, is as follows: About 0.2 gm. of the sample of morphine to be examined is dissolved in 30 cc. of standard hydrochloric acid (about N/20) in a 100 cc. flask, Wagner's reagent (containing about 1 per cent of free

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[a] Apoth. Zeit., **22,** 1117 (1907).
[b] Arch. Pharm., **241,** p. 106 (1903).
[c] Zeit. angew. Chem., **16,** 444–450 and 468–477 (1903).
[d] Proc. A. Ph. A., **54,** 441 (1906).
[e] Proc. A. Ph. A., **54,** 454 (1906).
[f] U. S. Pharmacopœia (1905), pp. 373–4.
[g] Zeit. anal. Chem., **39,** 205 (1900).
[h] Loc. cit.

iodine and 1.5 per cent of potassium iodide) added gradually, the whole well shaken after each addition until further addition of the reagent produces no further precipitate, when the contents are diluted to 100 cc. and again well shaken. After the precipitate has settled thoroughly the liquid is filtered, 50 cc. of the red filtrate decolorized by the gradual addition of 10 per cent sodium thiosulphate, a few drops of phenolphthalein added, and the excess of acid titrated with N/20 potassium hydroxide. It is found that 1 cc. of the acid is removed by 0.0137 gm. of morphine, from which the proper calculation will show the percentage of alkaloid in the specimen examined. This method is, of course, quite simple, and the possibility of using phenolphthalein as the indicator is an advantage hardly to be overestimated, and which advantage the author rightly considers so important as to bring it out in the title[a] of the communication in which the method is described. Unfortunately, however, it has been found that the amount of acid carried down by the precipitate formed on addition of Mayer's or Wagner's reagent is not a true measure of the amount of alkaloid in the solution. Thus Kippenberger,[b] who has investigated Gordin's method, states that his experiments show that the results obtained by this method, whether using Wagner's or Mayer's reagent, are so profoundly influenced by the proportion of free acid, as well as by that of the potassium iodide in the solution, that they are useless for quantitative purposes, being not only far too high, but also extremely irregular. Likewise, Gordin's suggestion that the acid should be standardized by a known quantity of morphine is also found by Kippenberger to be unserviceable, since equivalent quantities of morphine and strychnine titrated under identical conditions gave widely different results.

On the other hand, as early as 1887, Plugge[c] showed that, in general, in solutions of the salts of the alkaloids the free acid can be determined by titration with litmus while the total quantity of acid (free and combined) by titrating with phenolphthalein as indicator; since the latter indicator is neutral toward most of the vegetable alkaloids, and hence the acid combined with the alkaloid as salt acts toward it as if it were free acid. In adopting such a plan to the estimation of the free alkaloids, however, as is done by Barthe,[d] the advantage in the use of phenolphthalein is lost, since the result obtained is necessarily dependent on the accuracy with which the litmus indicator will show the actual amount of acid in excess of that which is combined with the alkaloid; and, in fact, the use of

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[a] The title of Gordin's paper is: Simple Alkalimetric Method for the Estimation of Salt-forming Alkaloids with the Aid of Phenolphthalein as Indicator.

[b] Zeit. anal. Chem., **42**, 101–108 (1903).

[c] Arch. Pharm. (3), **25**, 45–49 (1887).

[d] Compt. Rend., **115**, 512–514 (1892).

the phenolphthalein in such connection would be entirely superfluous, since the alkaloid could be dissolved in an excess but definite amount of acid in the first place, and the actual excess of acid remaining uncombined determined by means of the litmus indicator. Further, litmus as an indicator in alkaloidal titrations does not appear to yield highly satisfactory results. Thus, according to Kebler,[a] litmus is not as good an indicator in alkaloidal titrations as is cochineal, Brazil wood, or hæmatoxylin, the last giving the best results, while the shortcomings of even hæmatoxylin have already been pointed out above.

It thus appears from the literature that although it is known that the salts of most of the vegetable alkaloids can be estimated by means of phenolphthalein as indicator, no application of this fact to the estimation of the purity of the uncombined alkaloids, as found in commerce or as obtained in the course of the various pharmacopœial assays, has been made where the phenolphthalein is used as the only alkalimetric indicator, and hence as the controlling factor of the accuracy of the titration. It therefore occurred to the writer that in the course of the various pharmacopœial assays, if instead of dissolving the alkaloid in an excess of sulphuric acid,[b] as is usually done, an excess of a volatile acid be employed, such as acetic or hydrochloric acid for example, a definite amount of the acid, proportional to the amount of alkaloid present, would become fixed by combining with the alkaloid, and which combination of alkaloid and acid might be found stable at the temperature employed for volatilizing the excess of acid remaining free. If this were done, the amount of acid thus becoming fixed could in most cases be determined with the aid of phenolphthalein as indicator, and hence indirectly also the amount of alkaloid. And if hydrochloric acid[c] were chosen as the volatile acid to be thus employed, we could also estimate the alkaloids indirectly by determining the amount of chlorine in the alkaloidal residue by means of the well known and exact Volhard method; while the advantage thus to be gained in rendering it possible to estimate alkaloids indirectly by means of the Volhard method would perhaps be found of even wider application than the adaptation of phenolphthalein to their estimation alkalimetrically, since although phenolphthalein would be applicable as indicator in the titration of most of the alkaloids, yet in the cases of some of them, as, for example, morphine,[d] phenolphthalein does not show the end reaction very sharply. Again, it is evident that

[a] Loc. cit.

[b] U. S. Pharmacopœia (1905), pp. 28, 67, 107, 143, 146–147, 197, 200, 300, 340, 344.

[c] Hydrochloric acid is actually employed by the U. S. Pharmacopœia (1905) in a similar procedure for the *gravimetric* determination of coniine.

[d] Plugge: loc. cit.

where a change of color must be depended on for ascertaining the end of the reaction, the titration of colored liquids, which is not an infrequent occurrence in alkaloidal assaying, presents no little difficulty; and while phenolphthalein has in such cases the advantage over most other indicators in that the end reaction is not indicated simply by a change from one color to another, but from colorless to deep pink, still it must be conceded that the end reaction under such circumstances, even with phenolphthalein as the indicator, can not be obtained in all cases with as much precision as when a colorless liquid is titrated. If, however, we convert the estimation of the alkaloids into a simple chlorine determination, we can in many cases add to the solution containing the chlorine and acidified with nitric acid, a measured excess of a standard silver solution, filter, and determine the excess of silver in the filtrate: and if the latter happens to be too highly colored so as to obscure the action of the ferric indicator, it (or an aliquot portion) can be evaporated to dryness on the water bath and the organic matter, to which the color is usually due, gotten rid of by ignition, thus leaving the whole of the silver in condition to be taken up with nitric acid and titrated with standard thiocyanate in the usual way. In order to determine whether such a volumetric method would be practicable, the following experiments were carried out.

GENERAL MODE OF PROCEDURE.

It is evident that the practicability of such a method hinges almost entirely on whether or not the temperature employed for getting rid of the excess of the volatile acid, as well as the length of time such temperature is allowed to act, will always insure the complete removal of the free acid but leave the portion which has become fixed in a stable condition, and therefore also in constant proportion to the amount of alkaloid present. Hence in taking up any alkaloid it was first determined whether the combination of alkaloid and acid resulting from the evaporation to dryness of a given amount of the alkaloid dissolved in an excess of the volatile acid would remain stable, at the temperature chosen, for a time sufficiently long as to permit of its being considered stable at that temperature for the purpose in view. The temperature chosen was that of the boiling water bath (90–95 C.), since this is the most convenient and almost universally accessible, as well as requiring little or no attention in maintaining it at a reasonably constant temperature. Having found that such stability exists with the amount taken, quantities of the alkaloid varying between certain limits, which might conveniently be used in actual practice, were then similarly treated, keeping both the temperature and time constant. The general mode of procedure was as follows:

The theoretical amount of the pure alkaloid required to make a fiftieth-molar solution (one-fiftieth of the molecular weight in grams per liter) was carefully weighed out and dissolved in N/2 hydrochloric acid (in a few instances N/2 acetic acid was used instead), using sufficient of the latter to yield the required volume of solution. Equal volumes (10 or 20 cc.) of such solution were then transferred to porcelain dishes and the latter with their contents placed on the water-bath (90–95 C.), where they were kept until the liquid had completely evaporated, when the time was noted and thereafter, at intervals of half an hour, one of the dishes was removed and allowed to cool to room temperature. The residues in the dishes were then taken up with distilled water (usually 10 cc.), three drops of phenolphthalein indicator[a] added, and the acidity of the solution determined by means of N/10 sodium hydroxide. It was found that in the case of some of the alkaloids studied (e. g., quinine) when a drop of the phenolphthalein solution is added to the alkaloidal salt solution which had been titrated as just described until a light-pink color developed, that such additional drop of the phenolphthalein, especially when comparatively larger amounts of the alkaloid are present, causes the light-pink color to at once turn very deep pink. Hence at the end of each titration an additional drop of the phenolphthalein indicator was added in all cases, and when this caused the color to deepen very much, standard acid was added until only a light-pink color remained, an amount of alkali equivalent to the amount of acid thus used up being deducted from the total amount of alkali added in the first place, and the difference taken as the amount of standard alkali required to neutralize the acidity of the solution; it having been found that such procedure yielded results quite close to that theoretically required, whereas if the numbers representing the first titrations with the alkali were taken, the results were somewhat too high. After the acidity of the alkaloidal salt solution had been neutralized and the amount of alkali thus determined, the chlorine in the solution was determined by means of Volhard's thiocyanate method. And when it was found that a product of constant acidity is obtained under conditions of time varying, temperature and amount of alkaloid remaining constant, the experiments were then repeated with these conditions reversed, namely, amount of alkaloid varying while both time and temperature remained constant. The amount of hydrochloric acid becoming fixed being, likewise here, determined both alkalimetrically and by the Volhard method. The results obtained with the alkaloids cinchonine, quinidine, cinchonidine, quinine, and strychnine are given in the following tables:

[a] Prepared according to the U. S. Pharmacopœia, 1905, page 544.

CINCHONINE.

A M/50 cinchonine solution was prepared by dissolving 2.94 gm. of the pure anhydrous alkaloid in N/2 HCl and using the same acid to make up the total volume to 500 cc. The results obtained with this solution are given in Tables I and II.

TABLE I.

Effect of the temperature of the water bath (90–95 C.) on the stability of freshly-formed cinchonine hydrochloride.

Time remained on water bath after complete evaporation of liquid.	M/50 cinchonine taken.	N/10 NaOH required.	N/10 $AgNO_3$ required.	Amount of HCl found.[a]
Minutes.	*cc.*	*cc.*	*cc.*	*Gram.*
30	10	4.05	4.10	0.0148
60	10	4.00	4.04	.0146
90	10	4.00	4.02	.0146
120	10	3.95	4.00	.0144
150	10	4.00	3.98	.0146
180	10	4.03	4.00	.0147
210	10	3.98	3.96	.0145
240	10	4.00	3.94	.0146
270	10	3.95	4.00	.0144
300	10	4.02	4.00	.0147
330	10	3.95	3.98	.0144
360	10	4.00	3.96	.0146

Amount of HCl theoretically required for $C_{19}H_{22}N_2O.2HCl=0.0146$.

TABLE II.

Effect of varying the amount of cinchonine on the constancy of the proportion of HCl in the alkaloidal residue.

Temperature as in Table I; time, 3 hours.

M/50 cinchonine taken.	N/10 NaOH required.	N/10 $AgNO_3$ required.	Ratio between cc. cinchonine sol. taken and cc. N/10 NaOH required.[a]
cc.	*cc.*	*cc.*	
5	2.00	1.96	2.50
10	3.98	3.96	2.51
20	8.10	8.04	2.47
30	12.10	11.94	2.48
40	16.15	16.10	2.48
50	20.20	20.06	2.48

[a] All calculations were based on the results obtained on titration with the N/10 NaOH.

QUINIDINE.

A M/50 quinidine solution was prepared by dissolving 3.24 gm. of the pure anhydrous alkaloid in N/2 HCl and using the same acid to make up the total volume to 500 cc. The results obtained with this solution are given in Tables III and IV.

Table III.

Effect of the temperature of the water bath (90–95 C.) on the stability of freshly-formed quinidine hydrochloride.

Time remained on water bath after complete evaporation of liquid.	M/50 quinidine taken.	N/10 NaOH required.	N/10 $AgNO_3$ required.	Amount of HCl found.
Minutes.	*cc.*	*cc.*	*cc.*	*Gram.*
30	10	4.08	4.04	0.0149
60	10	4.03	4.00	.0147
90	10	4.00	3.96	.0146
120	10	4.02	3.98	.0147
150	10	3.98	4.06	.0145
180	10	4.00	3.98	.0146
210	10	3.95	3.98	.0144
240	10	4.02	4.04	.0147
270	10	4.00	4.00	.0146
300	10	3.95	3.96	.0144
330	10	4.00	3.98	.0146
360	10	3.98	3.96	.0145

Amount of HCl theoretically required for $C_{20}H_{24}N_2O_2 2HCl = 0.0146$.

Table IV.

Effect of varying the amount of quinidine on the constancy of the proportion of HCl in the alkaloidal residue.

Temperature as in Table III; time, 3 hours.

M/50 quinidine taken.	N/10 NaOH required.	N/10 $AgNO_3$ required.	Ratio between cc. quinidine sol. taken and cc. N/10 NaOH required.
cc.	*cc.*	*cc.*	
5	1.95	1.96	2.56
10	3.95	3.90	2.53
20	8.10	7.96	2.47
30	12.15	12.16	2.47
40	16.25	16.32	2.46
50	20.28	20.36	2.47

CINCHONIDINE.

A M/50 cinchonidine solution was prepared by dissolving 2.94 gm. of the pure anhydrous alkaloid in N/2 HCl (or N/2 acetic acid) and using the same acid to make up the total volume to 500 cc. The results obtained with these solutions are given in Tables V, VI, and VII.

TABLE V.

Effect of the temperature of the water bath (90–95 C.) on the stability of freshly-formed cinchonidine hydrochloride.

Time remained on water bath after complete evaporation of liquid.	M/50 cinchonidine taken.	N/10 NaOH required.	N/10 $AgNO_3$ required.	Amount of HCl found.
Minutes.	*cc.*	*cc.*	*cc.*	*Gram.*
30	10	4.10	4.06	0.0150
60	10	4.08	4.00	.0149
90	10	4.00	3.98	.0146
120	10	4.05	4.00	.0148
150	10	3.98	4.00	.0145
180	10	4.03	3.96	.0147
210	10	3.95	4.04	.0144
240	10	4.00	4.02	.0146
270	10	4.00	3.96	.0146
300	10	3.98	3.96	.0145
330	10	4.00	3.98	.0146
360	10	3.95	4.00	.0144

Amount of HCl theoretically required for $C_{19}H_{22}N_2O.2HCl=0.0146$.

TABLE VI.

Effect of varying the amount of cinchonidine on the constancy of the proportion of HCl in the alkaloidal residue.

Temperature as in Table V; time, 3 hours.

M/50 cinchonidine taken.	N/10 NaOH required.	N/10 $AgNO_3$ required.	Ratio between cc. cinchonidine sol. taken and cc. N/10 NaOH required.
cc.	*cc.*	*cc.*	
5	1.95	1.98	2.56
10	4.03	3.96	2.49
20	8.05	8.00	2.48
30	12.15	12.16	2.47
40	16.17	16.10	2.47
50	20.25	20.18	2.47

TABLE VII.

Effect of the temperature of the water bath (90–95 C.) on the stability of freshly-formed cinchonidine acetate.

Time remained on water bath after complete evaporation of liquid.	M/50 cinchonidine taken.	N/10 NaOH required.	Amount of acetic acid found.
Minutes.	*cc.*	*cc.*	*Gram.*
30	10	3.80	0.0228
60	10	3.33	.0200
90	10	3.20	.0192
120	10	3.03	.0182
150	10	3.00	.0180
180	10	3.00	.0180
210	10	2.85	.0171
240	10	2.62	.0157
270	10	2.60	.0156
300	10	2.33	.0140
330	10	2.00	.0120
360	10	2.15	.0129

Amount of $C_2H_4O_2$ theoretically required for $C_{19}H_{22}N_2O.C_2H_4O_2$=0.0120 gm.

QUININE.

A M/50 quinine solution was prepared by dissolving 3.24 gm. of the pure anhydrous alkaloid in N/2 HCl (or N/2 acetic acid) and using the same acid to make up the total volume to 500 cc. The results obtained with these solutions are given in Tables VIII, IX, and X.

TABLE VIII.

Effect of the temperature of the water bath (90–95 C.) on the stability of freshly-formed quinine hydrochloride.

Time remained on water bath after complete evaporation of liquid.	M/50 quinine taken.	N/10 NaOH required.	N/10 $AgNO_3$ required.	Amount of HCl found.
Minutes.	*cc.*	*cc.*	*cc.*	*Gram.*
30	10	4.10	4.08	0.0150
60	10	4.03	4.00	.0147
90	10	4.05	4.00	.0148
120	10	4.00	3.96	.0146
150	10	3.95	3.96	.0144
180	10	4.02	4.00	.0147
210	10	4.00	3.98	.0146
240	10	3.98	3.94	.0145
270	10	4.00	3.98	.0146
300	10	3.95	4.00	.0144
330	10	4.00	3.98	.0146
360	10	3.98	3.96	.0145

Amount of HCl theoretically required for $C_{20}H_{24}N_2O_2.2HCl$=0.0146.

TABLE IX.

Effect of varying the amount of quinine on the constancy of the proportion of HCl in the alkaloidal residue.

Temperature as in Table VIII; time, 3 hours.

M/50 quinine taken.	N/10 NaOH required.	N/10 $AgNO_3$ required.	Ratio between cc. quinine sol. taken and cc. N/10 NaOH required.
cc.	*cc.*	*cc.*	
5	1.98	1.96	2.52
10	4.02	3.98	2.49
20	8.15	7.96	2.45
30	11.98	12.00	2.50
40	16.10	15.94	2.48
50	20.17	20.10	2.48

TABLE X.

Effect of the temperature of the water bath (90–95 C.) on the stability of freshly-formed quinine acetate.

Time remained on water bath after complete evaporation of liquid.	M/50 quinine taken.	N/10 NaOH required.	Amount of acetic acid found.
Minutes.	*cc.*	*cc.*	*Gram.*
30	10	2.48	0.0149
60	10	2.50	.0138
90	10	2.48	.0149
120	10	2.00	.0120
150	10	2.00	.0120
180	10	2.18	.0131
210	10	2.00	.0120
240	10	1.70	.0102
270	10	1.90	.0114
300	10	2.05	.0123
330	10	2.07	.0124
360	10	1.75	.0105

Amount of $C_2H_4O_2$ theoretically required for $C_{20}H_{24}N_2O_2$. $C_2H_4O_2$=0.0120 gm.

STRYCHNINE.

A M/50 strychnine solution was prepared by dissolving 3.34 gm. of the pure anhydrous alkaloid in N/2 HCl (or N/2 acetic acid) and using the same acid to make up the total volume to 500 cc. The results obtained with these solutions are given in Tables XI, XII, and XIII.

TABLE XI.

Effect of the temperature of the water bath (90–95 C.) on the stability of freshly-formed strychnine hydrochloride.

Time remained on water bath after complete evaporation of liquid.	M/50 strychnine taken.	N/10 NaOH required.	N/10 $AgNO_3$ required.	Amount of HCl found.
Minutes.	*cc.*	*cc.*	*cc.*	*Gram.*
30	20	4.05	4.02	0.0148
60	20	4.00	3.96	.0146
90	20	3.98	3.96	.0145
120	20	4.00	3.98	.0146
150	20	3.95	4.00	.0144
180	20	4.00	4.02	.0146
210	20	4.00	3.96	.0146
240	20	3.95	3.94	.0144
270	20	3.98	3.98	.0145
300	20	4.02	4.00	.0147
330	20	3.95	3.96	.0144
360	20	3.95	3.94	.0144

Amount of HCl theoretically required for $C_{21}H_{22}N_2O_2HCl = 0.0146$.

TABLE XII.

Effect of varying the amount of strychnine on the constancy of the proportion of HCl in the alkaloidal residue.

Temperature as in Table XI; time, 3 hours.

M/50 strychnine taken.	N/10 NaOH required.	N/10 $AgNO_3$ required.	Ratio between cc. strychnine sol. taken and cc. N/10 NaOH required.
cc.	*cc.*	*cc.*	
5	0.95	0.98	5.26
10	1.95	1.96	5.13
20	4.00	3.94	5.00
30	5.90	5.88	5.08
40	7.80	7.82	5.13
50	9.90	9.80	5.05

TABLE XIII.

Effect of the temperature of the water bath (90–95) on the stability of freshly-formed strychnine acetate.

Time remained on water bath after complete evaporation of liquid.	M/50 strychnine taken.	N/10 NaOH required.	Amount of acetic acid found.
Minutes.	*cc.*	*cc.*	*Gram.*
30	20	4.25	0.0255
60	20	1.70	.0102
90	20	1.50	.0090
120	20	.50	.0030
150	20	.15	.0009
180	20	.05	.0003
210	20	.02	.0001
240	20	.02	.0001
270	20	.02	.0001
300	20	.00	.0000
330	20	.00	.0000
360	20	.00	.0000

Amount of $C_2H_4O_2$ theoretically required for $C_{21}H_{22}N_2O_2.C_2H_4O_2 = 0.0120$ gm.

In the following table (Table XIV) are given the results obtained with solutions of varying amounts of alkaloid dissolved in an excess of hydrochloric acid, the amount of alkaloid, as well as the amount of hydrochloric acid being both unknown to the writer at the time when the determinations were carried out.

TABLE XIV.

(Unknown solutions submitted by Prof. J. H. Kastle.)

Showing the proportionality between the N/10 NaOH equivalent of the amount of alkaloid given and that found to require.

Temperature, 90–95 C.; time, 6 hours.

Name of alkaloid.	Amount given.	N/10 NaOH equivalent of amount given.	N/10 NaOH required.	Ratio between the N/10 NaOH equivalent and that found to require.
	Gram.	*cc.*	*cc.*	
Cinchonine	0.0770	5.24	5.20	1.01
Do	.1294	8.80	8.76	1.00
Do	.2646	18.00	18.00	1.00
Do	.0676	4.60	4.43	1.04
Do	.1940	13.20	13.10	1.01
Do	.1617	11.00	10.89	1.01
Strychnine	.1403	4.25	4.09	1.04
Do	.2939	8.80	8.76	1.00
Do	.0835	2.50	2.42	1.03
Do	.1937	5.80	5.59	1.04
Do	.2505	7.50	7.18	1.04
Do	.1136	3.40	3.26	1.04
Average				1.02

The results given in the accompanying tables appear, in the cases where hydrochloric acid was used as the volatile acid, sufficiently close to what is theoretically required as to justify the conclusion that the small deviation from the theory in some cases is due largely to experimental error, and hence that the alkaloids, the study of which is here presented, may be estimated with fairly close results by the procedure here adopted, whether we use the alkalimetric method with the aid of phenolphthalein or determine the chlorine by means of Volhard's thiocyanate method. For estimating the actual amount of alkaloid in specimens of these alkaloids we may therefore adopt the following procedure:

About 0.2 gm. of the specimen to be examined is dissolved in an excess of dilute hydrochloric acid (about 20 cc. of 4 per cent), the liquid completely evaporated, any film which may have formed over the surface is broken up with the aid of a glass rod, and the residue allowed to remain on the water bath (90–95 C.) for three hours. It is then taken up with distilled water (10–20 cc.), phenolphthalein (3 drops) solution added, and titrated with standard sodium hydroxide until the solution develops a light pink color. At this point one

more drop of the phenolphthalein indicator is added, and if the solution at once assumes a very deep pink color, standard acid is added until the color remaining is only light pink, the alkali equivalent of the acid thus added being deducted from the total alkali added first. Knowing that in the case of quinine, quinidine, cinchonine, or cinchonidine two molecules of the sodium hydroxide are equivalent to one molecule of these alkaloids, we can of course calculate the actual amount of alkaloid in the specimen under examination. Similarly, in the case of strychnine the calculation is made on the basis of one molecule of the sodium hydroxide being equivalent to one molecule of the strychnine. The result thus obtained may also be confirmed by determining the chlorine in the solution by means of the Volhard thiocyanate method. In the case of other alkaloids, no complete systematic investigation has yet been made, but it is highly probable that with perhaps some slight modification such procedure will be found applicable to most, if not all, the alkaloids usually met with in practice. In fact, some preliminary work with a number of other alkaloids would seem to indicate that such is really the case.

It is interesting to note in this connection that the results which might be obtained from a study of the fixing power of the alkaloids on volatile acids under exactly equal conditions and using the same indicator (instead of a large number of *different* indicators) in the titrations of all the alkaloids compared, as is here done for several of them, should furnish conclusive evidence as to the exact relation of the various alkaloids to acids, i. e., their combining power with acids, a subject which appears to still remain, at least as far as some of the alkaloids are concerned, surrounded by uncertainty. Thus Schimpf [a] expresses himself in this respect with reference to the cinchona alkaloids as follows:

> In the titration of cinchona alkaloids such anomalous results are obtained that there is some doubt as to whether the relation of these alkaloids to acids is thoroughly understood.

Further, as examples of monacid alkaloids, Schimpf [b] enumerates quinine, morphine, and strychnine. Similarly, the U. S. Pharmacopœia, as may be seen from the tables [c] of equivalents in which the alkaloids are enumerated, apparently regards such alkaloids as quinine and strychnine, for example, as being equal in their combining power with acids, assigning to both strychnine and quinine a monacid value. This same view is also manifested in the U. S. Pharmacopœia by the nomenclature which it adopts for the salts of such alkaloids as quinine. Thus the combination of one molecule of quinine and one

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[a] Schimpf: Manual of Volumetric Analysis, 5th ed., p. 502 (1909).
[b] Ibid., p. 498.
[c] U. S. Pharmacopœia (1905), pp. 555, 567, 568.

molecule of HCl, ($C_{20}H_{24}N_2O_2$.HCl), is given the name of a normal salt,[a] while the combination of one molecule of quinine with one molecule of sulphuric acid ($C_{20}H_{24}N_2O_2.H_2SO_4$) is called an acid salt[b] and that of two molecules of quinine to one of sulphuric acid ($C_{20}H_{24}N_2O_2)_2.H_2SO_4$, is given the analogous name[c] (quinine sulphate) of the corresponding strychnine salt,[d] strychnine sulphate, ($C_{21}H_{22}N_2O_2)_2.H_2SO_4$. In the light, however, of the results here recorded we can hardly look upon quinine, for example, as a monacid alkaloid, certainly not as having no greater combining power with acids than strychnine. For we here see that under the identical conditions (see Table XI) under which strychnine yields a stable combination ($C_{21}H_{22}N_2O_2$.HCl) of one molecule of strychnine to *one* molecule of HCl, one molecule of quinine (see Table VIII) requires *two* molecules of HCl to form a stable combination ($C_{20}H_{24}N_2O_2$.2HCl). And not only is this difference seen in the case of hydrochloric acid, but the same difference in their combining power with acids is shown also in the case of acetic acid. In the latter case, although it would seem at first (Table X, 180 to 330 minutes) as showing that quinine is a monacid alkaloid since what appears as the comparatively stable product under these circumstances contains only one molecule of the monobasic acid (acetic acid) to one molecule of the quinine, yet this very fact may be used in illustrating the difference between the acid combining powers of quinine and strychnine and as pointing to quinine being a diacid alkaloid. For when we examine the corresponding comparatively stable product of strychnine under the same conditions (Table XIII, 180 to 330 minutes), we see that the difference of one molecule of the monobasic acid still holds, since the stable strychnine product under the same conditions still has *one molecule of the monobasic acid less* than the corresponding quinine combination. In other words, we see that the *same procedure* (Table XI) which yields what is conceded to be the *normal* hydrochloride of strychnine ($C_{21}H_{22}N_2O_2$.HCl) gives in the case of quinine (Table VIII) a salt of the alkaloid ($C_{20}H_{24}N_2O_2$.2HCl) having two molecules of the HCl to one of the alkaloid; and similarly, the same procedure which yields in the case of strychnine (Table XIII, 180 to 330 minutes) what must be conceded to be a *basic* product (since it is practically the pure alkaloid) yields in the case of quinine (Table X, 180–330 minutes) a comparatively stable product whose composition corresponds closely to that of one molecule of the monobasic acid and one of the alkaloid ($C_{20}H_{24}N_2O_2.C_2H_4O_2$). Therefore it would seem more in harmony with the results here presented if we regarded the salts of quinine, for example, in which the latter acts as a diacid

[a] U. S. Pharmacopœia (1905), p. 376.
[b] Ibid., p. 374.
[c] Ibid., p. 377.
[d] Ibid., p. 427.

alkaloid, as the normal salts, while those in which only one of the acid affinities of the alkaloid is satisfied, as *basic* salts.

SUMMARY.

1. The literature is briefly reviewed and it is shown that although it is known that the salts of most of the alkaloids can be estimated by means of phenolphthalein as indicator, no application of this fact to the estimation of the purity of the uncombined alkaloids as found in commerce or as obtained in the course of the various pharmacopœial assays, has been made where the phenolphthalein is used as the only indicator and hence as the controlling factor of the accuracy of the titration.

2. It is therefore pointed out that in the course of the various pharmacopœial assays, if instead of dissolving the alkaloid in an excess of sulphuric acid, as is usually done, an excess of a volatile acid be employed, such as hydrochloric acid, a definite amount of the acid proportional to the amount of alkaloid present would become fixed by combining with the alkaloid and which combination of alkaloid and acid might be found stable at the temperature employed for volatilizing the excess of acid remaining free. In the present communication the alkaloids quinine, quinidine, cinchonine, cinchonidine, and strychnine are taken up, and it is shown that fairly close results may be obtained by such procedure, whether we use the alkalimetric method with the aid of phenolphthalein or determine the chlorine in the solution by means of Volhard's thiocyanate method.

3. It is further pointed out that the results which might be obtained from a study of the fixing power of the alkaloids on volatile acids under exactly equal conditions and using the same indicator (instead of a large number of *different* indicators) should furnish conclusive evidence as to the exact relation of the various alkaloids to acids, i. e., their combining power with acids, a subject which appears to still remain, at least as far as some of the alkaloids are concerned, surrounded by uncertainty. Thus the results obtained with strychnine and quinine, for example, show that the view that both quinine and strychnine are monacid alkaloids is not in entire harmony with these facts, and that it is perhaps more rational to look upon the salts of quinine, for example, in which the latter acts as a diacid alkaloid as the normal salts (instead of acid salts), while those in which only one of the acid affinities of the alkaloid is satisfied (at present generally regarded as the normal salts), as *basic* salts.

LIST OF HYGIENIC LABORATORY BULLETINS OF THE PUBLIC HEALTH AND MARINE-HOSPITAL 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 etheral 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 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 *Tænia 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.

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TREASURY DEPARTMENT
Public Health and Marine-Hospital Service of the United States

HYGIENIC LABORATORY.—BULLETIN No. 55
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QUANTITATIVE PHARMACOLOGICAL STUDIES:

ADRENALIN AND ADRENALIN-LIKE BODIES

By
W. H. SCHULTZ

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QUANTITATIVE PHARMACOLOGICAL STUDIES—ADRENALIN AND ADRENALIN-LIKE BODIES.[a]

By W. H. Schultz,

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The active principle of the adrenal gland, like some of the other internal secretions, is unique in its action. It is supposed to fulfill a function both in normal and in pathological conditions of the human body that makes it a substance of the greatest interest. But aside from the importance that attaches to it because of this, adrenalin has come to hold a place in therapeutics which, until recently, could not be filled by any other known compound. Soon after the discovery of its physiological action, adrenal extract came into extensive use as a styptic and, to a more limited extent, for other purposes. With the discovery of methods for the isolation of the pure principle of the gland, a still wider field of usefulness was found for it as a drug, whereupon numerous preparations were placed upon the market and manufacturing firms widely advertised the importance of the pure product. The different preparations, however, varied greatly in physiological activity—some even being worthless, this being due partly to a lack of care in the process of preparation and partly to the nature of the container and solvent used in bottling the extract. Intravenous injections of the active principle have been suggested in cases of surgical shock, and for such it is a matter of the greatest moment that the solution be of a known concentration. Obviously, too weak a preparation would fail to produce the desired effect and too strong a solution might not only throw too great a strain upon an already weak heart, but cause an after depressing effect no less dangerous.

It is evident, therefore, that every container should be so stamped as to indicate not only the actual strength of the solution in terms of pure base, but it should be standardized pharmacologically against a preparation of known purity, and each package so labeled and dated. At least this should be done with solutions intended for

[a] Manuscript submitted for publication April, 1909.

purposes of injection, so that the physician or surgeon might be insured against the possibility of securing a solution of unknown strength.

Deteriorated solutions and preparations made from the natural base of uncertain purity have not been the only sources of trouble. Not long since, synthetic dl-adrenalin was placed upon the market and the most positive claims made in its behalf. It was represented as having even a little more pronounced action than natural l-adrenalin, and these claims were supported by what seemed to be researches by expert pharmacologists. Herein will be found data which fail to coincide with the claims made for dl-adrenalin. The investigation has been carried on in view of the fact that physiological methods are necessary in assaying adrenalin-like bodies. A comparison of the relative values of various methods has also been made, together with an effort to improve if possible these methods.

HISTORICAL.

The early discovery of the vaso-constrictor action of the adrenal extract by Oliver and Schäfer (61) and by Szymonowicz and Cybulskiego (70 and 71) introduces an epoch in physiological science, the development of the chemistry and physiology of which is scarcely less interesting than that represented by the modern theory of physiological oxidations.

Abel and others, by their extensive chemical research, opened up the way for the discovery of the active principle which was finally separated by Takamine and by Aldrich. Soon after enough data had accumulated to warrant an attempt at synthesizing adrenalin. Stolz succeeded in making the dl-product which was found somewhat different from the natural base, and, finally, Flächer split the dl-compound into its dextro- and levo- components, the latter of which is said to be identical in every respect with the levoadrenalin obtained from the adrenal gland.

The physiology of the adrenal glands alone covers a field of no mean proportions, and it is more or less closely associated with a still larger number of articles on the therapeutic and toxic action of adrenalin. Since, however, the studies to be described are for the most part quantitative in character, it seems advisable to limit references as far as possible to methods and results that are more or less quantitative which deal with the physiological action of adrenalin and closely allied compounds. Comprehensive résumés of the literature dealing with the adrenals, adrenalin and its homologues, may be found in the excellent papers by Hultgren and Anderson (40), Abel (2 and 3), Aldrich (4), Möller (58), Davis (22), Vincent (74), Rolleston (64), and Schäfer (65).

The early anatomists observed that the juice of the medullary substance of the *capsulæ atrabilianæ* (suprarenals) darkened upon exposure to the air, and called it *atra bilis*. It was not, however, until the nineteenth century that light was shed upon the cause of this color change. In 1856 Vulpian (75) observed that the medullary juice of the suprarenals turned emerald green and rose carmine when brought into contact with ferric chloride and iodine, respectively. These reactions were characteristic of this organ, and at once led to the surmise that the medulla of the gland contained a substance of physiological importance.

Pellacani (62) as early as 1879 performed in Foa's laboratory a series of very interesting experiments with extracts of the fresh organs, among which was a series of injections of adrenal extract into various animals. The capsules were excised, ground up in a mortar, and to this mass was added distilled water sufficient to filter, but not exceeding the amount needed for the injection. This liquid extract was filtered through linen and then paper, after which it was immediately injected. The greatest care was exercised both in the making of the extracts and in the preparation of the instruments, the latter being immersed in strong alcohol. Subcutaneous injections were made into dogs, cats, rabbits, guinea pigs, and frogs. A rise of temperature did not always occur, and in some of the animals there was a lowering of temperature, followed by death in from twelve to thirty hours for the more rapid cases of intoxication. The symptoms were general excitement, followed by paralysis, both sensory and motor, increasing weakness of the heart, lowering of temperature, 4–5°, followed by death. It is strange that Pellacani should have secured such characteristic adrenalin effects and yet observed that subcutaneous injections were more toxic than intra-peritoneal ones, and that both of these methods of injection caused more pronounced and quicker effects than intravenous injections. Although he used extracts of organs other than the adrenals, he found the latter to be more toxic and the following organs to be progressively less active: Muscle, liver, kidney, brain, milt. Five grams of clear extract of two suprarenals of rabbits injected into a 770-gram guinea pig caused death after twelve hours. Eighteen to twenty cubic centimeters of extract of cat or guinea-pig adrenals proved very toxic for rabbits, whereas a higher dose, 1 gram, of lamb or heifer suprarenal extract was necessary to cause a like degree of toxicity.

Mattei (53) repeated Pellacani's experiments, but arrived at very different conclusions. In his conclusions he states that water extracts of fresh organs injected into different animals does not cause any toxic action. The animals that die do so because of the after effects of the soluble organic matter of the decomposed tissues, which later cause septicemia.

Foa and Pellacani (32) in 1883 confirmed Pellacani's earlier results and removed, as they supposed, the influence of fibrin ferment by heating the extracts to 60°. They found the toxic substance to be very soluble in water and alcohol, but rather insoluble in ether and chloroform. Whereupon they purified the water extract as follows: The capsules were minced and put in boiling water; after a time the water was decanted, evaporated to dryness, and the residue treated with alcohol, the almost colorless alcohol solution evaporated to dryness and again taken up with water, thus leaving behind most of the impurities. Upon evaporating this solution a dark residue of a peculiar odor and of very acid reaction was obtained. One gram of this substance injected into dogs caused death, whereas the extracted pulp of the capsule was relatively inert. These investigators came to the conclusion that there is an active poison in the adrenal gland independent of fibrin ferment which causes extreme prostration, collapse, motor and sensory paralysis, and death from asphyxia because of paralysis of the medulla oblongata, especially the respiratory center. After examining the original articles of these early writers it seems that more credit should be given to their work than is usually found in literature, for they certainly described symptoms of poisoning very characteristic of adrenalin.

Krukenberg (44) (1885) assumed that the substance giving color with ferric chloride is not the chromogenic substance of Vulpian, but more likely pyrocatechin accompanying the chromogen.

Marino-Zuco (52) (1888) after freeing 50 capsules from fat, ground and macerated them in 1,000 c. c. of distilled water heated in a water bath for several hours. The mixture was strained, evaporated on a water bath, and filtered, the resulting solution being slightly acid and red in color. This extract, diluted to 200 c. c., and 1 c. c. injected subcutaneously into rabbits, caused death in five minutes. If, however, the extract were treated with acid or alkali, it was ineffective, even in large doses. The presence of neurine alone does not explain the toxic action of the pure extract, but in combination with very acid phosphates it may, at least when made artificially, prove very toxic.

Guarnieri and Marino-Zuco (35) (1888) made an extract of 10 beef suprarenals in 60 c. c. of water and injected 1 c. c. into medium-sized rabbits, causing death in a very short time. Treatment of the extract with acids and other reagents lessened the activity of the extract when intravenously injected. From these results they concluded the toxicity of the aqueous extracts was due primarily to neurine (which I believe is now generally conceded to have been choline) and organic phosphates.

It is interesting to know that Dzierzgowski (27 and 28) as early as 1893 synthesized a number of catechol derivatives closely related

to the ones discussed in this paper. The work, however, did not attract much attention at the time and seems to have been lost sight of, their physiological activity not having been tested by him. Even Stolz, who was familiar with the work, does not give Dzierzgowski and his contemporaries the credit they deserve for their pioneer work with these compounds. Among some of the compounds synthetized by Dzierzgowski are dimethyl-amido-aceto-catechol, anilido-aceto-catechol, methyl-anilido-aceto-catechol, quinolin-, pyridin-, and piperidin- aceto-catechol, aceto-chloro-aceto- and chlorpropio-catechol.

Gluziński (34) (1895) removed aseptically the adrenals of recently killed oxen, calves, hogs, rabbits, and dogs; weighed and ground them up with broken glass. One part of the pulp was allowed to macerate eight to twelve hours in a cold 50 per cent aqueous solution of glycerine. The solution was filtered through sterilized glass wool and the clear extract injected from a sterilized syringe into the ear vein of a rabbit. In one minute 0.3 to 1 gm. killed a 1,500 gm. rabbit. The extract heated to 100° for one hour retained its toxicity, causing hemiplegia, loss of sensibility, cramps of anterior part of body, opisthotonus, rapid breathing, dilation of the pupil, and, finally, symptoms of dyspnœa, general paralysis, and, unless artificial respiration were administered, death from asphyxia.

Moore, B. (59) (1895), basing his opinion on experiments which indicated that such chemical operations as destroy the color reactions by oxidizing the reducing agent likewise alter the physiological activity of the extract, concluded that Vulpian's chromogen and the active principle are identical.

Dubois (25) (1896) used an extract of fresh suprarenals of rats ground in equal volumes of alcohol and distilled water and allowed to remain twenty-four hours in glycerine. He concludes that such extracts contain two substances, one soluble in alcohol at 90°, causing vaso-dilation and congestion; the other, very soluble in this reagent, causing paralysis, depression of the heart, and death from asphyxia.

Fränkel (33) (1896) separated what he supposed to be the active principle, calling it "sphygmogenin," and observed that a close relation existed between the ease with which the substance is oxidized and its physiological activity. He suggested that "sphygmogenin" is a pyrocatechin derivative, and although he and Krukenberg were in a measure correct it is now known that Fränkel's product was a mixture.

Vincent, S. (73) (1897), performed a series of 80 experiments with rabbits, guinea pigs, rats, mice, frogs, and toads. The extract was made by boiling a short time and filtering the chopped-up gland, although a dried gland, glycerine, or alcoholic extract was some-

times used. Five-tenths gram of the fresh gland in the form of a glycerine extract injected into the dorsal lymph sack of the frog caused immediate paralysis, from which the animal soon recovered, doses up to 3 gm. not proving fatal. With toads much larger doses were required to produce corresponding symptoms. Doses equivalent to 1.5 and 3 gm. of fresh gland caused in rats the usual cardiac, respiratory, and nervous symptoms, some of the animals recovering, others dying from failure of respiration. Guinea pigs of average weight were injected with an equivalent of 6 gm. of fresh gland, the symptoms being practically the same as for cats and mice, except that the urine of the former was more blood colored (with or without corpuscles). The fatal dose for rabbits can not be stated, since they vary so much in reaction. Unlike the guinea pig, which becomes very restless, the rabbit grows drowsy and listless. Small initial doses followed by one that was usually fatal for fresh rabbits no longer proved to be so. Vincent thinks that a partial immunity (tolerance) is established toward the toxic action of the extract, which passes off after a few weeks.

In the meanwhile Abel (1899), Von Furth, and others had been working upon the chemistry of the active principle. The former isolated a substance which he called "epinephrin;" the latter a substance which he called "suprarenin." A controversy arose as to which was the active principle. As a matter of fact, neither chemist seemed to be working with the pure substance. They did, however, throw much light upon the chemistry of the compound, and prepared the way for its separation by Takamine (72) (1901) and by Aldrich (4).

Moore and Purinton (60) (1899) criticized the idea of epinephrin (Abel), suprarenin (Von Furth), and the other so-called pure products being the active principle. They record a rise of blood pressure after intravenous injections of the crude medullary extract in doses ranging from 0.245 to 24 millionths of a gram per kilo. This, they maintain, represents a physiological activity far in excess of any of the so-called active principles.

In reply to Moore and Purinton's criticism, Hunt (41) determined the minimal amounts of Abel's epinephrin sulphate necessary to cause a rise of blood pressure, finding that even so small an amount as 0.083 millionths of a gram per kilo body weight caused a rise of 5 mm. of mercury, and 0.23 millionths of a gram per kilo, a rise of 7 mm. The duration of the injection period (in this case two to five seconds) was found a very important factor in determining the degree of vaso-constriction resulting from a given dose of adrenalin. One and one-tenth millionths of a gram per kilo injected rapidly might cause a rise of blood pressure equal to 14 mm. of Hg., whereas double this dose injected slowly caused a rise equivalent to but 8 mm.

of Hg. Hunt maintains that these results justify Abel's contention that epinephrin is the active principle.

Cybulskiego (19) found that 1 c. c. of a 10 per cent solution of the extract injected into the vein of a rabbit caused death, but if this solution were diluted ten to twenty times the same dose of adrenalin was borne without any untoward effects.

Bouchard and Claude (14) (1902) experimented with a small number of animals, finding that the lethal dose may be only 0.5 or 0.2 mg. per kilo, an animal occasionally withstanding an intravenous injection of as much as 1 mg., 2 mg. usually proving fatal. The lethal dose for rabbits therefore lies between 1 and 2 mg. per kilo. Provided there was a gradual increase of each successive dose until a maximum was reached, they could inject as much as 4 mg. per kilo without any untoward effects other than temporary paresis such as is brought on by an initial dose of 1 mg.

Battelli (7) (1902), using a slight modification of Takamine's method, claims to have secured adrenalin even more pure than that obtained by Takamine. Battelli (9) injected this preparation subcutaneously into rabbits, guinea pigs, and frogs with the following results: With a corresponding number of guinea pigs 10 mg. per kilo proved to be the lethal dose. On the other hand, frogs were ten times more resistant than rabbits, 1,000 mg. killing only three out of four.

Dose per kilo.	Number of rabbits injected.	Number of animals died.
Mg.		
2	5	0
10	6	5
20	3	3

He also (8) (1902) notes that Gluziński in 1895 called attention to the fact that intravenous injections were more toxic than subcutaneous ones. Battelli experimented with what he considered a very pure product, using adrenalin base dissolved in water acidulated with hydrochloric acid and neutralized with Na_2CO_3 just before injecting into the femoral vein, and obtained the following results:

Rabbits—0.1 mg. per kilo, not lethal.
0.2 mg. per kilo, 1 out of 5 died.
0.4 mg. per kilo, 3 out of 4 died.
0.6 mg. per kilo, always lethal.

Guinea pigs—0.05 mg. per kilo, not lethal.
0.10 mg. per kilo, 2 out of 5 died.
0.20 mg. per kilo, always lethal.

The toxic dose for the rabbit and guinea pig was about the same when the injection was made into the jugular vein of the rabbit

and into the femoral vein of the guinea pig, death ensuing from œdema of the lungs or fibrillation of the heart. Summing up his work, he concludes that intravenous injections are about forty times as potent as subcutaneous ones.

Eeckhout (26) is quoted as finding the lethal dose of adrenalin to be 0.08 to 0.06 mg. per kilo. In the original paper, however, I find that this lethal dose is calculated from doses that accidentally caused death in animals previously injected with morphine and atropine and anæsthetized with chloroform, which, according to later writers, renders the animals less resistant to adrenalin.

Amberg (5 and 6) (1902) compared the toxicity of Abel's epinephrin with the commercial product made by Takamine's method, and found that Abel's sample dissolved completely, whereas only 517.7 mg. of the commercial product dissolved in 18 c. c. of H_2O, leaving behind 4.6 mg. of sediment. After studying the effect of subcutaneous injections upon 9 dogs and intravenous injections upon 16, he concluded that the lethal intravenous dose lay between 0.99 and 2 mg. per kilo, subcutaneous injections of 4.9 mg. per kilo not proving lethal, though 6 mg. or more per kilo were.

Lesage (47, 48, and 49) (1904) does not state by what process his adrenalin was made, but judging from the size of the lethal dose it must have been a very good one. From a stock solution containing 0.04 gm. adrenalin, 40 gm. H_2O, and 1 drop of HCl he made a 1:20,000 solution to be used for intravenous injection.

Rabbits—0.05 mg. per kilo, signs of intoxication.
0.20 mg. per kilo, lethal (4 animals).
Dogs—0.05 mg. per kilo, not toxic.
0.12 mg. per kilo, sometimes lethal.
0.20–0.25 mg. per kilo, usually lethal (4 out of 6).
Cats—0.50–0.81 mg. per kilo, lethal (6 animals survived after 5 injections of 0.25 mg. and 1 of 0.50 mg. per kilo).

With the larger doses, 0.26 mg. per kilo, he observed that the dogs usually died from asphyxia, but when 0.20 mg. per kilo was fatal they usually died from heart failure. In general, anesthetics augmented the toxic action and sublethal doses of adrenalin rendered the animals resistant to doses that ordinarily proved fatal for normal animals. He concludes that there is a considerable variation in the susceptibility of one individual as compared with another, and a still greater variation for different species.

Baylac, J. (10) (1905), working with a 1:1,000 solution of adrenalin, found that the lethal dose varies with the species and according to the manner of its injection. The lethal subcutaneous dose as determined on 6 guinea pigs and 6 rabbits is estimated at 100 mg. per kilo and 20 mg. per kilo, respectively. On the other hand, he estimated

from experiments with 5 rabbits that the lethal intravenous dose is about 0.06 mg. per kilo, although 0.03 mg. per kilo may prove very toxic. An intrapleural injection of 2 mg. per kilo or the same amount injected intraperitoneally into guinea pigs may prove fatal.

Up to this time toxicity experiments and blood pressure determinations were primarily qualitative in nature, yet they furnish a most reliable index of the relative purity and activity of adrenal extracts, and of the pure products of the active principle thus far made.

Houghton (38), however, in 1901, emphasized the usefulness of quantitative determinations by the blood-pressure method and published records illustrating the relative effect of different volumes of the same solution injected subcutaneously. In 1902 (39) he proved this method one of the most accurate means of assaying adrenalin. Three solutions, A, B, and C, were used, one containing 85, one 40, and another 130 per cent of adrenalin in a given solution. An assistant reported that A contained 80, B 40, and C 135 per cent of the amount in the known solution. It was also found that Takamine's crystalline product was from 600 to 800 times as active as the best aqueous extracts of freshly prepared glands.

Läwen (46) in 1903 studied the quantitative effect of adrenalin upon the blood vessels of frogs. The brain and spinal cord were destroyed and, by measuring the outflow from the vessels subjected to a given pressure with a known solution, he determined the relative vaso-constrictor action of the different adrenalin solutions. With a pressure equivalent to 30 c. c. of H_2O the frogs lasted about two hours. Two ten-thousandths of a milligram of suprarenin in a concentration of 2:100,000,000 constricted the blood vessels of a 50-gram frog so that the outflow was reduced 20 to 37 per cent. In other experiments it was determined what pressure in excess of the minimum was necessary to overcome the increased resistance due to vaso-constriction. By these experiments Läwen not only could study solutions quantitatively, but could even detect the differences between fresh solutions and those that had been standing for some time.

Up to this time little attention had been given to the possibility of using particular organs for assaying adrenalin, but observations made by the following writers eventually led to the idea that possibly the eye or separate strips of muscle might prove serviceable.

In 1904 Meltzer and Auer (54), Lewandowsky (50), Boruttau (13), and Langley (45) confirmed Foa's observation that adrenalin extract intravenously injected causes dilation of the pupil and that subcutaneous injections have no effect. It is assumed that the extract is oxidized in the lymph spaces before it reaches the neuro-muscle apparatus and effects dilation. Meltzer (54) states that his extensive

experience with subcutaneous injections of adrenalin in normal rabbits warrants the conclusion that dilation does not occur unless the dose is large enough to cause asphyxia. If, however, the superior cervical ganglion is excised, even a moderate dose, 0.6 c. c. of a 1:1,000 solution, will cause the pupil on the side from which the ganglion is removed to become dilated *ad maximum*. Radziejewski (63) was one of the first to claim that adrenalin instilled into the eye exerts no effect upon the pupil, and this idea is supported by Lewandowsky (1889), Boruttau, Meltzer, Loewi, Ehrmann, etc. Meltzer (55), however, finds that removal of the superior cervical ganglion and instillations of adrenalin twenty-four hours later made the pupil dilate in proportion to the amount of adrenalin instilled. After four or five instillations, two drops every two or three minutes, the dilation can be *ad maximum*, lasting several hours in rabbits and cats (54).

Meltzer and Auer (56) in a later series of experiments showed that adrenalin subcutaneously injected into frogs or instilled into the eye dilated the pupil widely, the vertical axis being affected most. Three drops of a 1:1,000 solution instilled by pushing a fine pipette between the bulbus and the lid caused very marked dilation in three to seven minutes, which lasted as long as if subcutaneously injected. To eliminate the possibility of the drug being absorbed by the skin the bulbus was excised and adrenalin dropped upon the corneal surface. There was prompt dilation, which lasted many hours. As a result of this work the writers suggested that "the frog's eye excised or *in situ* might prove to be a better reagent than the blood pressure to demonstrate the efficiency of a suprarenal preparation."

Wessely (76) (1905–6) reinvestigated the action of adrenalin upon the intraocular pressure and upon the pupil. He found that instillations of adrenalin into the eyes of vertebrates causes dilation, provided the strength of the solution is increased to suit the animal experimented with. For man a 1 per cent solution is accompanied by danger, and a 0.1 per cent solution is too weak to cause mydriasis.

Schultz (66) also showed that adrenalin instilled into the eyes of mammals always causes dilation of the pupil. The degree of mydriasis resulting from a given amount of adrenalin depends, however, upon the intensity of light stimuli and upon the kind of animal used. There is, so to speak, a kind of antagonism between the processes set up by the light stimuli and those initiated by adrenalin. So that animals with a sensitive and more highly developed light-accommodating mechanism require a longer period of instillation and a greater amount of adrenalin to cause mydriasis than those with eyes less sensitive to light.

Ehrmann (29) (1905) used the method suggested by Meltzer (56), and thinks to have ruled out the influence of the sympathetic im-

pulses, the presence of which is held to prevent mydriasis. The enucleated frog's bulbus placed in a small vessel of 5 c. c. capacity with a known amount of adrenalin was compared with controls in physiological saline. He mentions that there is considerable individual variation, but in the subsequent paragraphs and the reviews of his article this factor is lost sight of. The pupil dilated to a maximum in a solution of 0.001 mg. and 0.0001 mg. per c. c. produced distinct dilation. Having, as he thinks, proved the delicacy of the test object, he then determines the adrenalin content not only of the blood after an intravenous injection of adrenalin, but even determines that the adrenals secrete into the blood a perceptible amount of adrenalin. He overlooks, however, the fact that in the blood there are other substances that cause the pupil to dilate, not to mention certain factors discussed later which enter in to justify the severest criticism of his technique and conclusions.

Meyer (57) (1906), by suspending strips of beef's subclavian and carotid arteries in adrenalin solutions, concludes that with:

1 gm. of adrenalin to one thousand million c. c. of oxygenated Ringer, contraction may occur.
1 gm. of adrenalin to one hundred million c. c. of oxygenated Ringer, contraction usually but not always occurs.
1 gm. of adrenalin to fifty thousand c. c. of oxygenated Ringer, maximal contraction usually occurs.
1 gm. of adrenalin to one hundred c. c. of oxygenated Ringer, maximal contraction may occur.

He maintains that the method is a quantitative one, and along with other interesting statements says that strips exposed to rather concentrated solutions of adrenalin (1:10,000) for eight minutes, removed, wiped dry, and then hung in 20 c. c. of fresh Ringer solution for five minutes diffuses sufficient adrenalin into the new solution to stimulate fresh strips quantitatively equivalent to a one to twenty million solution.

By this time sufficient chemical data had accumulated to warrant attempts to synthesize adrenalin. Stolz (68) and Dakin (21) succeeded, independently, in making substances closely allied to this product, among which proved to be dl-adrenalin. The discovery of these interesting compounds resulted in a new series of pharmacological experiments.

The physiological testing of synthetic substances by Dakin (21) and by Loewi (51) and Meyer are hardly quantitative in nature, and their results, though roughly comparable with each other, give only a general idea of the activity of the compounds. Biberfeld and German writers in general seem, as pointed out, to have been misled by some of the statements of Loewi and Meyer, based upon quali-

tative rather than quantitative studies of such compounds as aminoketone, ethyl- and methyl- aminoketone, and ethyl- and methyl-aminoalcohol. Dakin (20, 21) examined analino, o-toluidino, and α-naphthylamino-acetylcatechol, finding that small quantities cause no rise of blood pressure; piperidine does, though its compounds are less active than piperidine itself. He arrives at the generalization that the catechol nucleus is necessary to produce a physiologically active substance of the type of adrenalin; that it is of importance that the hydrogen atoms of both hydroxyl groups in the catechol nucleus be unsubstituted. He thinks also that an alkyl group of low molecular weight attached to the nitrogen atom tends to produce a more active substance than when an aromatic group is attached, whereas derivatives of piperidine, heptylamine, and benzylamine occupy an intermediate position, and, finally, that there seems to be a close connection between chemical instability and physiological activity, and *vice versa.*

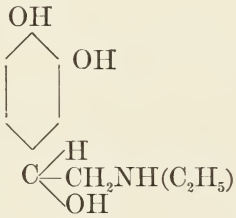
One of the first and most strikingly practical applications of quantitative determinations of adrenalin found in literature is that of Hunt (42) (1906). Samples were sent to the Hygienic Laboratory for the determining of their relative physiological activity. He found that the rise of blood pressure from equal amounts of samples A and B were practically the same, about 1.4 to 1.8 times that of C; in order to record the same rise of blood pressure from equal volumes of C and of A and B it was necessary to increase the strength of C by about 1.7 times. In a second series four preparations were tested; three of these were labeled "1:1,000 solutions of the active principle" and one "dried powdered gland." Preparations C and A, from the manufacturers of the first series, were about equal in strength and approximately five times as strong as D. It was possible to prepare from an ounce of the dried suprarenal gland about 15 fluid ounces of a decoction as potent as the active principle labeled 1:1,000. To further emphasize the accuracy of the blood-pressure method I quote an interesting paragraph from the same paper: "Abel calculated that fresh beef's suprarenals contain at least 0.3 per cent of the active principle. One part of the dried gland corresponds, according to the United States Pharmacopœia, to approximately six parts of the fresh gland; hence, according to Abel's experience * * * 1 gm. of the dried gland should contain 0.018 gm. of the active principle * * * and should yield about 18 c. c. of a solution corresponding to 1:1,000 of the active principle. As a matter of fact, I found that it yielded about 15 c. c. [from blood-pressure data] of such a solution, and it is improbable that the gland was completely exhausted in my experiments."

Sollmann (67) (1906) also examined a series of commercial suprarenal preparations and found the relative efficiency to vary consider-

ably. At least two samples, *a* and *b*, made by the same firm, were bought on the open market and tested. In order to eliminate all bias the solutions to be tested were made by a second person. The relative efficiency of the original solutions was thus estimated and found to be as follows: 1a = 70, 1b = 70; 2a = 100, 2b = 86; 3a = 86, 3b = 63; 4a = 95, and 4b = 0.

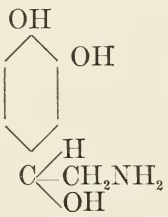
As already pointed out, Dakin and Stolz had synthesized compounds thought to be allied to adrenalin. Biberfeld (11) (1906) compared methylamino alcohol (synthetic dl-adrenalin) with natural adrenalin and found them physiologically identical. A 1:500,000 solution causes dilation of the frog's pupil; 2 mg. injected subcutaneously into a rabbit causes diuresis and glycosuria; 0.001 of a milligram injected intravenously causes a rise of blood pressure. The lethal dose for rabbits is given as 0.1 to 0.2 mg. per kilo for intravenous injections and 4 mg. per kilo when injected subcutaneously.

The reduction product of the aminoketone, ethylaminoalcohol represented by the formula



is said to act more strongly upon the vagus, but to affect the blood pressure in a less degree than natural adrenalin. Diethylamino alcohol was also found to be much less active.

The amino alcohol represented by the formula



produced a rise of blood pressure equal to that caused by natural adrenalin. In one case a rabbit withstood a dose of arterenol two to three times as great as the lethal dose of adrenalin without showing any very untoward symptoms of intoxication.

Gunn and Harrison (36) (1908) dissolved samples of adrenalin in various kinds of media, preserving the 1:1,000 solutions in different kinds of containers and under various conditions, their object being

to determine the effect of various factors upon their rate of deterioration. Each of the set of about twenty samples was marked by letter or number and submitted to Dixon for physiological testing. The results when examined by Gunn and Harrison revealed that the fresh samples of natural and synthetic adrenalin, though of the same concentration, did not possess the same physiological activity, the natural product being approximately one-half that of the synthetic. These writers in referring to Dixon's tests conclude that since "the natural substance is lævorotatory and the synthetic is optically inactive, it would appear that only one of the two isomers possesses physiological activity." This is the first published statement of the kind found that is supported by experimental data of so reliable a character, but Dixon (23) in a note in the Pharmaceutical Journal states that "it was Cushny who first suggested at a meeting of the Physiological Society that the synthetic adrenalin might be a mixture of the two optical isomers of which the lævo variety alone was markedly active."

Biberfeld (12) repeated his work of 1906 and not only confirmed to his own satisfaction his earlier results but also criticised unjustly the work of Dixon described by Gunn and Harrison. He weighed equal quantities of the natural and synthetic base, dissolved each in 0.8 per cent sodium chloride solution acidified with the calculated amount of hydrochloric acid, and allowed them to stand for eighteen days when their vasoconstrictor action was tested upon a rabbit weighing 2 kilograms. The rise of blood pressure resulting from intravenous injections of these compounds is given as follows:

0.001 mg. natural, rise of 8.5 m. m.	0.001 mg. synthetic, rise of 7.0 m. m.
0.002 mg. natural, rise of 15.0 m. m.	0.002 mg. synthetic, rise of 11.0 m. m.
0.008 mg. natural, rise of 18.0 m. m.	0.008 mg. synthetic, rise of 19.0 m. m.
0.016⅔ mg. natural, rise of 27.0 m. m.	0.016⅔ mg. synthetic, rise of 27.0 m. m.

This is certainly insufficient data upon which to base such important conclusions, and more especially is this true when part of the data given in itself seems open to criticism. One and five-tenths milimeters difference in such small blood-pressure changes as occur in the first reading and 4 m. m. difference in the second indicate something other than mere experimental error.

Stolz and Flächer (69) (1908), in criticising Gunn and Harrison's article, state that "the two products, synthetic and natural adrenalin are not isomeric substances but identical structurally. The suggestion that synthetic suprarenin is only half as active as the natural lævorotatory suprarenin is incorrect, as otherwise in logical sequence the dextrorotatory suprarenin would be entirely inactive. We made by synthesis a preparation composed of one-half of dextrorotatory and the other half racemic suprarenin. The latter, which

is optically inactive, consists of equal quantities of the lævorotatory and dextrorotatory modification, so that this substance consisted of three-quarters dextro and one-quarter lævorotatory suprarenin. If the supposition were correct that the dextro-rotatory suprarenin is inactive, then this mixture could only possess one-fourth the activity of the natural suprarenin. Careful experiments upon animals with the kymograph demonstrate, however, the complete equivalence of this preparation with the natural substance." These writers are not content with this statement, but even make it appear as if a commercial product in which they are deeply interested is, according to "a great number of clinicians and pharmacologists, not only equal to that obtained from the adrenal organs but its activity is even greater than the latter, which must be attributed to the absolute purity of the synthetic preparation."

Cushny (16) (1908), in a preliminary report, states that he examined the products just mentioned, finding that the strength of the three substances—natural base, synthetic base, and the three-fourths dextrorotatory base—bear the relation 4:2:1. He also finds Biberfeld's results divergent from his own, and offers as a possible explanation the use of rabbits which, Cushny thinks, acquire a tolerance for adrenalin.

Cushny (17) (1908) compared a sample of natural adrenalin the optical activity of which was found to be $-42.25°$ with a synthetic sample optically inactive. Instead of trying, as did Biberfeld, the relative activity by comparing the minimal doses necessary to cause a rise of blood pressure he checked 1 c. c. of 1:100,000, 1:50,000, and 1:25,000 of adrenalin base with corresponding solutions of the synthetic product. Then by decreasing the dose of the stronger preparation and increasing that of the weaker until the rises of blood pressure approximated each other he was able to determine the relative physiological activity of each. As a result of his experiments upon dogs anesthetized with paraldehyde with both vagi cut and under conditions of artificial respiration he concludes that the "natural lævoadrenalin acts twice as strongly on the blood pressure as synthetic or racemic adrenalin, and presumably also upon the other organs affected by adrenalin. From this it is inferred that dextroadrenalin is devoid of action on these tissues, and this is confirmed by the examination of a partially isolated d-adrenalin."

Flächer (31) (1908) succeeded in splitting dl-adrenalin into its d- and l- components. He concluded that the physico-chemical properties of the synthetic l-adrenalin and the natural l-adrenalin are identical. Both melt at 211° to 212° C. (uncorrected). They form oxalates and chlorides that do not crystallize. The synthetic product purified from the bitartrate and dissolved in hydrochloric

acid shows an optical activity of $[\alpha]_D^{19.6} = -51.40°$ and that of the pure natural l-adrenalin obtained from the bitartrate showed an optical activity of $[\alpha]_D^{19.8} = -51.40°$. The d-adrenalin dissolved in weak hydrochloric acid showed an optical activity of $[\alpha]_D^{19.8} = +51.80$ and, like its optical isomer, melts at 211° to 212° C. (uncorrected), and it forms oxalates and chlorides which do not crystallize.

The physiological activity of these new products was tested by Abderhalden (1) (1908) and Müller, and reported in a very unsatisfactory manner. The l-adrenalin was found to be fifteen times more active than the d-isomer.

Cushny (18) (1908) in a very brief report states that he tested the synthetic d- and l- adrenalin prepared by Flächer, finding the synthetic l-adrenalin equal in potency to the l-adrenalin obtained from glands. It was found difficult to determine the absolute relative physiological activity of the synthetic d- and the synthetic l- adrenalins, but in general ten times more synthetic d-adrenalin was required than of the synthetic l- to produce a given rise of blood pressure. It was calculated that the relative physiological activity of the d- and l- products were in the ratio of 12:1. In the light of these experiments the relative activity of the natural l-adrenalin and the synthetic dl-adrenalin may be expressed by the ratio 24:13.

Although Emmert's work (30) (1908) has to deal primarily with histological changes ensuing from repeated injections of sublethal doses of adrenalin, some interesting toxicological data is recorded. The number of mice used by him does not seem to have been large, and hence the dose given as lethal must not be taken too seriously. It is stated that 0.1 mg. generally caused death in from one minute to sixty hours. In one case he was able to increase gradually the dose to as much as 0.5 mg. before acute poisoning set in. In another case three mice were injected with 0.1 mg. once or twice a day for 9, 24, and 39 days. In still another experiment mice were observed for 109 days, fifty-six 0.033 mg. doses being first injected, and later twenty-five 0.1 mg. doses. Only three mice lived after doses of 0.1 to 0.15 mg., and these were always prostrated by each of the twenty-five injections given in the course of 24 days. For the object of studying lesions caused by repeated injections his experiments are perhaps sufficient, but for purposes of determining the lethal dose they are not.

THE RELATIVE ACTIVITY OF ORTHO-DIOXY-PHENYL-ETHANOL-METHYL-AMIN (NATURAL L- AND SYNTHETIC DL- ADRENALIN), OF ORTHO-DIOXY-PHENYL-ETHANOL-AMIN ("ARTERENOL"), AND ETHYL-AMINO-DIOXY-ACETO-PHENON ("HOMORENON") AS DETERMINED BY BLOOD PRESSURE.

Soon after synthetic dl-adrenalin was placed upon the market samples of it were submitted to the Division of Pharmacology of the Hygienic Laboratory for comparison with the natural l-base. A few preliminary tests showed the synthetic substance to be only one-half to two-thirds as active as the natural. Upon noting this, it was decided to make a study of certain catechol derivatives and also to examine into the best methods for standardizing them. Of the several methods proposed it was found that on the whole that of blood pressure, of the pupil, and of subcutaneous injections was most satisfactory. The pupil method as used by Meltzer and by Ehrmann is adequate for qualitative but not for quantitative testing. Hence this method was changed to eliminate as far as possible the most serious errors that could arise, which has now made the pupil method, though not quite so delicate, almost as reliable as that of blood pressure. Likewise the toxicity data of adrenalin literature, though in a general way supplementing the qualitative and quantitative results of their period, are, on the whole, unsatisfactory, being unsuited for comparison with more recent experiments with pure compounds. For this reason a series of experiments was carried on under conditions whereby the members of one series could be compared with those of another.

A glance over the literature on adrenalin reveals at once how prominent a place the blood-pressure method occupies in testing this drug both in a qualitative and quantitative manner, and it would seem to be the most consistent test for catechol derivatives, these substances being primarily vaso-constrictors. Because of this the relative pharmacological action of the compounds already mentioned will first be considered in terms of rise of blood pressure and all subsequent results by other methods will be referred to this as a standard.

One of the first difficulties encountered was the variation in the activity of adrenalin found upon the market. In order to eliminate this factor the Hygienic Laboratory purchased direct from a manufacturing firm 19.4 grams of natural l-adrenalin base. This they claimed to contain 15 grams of pure adrenalin, their basis for calculation being that incineration left 22.6 per cent ash, which, as will be seen later, was erroneously inferred to represent all the impurities present. To make sure of a good preparation, however, the sample was repurified by Taveau, chemist in the Division of Pharmacology, who has done so much valuable work in synthesizing compounds of this character both in Abel's laboratory and in the Division

of Pharmacology of the Hygienic Laboratory. He obtained upon purifying the 19.4 grams of adrenalin 5.5 grams of a fine crystalline ash-free base of unusual physiological activity with an optical activity of $[\alpha]_D^{26.4°} = -53.40°$.[a] An additional one-half gram of practically ash-free base made a total of 6 grams. There was, of course, some loss in the process of purifying, but that this unusual loss was one due not merely to chemical manipulation but rather to the impurities eliminated is shown by physiological testing. The original sample was checked against the repurified sample and shown by the blood-pressure method on cats to be only one-half to one-third as active. These findings were eventually confirmed by the physiologist of the manufacturers and concurred in by them, as is evidenced by their voluntarily sending a bill for 6 instead of for 15 grams of adrenalin base.

Having secured an unusually pure sample of adrenalin base to be used as a standard, the next problem was to find an anesthetic that would not increase the secondary depressing action of adrenalin upon the heart and yet maintain a constant state of anesthesia. An animal in order to yield a uniform blood-pressure record must of course be so anesthetized as to maintain the irritability of the parts affected by the drug at not too high nor too low a threshold value, at a level where absence of pain is assured and yet where motor disturbances are removed.

It may not be amiss to speak for a moment of anesthetics that under certain conditions seem to fail in these requirements, themselves bringing about results which at first glance might be attributed to the effect of adrenalin itself. Anesthesia from urethane and chloral, chloretone, and paraldehyde of course have advantages, but the stomach puts beyond the control of the operator all subsequent adjustment of the degree of narcosis. And I am inclined to think that in the latter part of long experiments there may be present a condition of too low an irritability, so that small doses of adrenalin at first effective are now no longer so. This condition seemed to be present in cats anesthetized with urethane chloral, but not when under light ether anesthesia. According to Alexander-Lewin animals may be chloralized to the extent of annulling all action of adrenalin but still leaving the vaso-motor apparatus sensitive to camphor. It is reasonable to suppose that drugs with a chloral-like action upon smooth an l heart muscle even in doses much smaller than used by Alexander-Lewin might so depress the irritability of

[a] The optical activity of this preparation was determined by Mr. Taveau and myself and compares very well with the best measurements that have ever been made, viz, those of Korndörfer (43) made for Flächer. Some of the other readings given in literature are $[\alpha]_D = -32.6°$ (Jowett), $[\alpha]_D^{23.5°} = -43°$ (Pauly), $-42.25°$ (Cushny), $[\alpha]_D^{20°} = -50.72°$ (Abderhalden and Guggenheim) and $[\alpha]_D^{19.8°} = -51.40°$ (Korndörfer) (43).

the vaso-constrictor muscle as to render small doses of adrenalin ineffective. So it may not be far wrong to attribute the loss of sensitiveness to small doses of adrenalin in cats and rabbits to a loss of irritability in the more advanced stages of chloral or paraldehyde anesthesia instead of to so-called acquired immunity (tolerance). May not Cushny's results with rabbits have been of this nature? Hunt not only observed no loss of irritability toward small doses of adrenalin but secured with rabbits some of his best results.

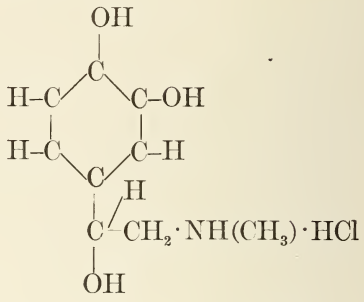
After trying different anesthetics it was found that ether on the whole was the most satisfactory, and so it was used in all of my later experiments. As is well known the chief objection to this anesthetic is the difficulty of maintaining a uniform condition of anesthesia. But this is practically removed by using a modification of the drop method suggested by Porter. In this way even cats, proverbial for their vasomotor "storms," yielded results that were very consistent. On the whole I prefer morphine along with ether for dogs and ether alone for cats. When working with small doses of adrenalin the best results are assured by an anesthesia just sufficient to maintain a condition of unconsciousness so that the animal has no pain, with only enough curare to render it free from muscle tremors. The amount of curare given must of course be determined by experience, since not only different samples vary but individual animals also vary to a certain extent in their reaction to this drug. Indeed large doses of curare should be avoided. Being a depressant, it lowers the blood pressure and interferes with the action of adrenalin, often making consistent results impossible, even with dogs, which in my experience yield the most reliable results.[a]

In all the later experiments the injections were made from a standardized 1 c. c. syringe graduated in five-hundredths of a c. c. The cannulæ were of small bore, provided with very short connections allowing a minimum amount of dead space. There was one injection set for each of the two solutions to be compared so that one solution might be injected into the right and the other into the left saphenous vein. When constant results were thus obtained the solution formerly used for the left vein was now injected as a check into the right vein from the right injection set and *vice versa*.

The following blood-pressure tables are results selected as typical from 21 animals (cats and dogs). Tables I to VI inclusive represent in a general way the relative physiological activity of synthetic dl-adrenalin and natural l-adrenalin. The latter of these compounds has already been described. The synthetic dl-adrenalin manufactured

[a] This animal even at the end of a nine-hour experiment yielded with 1 c. c. injections of a 1:100,000 adrenalin solution a rise of blood pressure equivalent to that from the first injections of this amount, showing in no case a diminished sensitiveness to l-natural and dl-synthetic adrenalin unless too much ether was administered.

by Meister, Luscius, and Brüning is said to be a methyl-amino-alcohol or, more specifically, ortho-dioxy-phenyl-ethanol-methyl-amino-hydrochloride represented by the formula



It is a fine, granular, almost white, crystalline powder, easily soluble in water or normal saline. The solution decomposes much more readily than does a similar solution of the natural l-adrenalin. It turns cherry-red, later brownish, and finally deposits a brown precipitate, whereupon the solution loses its characteristic color and likewise its physiological activity. It is optically inactive and possesses the chemical properties generally attributed to it.

The data of Table I show distinctly that the natural l-adrenalin is more active than the synthetic dl- since equal volumes of 1:100,000 of the two solutions result in rises of blood pressure, which with few exceptions are greater for the natural product than for the synthetic. Although this table shows only a few results with 1:100,000 solutions, a great number of similar injections were made with solutions of them, maintaining the ratio 1 l : 1 dl, but varying in concentration from 1:5,000 to 1:200,000, with the result that the natural base nearly always caused the greatest rise of blood pressure. So that other sets might be chosen to illustrate the same point as does Table I. If instead of the effect of the equal volume of solution of like concentration, a comparison is made of the equal volume of solution with twice as much of the synthetic dl- substance as of the natural l-, the rise of blood pressure from the latter is nearly always less than that resulting from the synthetic dl-, as Table II will show. This table serves to illustrate results with solutions of higher or lower concentration with the ratio 1 l : 2 dl maintained. It is thus evident that with the ratio 1 : 1 the natural l- is too strong to cause like rises of blood pressure, with the ratio 1:2 the natural l- is too weak, and that the right ratio lies between these two. With a gradual increase of the concentration of the weaker or a decrease of the stronger a ratio was found that yielded the greatest number of like rises of blood pressure, which is 2 l:3 dl. Tables III and V, respectively, show that a 1:60,000 solution of natural l- is the equivalent of a 1:40,000 solution of synthetic dl-adrenalin. Table IV shows that like rises of

blood pressure may be obtained with equal volumes of 1:30,000 and 1:20,000 solutions of natural l- and synthetic dl-adrenalin, respectively. Table VI illustrates the same thing for cats.

It is also true that isolated cases might be chosen to show that both Biberfeld and Cushny were correct, the former maintaining that these two products are equal in activity, the latter contending that the synthetic dl-product has only one-half the activity of the natural base. For in a large number of experiments one may occasionally obtain equal rises of blood pressure when comparing 1:50,000 of synthetic dl- with 1:100,000 natural l-adrenalin or when comparing solutions of equivalent concentrations. But there is no such agreement after successive sets of readings, as indicated in the accompanying tables that have a ratio of concentration 2:3, instead of 1:1 according to Biberfeld, or 1:2 according to Cushny. Cushny is, however, more nearly correct than Biberfeld, and his experimental data, though inadequate, carries greater weight than any hitherto published. Finally, my results indicate that the ratio of activity of the natural l-base and the synthetic dl-product are to each other as 2:3. These results are in accordance with my first determinations, made before the publication of Cushny's first paper, and I am inclined to think them more nearly correct than his, which made the ratio 1:2.

TABLE I.—*The relative activity of natural l-adrenalin base and synthetic dl-adrenalin hydrochloride determined by blood pressure.*

Blood-pressure experiment No. 16, January 11, 1909.

Dog, 7,660 gm. weight, subcutaneous injection of 76.6 mg. of morphine sulphate, ether anesthesia, small doses of curare from time to time. Both vagi cut. Artificial respiration of warmed air. Solutions injected into femoral veins. Five milligrams of adrenalin base dissolved in 5 c. c. Ringer 11.50 a. m. Six milligrams synthetic dl-adrenalin hydrochloride crystals dissolved in 5 c. c. Ringer solution 11.55 a. m.

	Injection.	Base.	Ringer.	Time of injection.	Blood pressure before.	Blood pressure after.	Rise of blood pressure.
	C. c.	*Gram.*	*C. c.*	*P. m.*	*M. m.*	*M. m.*	*M. m.*
Natural l-adrenalin base	1	1	100,000	1.22	104	171	67
Synthetic dl-adrenalin	1	1	100,000	1.24	104	155	51
Natural l-adrenalin	1	1	100,000	1.36	106	170	64
Synthetic dl-adrenalin	1	1	100,000	1.33	105	162	57
Natural l-adrenalin	1	1	100,000	1.52	97	156	59
Synthetic dl-adrenalin	1	1	100,000	1.56	93	134	41
Natural l-adrenalin	1	1	100,000	2.26	106	160	54
Synthetic dl-adrenalin	1	1	100,000	2.28	106	152	46
Natural l-adrenalin	1	1	100,000	2.34	104	147	43
Synthetic dl-adrenalin	1	1	100,000	2.31	104	143	39
Natural l-adrenalin	1	1	100,000	2.40	104	140	36
Synthetic dl-adrenalin	1	1	100,000	2.38	105	134	29

From the above data it will be observed that the pure adrenalin is stronger than the synthetic dl-; on the other hand, if one chooses such dilutions as to make the solutions of synthetic twice as concentrated (in terms of base) as the natural adrenalin, then this solution, as shown by the following tables, is the stronger of the two physiologically:

TABLE II. (See legend to Table I.)

	Injection.	Base.	Ringer.	Time of injection.	Blood pressure before.	Blood pressure after.	Rise of blood pressure.
	C.c.	*Gram.*	*C.c.*	*P.m.*	*M.m.*	*M.m.*	*M.m.*
Curare	1½						
Synthetic dl-adrenalin	1	1	50,000	12.56	97	181	84
Natural l-adrenalin	1	1	100,000	12.59	98	162	64
Synthetic dl-adrenalin	1	1	50,000	1.6	93	171	78
Natural l-adrenalin	1	1	100,000	1.3	94	153	59
Synthetic dl-adrenalin	1	1	50,000	1.12	100	180	80
Natural l-adrenalin	1	1	100,000	1.9	102	155	53
Synthetic dl-adrenalin	1	1	50,000	3.5	114	190	76
Natural l-adrenalin	1	1	100,000	3.9	110	180	70
Synthetic dl-adrenalin	1	1	50,000	3.11	114	186	62
Natural l-adrenalin	1	1	100,000	3.15	118	177	59
Curare				3.22			
Synthetic dl-adrenalin	1	1	50,000	3.38	112	187	75
Natural l-adrenalin	1	1	100,000	3.41	115	186	71
Synthetic dl-adrenalin	1	1	50,000	4.7	122	220	98
Natural l-adrenalin	1	1	100,000	4.9	124	214	90
Synthetic dl-adrenalin	1	1	50,000	4.11	122	218	96
Natural l-adrenalin	1	1	100,000	4.15	124	204	80
Synthetic dl-adrenalin	1	1	50,000	4.22	123	194	71
Natural l-adrenalin	1	1	100,000	4.19	122	178	56
Synthetic dl-adrenalin	1	1	50,000	4.28	127	218	91
Natural l-adrenalin	1	1	100,000	4.25	124	210	86
Synthetic dl-adrenalin	1	1	40,000	5.52	95	213	128
Natural l-adrenalin	1	1	80,000	5.49	96	216	120
Synthetic dl-adrenalin	1	1	40,000	6.3	103	197	94
Natural l-adrenalin	1	1	80,000	6.8	104	194	90
Synthetic dl-adrenalin	1	1	40,000	6.13	96	186	90
Natural l-adrenalin	1	1	80,000	6.12	98	168	70

If instead of the concentrations described in Tables I and II the ratio 2 of synthetic to 3 of natural be used, the values representing the rises in blood pressure from 1 c. c. more nearly approximate each other. It is indeed remarkable how different sets of readings agree with each other, how constant is the interval required for recovery of the vasomoter apparatus, and with what certainty one can forecast the height to which the blood pressure will ascend in response

to the second injection of a given set. The following tables bring this out more clearly:

TABLE III. (See legend to Table I.)

	Injection.	Base.	Ringer.	Time of injection.	Blood pressure before.	Blood pressure after.	Rise of blood pressure.
	C.c.	*Gram.*	*C.c.*	*P.m.*	*M.m.*	*M.m.*	*M.m.*
Synthetic dl-adrenalin	1	1	40,000	6.30	93	202	109
Natural l-adrenalin	1	1	60,000	6.32	94	200	106
Synthetic dl-adrenalin	1	1	40,000	6.41	84	197	113
Natural l-adrenalin	1	1	60,000	6.44	84	197	113
Synthetic dl-adrenalin	1	1	40,000	6.46	83	194	111
Curare	1½			7.30			
Synthetic dl-adrenalin	1	1	40,000	7.48	86	198	112
Natural l-adrenalin base	1	1	60,000	7.46	84	198	114
Synthetic dl-adrenalin	1	1	40,000	7.54	84	194	110
Natural l-adrenalin	1	1	60,000	7.56	84	198	114

TABLE IV. (See legend to Table I.)

	Injection.	Base.	Ringer.	Time of injection.	Blood pressure before.	Blood pressure after.	Rise of blood pressure.
	C.c.	*Gram.*	*C.c.*	*P.m.*	*M.m.*	*M.m.*	*M.m.*
Synthetic dl-adrenalin	1	1	20,000	8.37	64	194	130
Natural l-adrenalin	1	1	30,000	8.39	64	190	126
Natural l-adrenalin	1	1	30,000	8.43	64	198	126
Synthetic dl-adrenalin	1	1	20,000	8.45	70	198	128
Natural l-adrenalin	1	1	30,000	8.47	70	198	128
Synthetic dl-adrenalin	1	1	20,000	9.0	73	208	135
Natural l-adrenalin	1	1	30,000	9.2	73	207	134
Synthetic dl-adrenalin	1	1	20,000	9.4	72	192	120
Natural l-adrenalin	1	1	30,000	9.6	72	193	121
Curare				9.12			
Natural l-adrenalin	1	1	30,000	9.18	67	215	148
Synthetic dl-adrenalin	1	1	20,000	9.20	61	208	147
Natural l-adrenalin	1	1	30,000	9.23	64	197	133
Synthetic dl-adrenalin	1	1	20,000	9.25	61	196	135
Natural l-adrenalin	1	1	30,000	9.27	62	188	126
Synthetic dl-adrenalin	1	1	20,000	9.29	60	186	126

TABLE V.

Experiment 17, January 12, 1909.

Pregnant cat, weight 3,770 gm., ether anesthesia, curare. Artificial respiration with warm air. Five milligrams of adrenalin base dissolved in Ringer solution 9 a. m. Six milligrams synthetic dl-adrenalin hydrochloride dissolved in 5 c. c. Ringer solution 10 a. m. Vagi cut 1.12 p. m. Injection into femorals.

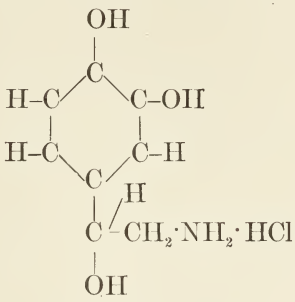
	Injection.	Base.	Ringer.	Time of injection.	Blood pressure before.	Blood pressure after.	Rise of blood pressure.
	C.c.	*Gram.*	*C.c.*	*P.m.*	*M.m.*	*M.m.*	*M.m.*
Natural l-adrenalin	1	1	90,000	12.18	132	152	20
Synthetic dl-adrenalin	1	1	60,000	12.19	130	149	19
Natural l-adrenalin	1	1	90,000	12.21	135	151	16
Synthetic dl-adrenalin	1	1	60,000	12.23	135	151	16
Natural l-adrenalin	1	1	90,000	12.38	140	157	17
Synthetic dl-adrenalin	1	1	60,000	12.41	141	158	17
Natural l-adrenalin	1	1	90,000	12.49	142	158	16
Synthetic dl-adrenalin	1	1	60,000	12.57	144	160	16
Vagi cut				1.12			
Curare	1½			1.21			
Natural l-adrenalin	1	1	60,000	1.32	142	168	26
Synthetic dl-adrenalin	1	1	40,000	1.34	143	168	25
Natural l-adrenalin	1	1	60,000	1.38	142	164	22
Synthetic dl-adrenalin	1	1	40,000	1.42	148	170	22
Natural l-adrenalin	1	1	60,000	1.44	146	170	24
Synthetic dl-adrenalin	1	1	40,000	1.46	149	172	23
Synthetic dl-adrenalin	1	1	40,000	1.49	146	172	26
Natural l-adrenalin	1	1	60,000	1.57	147	172	25

TABLE VI. (See legend to Table V.)

	Injection.	Base.	Ringer.	Time of injection.	Blood pressure before.	Blood pressure after.	Rise of blood pressure.
	C.c.	*Gram.*	*C.c.*	*P.m.*	*M.m.*	*M.m.*	*M.m.*
Natural l-adrenalin	1	1	30,000	3.12	122	156	34
Synthetic dl-adrenalin	1	1	20,000	3.15	122	153	31
Synthetic dl-adrenalin	1	1	20,000	3.29	124	148	24
Natural l-adrenalin	1	1	30,000	3.31	120	147	25
Synthetic dl-adrenalin	1	1	20,000	3.33	123	149	26

THE RELATIVE ACTIVITY OF ORTHO-DIOXY-PHENYL-ETHANOL-AMIN (ARTERENOL) AND NATURAL L-ADRENALIN DETERMINED BY BLOOD PRESSURE.

Another synthetic product of interest is the reduction product of amino-aceto-pyro-catechin, dioxy-phenyl-ethanol-amin. The chloride is known commercially as arterenol hydrocholride, the formula of which is given as—



It is a fine, granular, odorless, crystalline powder, easily soluble in water or normal saline. The deterioration of this product is not very evident, since there does not accompany it distinct coloration so easily noticed in adrenalin solutions. Just how rapid this process of deterioration is has not been determined, but it certainly is an important factor in determining the relative value of the substance. One solution received for purposes of testing was found to have an activity comparable to that of our natural l-adrenalin. So surprised was I to note this that the balance of the sample was preserved for a subsequent testing, but a few days later this preparation had deteriorated and a fresh solution had to be made. It can readily be seen that to the physician the commercial 1:1,000 solution may prove very disappointing if kept for subsequent use after once the original package has been opened. The fresh solution, however, has a remarkable vaso-constrictor action, and if it were more certain in yielding quantitative results throughout entire experiments, it would be a worthy rival of natural l-adrenalin itself. In the early parts of long experiments fairly constant results may be obtained with small doses, but in the latter parts, or after larger doses (1 c. c. of 1:60,000 or over), irregularities seemed to appear the exact meaning of which must be determined later. As a matter of fact, the following tables represent results taken from the beginning of experiments only.

Table VIII is interesting, since it shows that a 1 c. c. injection of adrenalin or arterenol in 1:100,000 solutions causes a like rise of blood pressure (27–28 m. m.) and for 1 c. c. injections of a 1:50,000 solution (38 m. m.). A 1:50,000 solution of arterenol, however, is uniformly more active than a 1:100,000 solution of natural l-base. Finally, consistent results are obtained by comparing the two substances each in a concentration of 1:80,000 or in one of 1:60,000.

TABLE VII.—*Physiological activity of arterenol hydrochloride compared with that of l-natural adrenalin base.*

Blood pressure experiment No. 21, March 22, 1909.

Female bull terrier, weight 10.4 kilograms; 10.20 a. m. subcutaneous injection of 100 milligrams morphine sulphate. Ether anesthesia; small intravenous injections of curare from time to time. Both vagi tied off. Artificial respiration with warmed air. Solutions injected into saphenous veins. 5 milligrams specially pure natural l-adrenalin base dissolved (10.35 a. m.) in 5 c. c. of Ringer solution acidulated with calculated amount of HCl. Six milligrams arterenol hydrochloride dissolved in 5 c. c. Ringer solution 11.50 a. m. These two solutions were then diluted as if they were the equivalents of a 1:1,000 solution of the base—that is, 1 c. c. of either one of these stock solutions diluted to 10 c. c. gave a 1:10,000 solution (base).

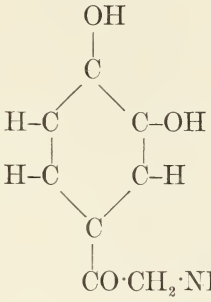
	Injection.	Base.	Ringer.	Time of injection.	Blood pressure before.	Blood pressure after.	Rise of blood pressure.
	C. c.	*Gram.*	*C. c.*	*P. m.*	*M. m.*	*M. m.*	*M. m.*
Natural l-adrenalin base	1	1	100,000	12.05	127	182	55
Arterenol hydrochloride	1	1	100,000	12.09	126	182	56
Natural l-adrenalin base	1	1	100,000	12.14	127	184	57
Arterenol hydrochloride	1	1	100,000	12.18	124	179	55
Curare				12.21			
Natural l-adrenalin base	1	1	100,000	12.28	130	193	63
Arterenol hydrochloride	1	1	100,000	12.31	121	184	63
Arterenol hydrochloride	1	1	100,000	1.08	131	152	21
Natural l-adrenalin base	1	1	100,000	1.12	131	150	19
Arterenol hydrochloride	1	1	100,000	1.16	134	156	22
Natural l-adrenalin base	1	1	100,000	1.20	134	155	21
Arterenol hydrochloride	1	1	100,000	1.28	132	154	22
Curare	2			1.44			
Arterenol hydrochloride	1	1	100,000	2.47	142	185	43
Natural l-adrenalin base	1	1	100,000	2.50	151	195	44

TABLE VIII. (See legend to Table V.)

	Injection.	Base.	Ringer.	Time of injection.	Blood pressure before.	Blood pressure after.	Rise of blood pressure.
	C. c.	*Gram.*	*C. c.*	*P. m.*	*M. m.*	*M. m.*	*M. m.*
Natural l-adrenalin base	1	1	80,000	6.16	157	187	30
Arterenol hydrochloride	1	1	80,000	6.20	153	184	31
Natural l-adrenalin base	1	1	100,000	7.24	123	150	27
Arterenol hydrochloride	1	1	50,000	7.26	124	162	38
Natural l-adrenalin base	1	1	100,000	7.30	126	154	28
Arterenol hydrochloride	1	1	50,000	7.35	126	164	38
Arterenol hydrochloride	1	1	60,000	7.39	124	152	28
Natural l-adrenalin base	1	1	60,000	7.42	128	155	27

THE RELATIVE ACTIVITY OF ETHYL-AMINO-ACETO-CATECHOL AND NATURAL L-ADRENALIN AS DETERMINED BY BLOOD PRESSURE.

A substance known commercially as homorenon hydrochloride was the last one tested. This is the hydrochloride of ethyl-amino-aceto-catechol represented by the formula:



It is a white powder composed of fine crystalline needles, easily soluble in normal saline, and, so far as I have been able to determine, keeps much better than any of the products already mentioned. Its optical activity is *nil*. As a vaso-constrictor it is inferior to all of the other substances tested, as illustrated in Tables IX and X. A 1 c. c. injection of a 1:160,000 solution of natural l-adrenalin causes a rise of blood pressure which with a like volume of homorenon is equaled only by a 1:2,000 solution. It is found that a 1 c. c. injection of a 1:80,000 solution of adrenalin base causes the same rise of blood pressure as a 1 c. c. injection of a 1:1,000 solution of homorenon. So that by the blood-pressure method, natural l-adrenalin base is eighty times as active as homorenon base, the base being calculated from the foregoing formula.

TABLE IX.—*Physiological activity of homorenon hydrochloride compared with that of l-natural adrenalin base.*

Blood-pressure experiment No. 20. March 16, 1909.

Female fox terrier, weight 6.8 kilograms. 10.15 a. m. subcutaneous injection of 68 mg. of morphine sulphate. Ether anesthesia, small intravenous injections of curare from time to time. Both vagi tied off. Artificial respiration of warmed air. Solutions injected into saphenous veins.

Five milligrams specially pure adrenalin l-base dissolved 10.23 a. m. in 5 c. c. of Ringer solution, acidulated with the calculated amount of HCl.

Six milligrams arterenol hydrochloride dissolved 6 p. m. in 5 c. c. Ringer solution.

118.6 mg. homorenon hydrochloride dissolved 11.30 a. m. in 10 c. c. of Ringer solution. These solutions were then diluted as if they were the equivalents of a 1:1,000 solution of adrenalin base.

	Injection.	Base.	Ringer.	Time of injection.	Blood pressure before.	Blood pressure after.	Rise of blood pressure.
	C. c.	*Gram.*	*C. c.*	*P. m.*	*M. m.*	*M. m.*	*M. m.*
Natural l-adrenalin base	1	1	160,000	3.49	180	204	24
Homorenon hydrochloride	1	1	2,000	3.51	178	202	24
Natural l-adrenalin base	1	1	160,000	3.55	177	202	25
Natural l-adrenalin base	1	1	160,000	4.6	160	186	26
Homorenon hydrochloride	1	1	2,000	4.9	150	175	25

TABLE IX.—*Physiological activity of homorenon hydrochloride compared with that of l-natural adrenalin base*—Continued.

	Injection.	Base.	Ringer.	Time of injection.	Blood pressure before.	Blood pressure after.	Rise of blood pressure.
	C. c.	*Gram.*	*C. c.*	*P. m.*	*M. m.*	*M. m.*	*M. m.*
Natural l-adrenalin base	1	1	160,000	4.31	156	178	22
Homorenon hydrochloride	1	1	2,000	4.36	154	177	23
Natural l-adrenalin base	1	1	160,000	5.15	172	186	21
Homorenon hydrochloride	1	1	2,000	5.19	173	195	22
Natural l-adrenalin base	1	1	160,000	5.22	172	194	22
Homorenon hydrochloride	1	1	2,000	5.25	169	191	22
Homorenon hydrochloride	1	1	2,000	5.32	155	176	21

TABLE X. (See legend to VI.)

	Injection.	Base.	Ringer.	Time of injection.	Blood pressure before.	Blood pressure after.	Rise of blood pressure.
	C. c.	*Gram.*	*C. c.*	*P. m.*	*M. m.*	*M. m.*	*M. m.*
Natural l-adrenalin base	1	1	80,000	1.57	138	180	42
Homorenon hydrochloride	1	1	1,000	2.1	138	180	42
Natural l-adrenalin base	1	1	80,000	2.28	158	208	50
Homorenon hydrochloride	1	1	1,000	2.33	156	205	49
Natural l-adrenalin base	1	1	80,000	2.39	166	215	49
Homorenon hydrochloride	1	1	1,000	2.56	164	200	36
Natural adrenalin base	1	1	80,000	3.02	162	198	36
Homorenon hydrochloride	1	1	1,000	3.04	156	193	37
Natural adrenalin base	1	1	80,000	3.09	160	198	38
Natural adrenalin base	1	1	80,000	3.39	174	228	54
Homorenon hydrochloride	1	1	1,000	.....	170	223	53

THE RELATIVE TOXICITY OF ORTHO-DIOXY-PHENYL-ETHANOL-METHYL-AMIN (NATURAL L- AND SYNTHETIC DL-ADRENALIN), OF ORTHO-DIOXY-PHENYL-ETHANOL-AMIN ("ARTERENOL"), AND ETHYL-AMINO-DIOXY-ACETO-PHENON ("HOMORENON") AS DETERMINED UPON MICE.

It is conceivable that coefficients of physiological activity of a series of closely related compounds, when determined by vasomotor effects, may be so arranged as to express one ratio, whereas those determined by toxic effects when similarly arranged may express a very different one. That is, a coefficient determined by the blood-pressure method may be relatively small when compared with that determined by the dose necessary to kill, say, more than 50 per cent of the animals injected. It would seem that a comparison of such coefficients determined in various ways might throw additional light on the relative safety and effectiveness of adrenalin-like bodies. But scarcely anything has been published from which can be derived the coefficients needed in making the above-

named comparisons. Hence, with a view of determining the relative toxicity of the compounds already compared by the blood-pressure method, the following experiments were undertaken. The test object chosen is the ordinary white mouse, reared in our own mousery under conditions well under control. As a rule these mice when placed in individual jars on a diet of oats and water lose in weight, but after the first day, upon becoming accustomed to their new surroundings, return to normal. Generally on a given day the mice were brought into the preparation room, weighed, and on the second day weighed again, whereupon the dose was calculated and unless otherwise stated the solution made up and injected at once. All mice were injected tailward underneath the skin of the back. The syringe used was calibrated in 0.05 c. c., standardized, and fitted at first with a steel needle. Since this needle, especially if tarnished, decomposed the solutions, a platinum-iridium needle was substituted.

The phenomena accompanying subcutaneous injections of this nature may be summarized as follows: Soon after being released the animal seeks a corner of the jar, under the chaff, or at once stretches itself lengthwise upon the belly with hind feet directed backward and planta turned upward. The favorite position is to lie upon the cool, moist oat jar with head downward, breathing at first very rapidly and later more or less irregularly. In this stage the animal may be highly excitable, its reflexes being probably intensified not only by the injected material but also by the fear aroused by the smarting of the needle's prick and by fright from handling. In case the dose is rather large the bulbus is protruded, and the glands of the eye are usually stimulated to excessive secretion.

In some mice thirty to sixty mintues after injection a small opalescent[a] disc appears in the eye. This later becomes opaque white and may be confined to only one or extended to both eyes. The phenomenon seems to be one of the lens and resembles cataract. In this respect it differs from the opaquing observed in the eyes of frogs, for in the latter case I have proved that the initial opaquing is due to coagulation of the outer membranes of the bulbus and that it can be removed by stripping off this membrane, whereupon the eye becomes perfectly clear. In the eye of the mouse the opaque disc, examined with a magnifying glass, seems to be underneath the cornea, of the size and position of the lens. The following protocol is taken from a mouse injected with arterenol and is typical of the cases observed with natural l- and synthetic dl-adrenalin:

Mouse 324, weight 22.22 gm. March 15, 12.20 p. m., injected subcutaneously 0.44 c. c. (0.88 mg.) arterenol hydrochloride.

[a] After this section had been written I noticed in a recent article by Emmert a reference to his observation of what seems to be a similar phenomenon.

12.26, lies flat, restless from time to time, hind leg stretched out, but when touched is jerked away.

12.30, forced breathing.

12.33, abundant salivation, leg still sensitive, but control of hip muscle imperfect.

12.37, lies flat; does not move when tail is pinched; feet and leg still sensitive, but reflex not so rapid as before.

1.34, opaque disc in center of left eye.

2.35, opaque disc of left eye much larger, opaque disc in right eye also.

March 16, 8.15 a. m. Both eyes cleared up, mouse prostrated.

March 17, 9 a. m. Mouse prostrated, eye clear, breathing shallow and slow.

12.20, found dead.

The saliva may or may not flow abundantly, but when it does the amount is so great as to drop from the lower jaw. In the males erection may follow and even be accompanied by semination. Should the dose be lethal the earlier stages are passed through quickly; in general the animal becomes very sluggish, largely on account of constriction of the blood vessels, causing anæmia not only of the muscles, but also of the cord and brain. Sometimes animals die very suddenly from doses that seem hardly to affect other individuals. I am inclined to think that in such cases some of the drug enters a vein. Otherwise there does not occur any explanation, unless it be due to heart failure, such as indeed has been noted in apparently vigorous human beings under the influence of great fear or excitement. When spasms result, the animal usually dies in one to two minutes; only one out of over four hundred recovered, and this one died at the end of four or five days. When once the lethal dose is exceeded death becomes more violent, accompanied by spasms of very short duration.

TOXICITY OF NATURAL L-ADRENALIN.

Judging from the remarkable activity of the natural l-adrenalin in causing vaso-constriction, it was presumed that the lethal subcutaneous dose would be small. The experimental data of Table I justifies this presumption, for 0.008 mg. per gram mouse is usually fatal. Certain mice may die from doses as small as 0.004 mg. per gram, but this is the exception, whereas others may survive as much as 0.017 mg. per gram, which is likewise unusual. Perhaps a greater number of experiments might reveal certain cases of even greater resistance, but in spite of these exceptions, as seen in Table XI, it may be said that 0.008 is the lethal dose for the average mouse.

TABLE XI.—*Toxicity of pure natural l-adrenalin.*

Number of mouse.[a]	Mg. base per gm.[b]	Body weight.	Injected.			Died.
			Total.		Time.	
		Grams.	*Mg.*	*C. c.*		
42	0.0005	16.45	0.008	0.04	Dec. 3, 9.28 a. m.	
43	0.00075	19.28	0.014	0.07	Dec. 3, 9.32 a. m.	
44	0.001	16.62	0.017	0.08	Dec. 3, 9.33 a. m.	
52	0.001	20.92	0.021	0.10	Dec. 4, 9.28 a. m.	
32	0.002	18.23	0.036	0.07	Dec. 2, 9.29 a. m.	
45	0.002	20.92	0.042	0.22	Dec. 3, 9.37 a. m.	
53	0.002	21.21	0.042	0.21	Dec. 4, 9.26 a. m.	
62	0.002	12.26	0.024	0.02	Dec. 5, 9.08 a. m.	
72	0.002	16.52	0.033	0.03	Dec. 7, 9.40 a. m.	
82	0.002	20.40	0.040	0.04	Dec. 7, 2.01 p. m.	
22	0.004	18.52	0.074	0.08	Nov. 30, 4.34 p. m.	Nov. 30, 4.48 p. m.
33	0.004	17.89	0.071	0.15	Dec. 2, 9.34 a. m.	Dec. 2, 11.35 a. m.
46	0.004	19.77	0.079	0.42	Dec. 3, 9.39 a. m.	
54	0.004	17.33	0.069	0.35	Dec. 4, 9.28 a. m.	
63	0.004	15.80	0.063	0.063	Dec. 5, 9.11 a. m.	
73	0.004	17.74	0.071	0.07	Dec. 7, 9.43 a. m.	
83	0.004	20.00	0.080	0.08	Dec. 7, 2.05 p. m.	
55	0.005	16.14	0.081	0.41	Dec. 4, 9.32 a. m.	
56	0.006	13.62	0.082	0.41	Dec. 4, 9.34 a. m.	
64	0.006	20.05	0.120	0.12	Dec. 5, 9.13 a. m.	
74	0.006	14.54	0.087	0.09	Dec. 7, 9.45 a. m.	
84	0.006	17.28	0.103	0.10	Dec. 7, 2.07 p. m.	
112	0.006	12.41	0.074	0.07	Dec. 9, 5.21 p. m.	
152	0.006	17.77	0.107	0.11	Jan. 4, 2.52 p. m.	
155	0.006	16.55	0.099	0.10	Jan. 4, 2.55 p. m.	Jan. 5, 12.15 a. m. (W.)
158	0.006	13.07	0.078	0.08	Jan. 4, 3.08 p. m.	
23	0.008	18.15	0.145	0.16	Nov. 30, 4.37 p. m.	Nov. 30, 4.43 p. m.
34	0.008	15.98	0.128	0.14	Dec. 2, 9.19 a. m.	Dec. 2, 12.34 p. m.
65	0.008	20.51	0.164	0.16	Dec. 5, 9.16 a. m.	Dec. 5, 10 p. m.
75	0.008	20.50	0.164	0.16	Dec. 7, 9.48 a. m.	Dec. 7, 6 to 7 p. m.
85	0.008	16.00	0.128	0.13	Dec. 7, 2.10 p. m.	
113	0.008	16.30	0.130	0.13	Dec. 9, 5.23 p. m.	Dec. 9, 8 p. m. (W.)
153	0.008	18.27	0.146	0.15	Jan. 4, 2.53 p. m.	
156	0.008	16.65	0.133	0.13	Jan. 4, 3.03 p. m.	
159	0.008	13.79	0.110	0.11	Jan. 4, 3.10 p. m.	
218	0.008	19.39	0.160	0.16	Jan. 9, 3.07 p. m.	Jan. 9, 3.20 p. m.
221	0.008	15.12	0.120	0.12	Jan. 9, 3.13 p. m.	Jan. 10, 10.30 a. m. (W.)
224	0.008	13.61	0.110	0.11	Jan. 9, 4.54 p. m.	Jan. 10, 4.30 a. m. (W.)
227	0.008	13.78	0.110	0.11	Jan. 9, 5.01 p. m.	Jan. 10, 10.30 a. m. (W.)
230	0.008	14.84	0.120	0.12	Jan. 9, 5.10 p. m.	Jan. 10, 3.30 a. m. (W.)
66	0.010	12.66	0.127	0.13	Dec. 5, 9.19 a. m.	Dec. 5, 4.20 p. m.
76	0.010	17.24	0.170	0.17	Dec. 7, 9.50 a. m.	Jan. 7, 2.26 p. m.
86	0.010	13.04	0.130	0.13	Dec. 7, 2.12 p. m.	
114	0.010	20.28	0.203	0.20	Dec. 9, 5.25 p. m.	
154	0.010	19.04	0.190	0.19	Jan. 4, 2.55 p. m.	Jan. 6, 11.05 a. m.
157	0.010	16.51	0.165	0.16	Jan. 4, 3.05 p. m.	Jan. 6, 12.45 a. m. (W.)
160	0.010	13.00	0.130	0.13	Jan. 4, 3.13 p. m.	
215	0.010	14.55	0.150	0.15	Jan. 9, 3.00 p. m.	Jan. 10, 12.55 p. m. (W.)
216	0.010	18.04	0.180	0.18	Jan. 9, 3.03 p. m.	Jan. 9, 3.22 p. m.

[a] Mice not marked "W" or "F" were observed by the author to die at time noted.

[b] These figures are only approximate. The actual dose is in the column "total milligrams injected."

W. Time noted by watchman.

TABLE XI.—*Toxicity of pure natural l-adrenalin*—Continued.

Number of mouse.	Mg. base per gm.	Body weight.	Injected.			Died.
			Total.		Time.	
		Grams.	*Mg.*	*C. c.*		
219	0.010	12.73	0.130	0.13	Jan. 9, 3.09 p. m.	
222	0.010	13.58	0.140	0.14	Jan. 9, 3.15 p. m.	
225	0.010	15.44	0.150	0.15	Jan. 9, 4.57 p. m.	Jan. 10, 3.30 a. m. (W.)
228	0.010	13.72	0.140	0.14	Jan. 9, 5.05 p. m.	Jan. 10, 5.14 p. m.
231	0.010	15.77	0.160	0.16	Jan. 9, 5.12 p. m.	Jan. 9, 5.28 p. m.
115	0.012	13.74	0.165	0.16	Dec. 9, 5.27 p. m.	Dec. 9, 6.30 p. m. (W.)
217	0.012	14.80	0.180	0.18	Jan. 9, 3.05 p. m.	
220	0.012	19.83	0.240	0.24	Jan. 9, 3.12 p. m.	Jan. 12, 8 a. m. (W.)
223	0.012	17.80	0.210	0.21	Jan. 9, 3.19 p. m.	Jan. 9, 3.35 p. m.
226	0.012	13.04	0.140	0.14	Jan. 9, 4.59 p. m.	Jan. 9, 5.11 p. m.
229	0.012	12.11	0.140	0.14	Jan. 9, 5.07 p. m.	Jan. 9, 5.26 p. m.
232	0.012	13.22	0.140	0.14	Jan. 9, 5.15 p. m.	Jan. 9, 5.22 p. m.
24	0.013	15.77	0.210	0.21	Nov. 30, 4.40 p. m.	Dec. 1, 9 p. m. (F.)
35	0.013	13.43	0.180	0.18	Dec. 2, 9.21 a. m.	Dec. 2, 11 a. m.
116	0.014	16.78	0.230	0.23	Dec. 9, 5.29 p. m.	Dec. 10, 12,15 a. m.
12	0.0147	18.27	0.270	0.27	Nov. 28	Nov. 28, 4.40 p. m. (W.)
7	0.017	18.20	0.318	0.32	Nov. 23, 1.18 p. m.	
8	0.017	17.09	0.298	0.30	Nov. 23	Nov. 25, 10 p. m.
13	0.017	20.18	0.350	0.35	Nov. 28, 3.18 p. m.	Nov. 28, 3.29 p. m.
25	0.017	17.94	0.300	0.30	Nov. 30, 4.43 p. m.	Nov. 30, 4.55 p. m.
26	0.0218	16.75	0.370	0.37	Nov. 30, 4.47 p. m.	Dec. 1, 1.45 a. m. (W.)
14	0.0218	21.52	0.470	0.47	Nov. 28, 3.22 p. m.	Nov. 28, 6.30 p. m. (W.)
36	0.022	17.97	0.410	0.41	Dec. 2, 9.23 a. m.	Dec. 2, 9.28 a. m.
9	0.026	12.38	0.320	0.32	Nov. 23, 10.54 a. m.	Nov. 27, 1.50 a. m. (F.)
10	0.035	14.40	0.500	0.50	Nov. 25, 11.23 p. m.	Nov. 26, 6 p. m. (F.)
15	0.035	19.26	0.590	0.59	Nov. 28, p. m.	Nov. 30, 8.55 p. m. (F.)

F. Found dead at time noted.
W. Time noted by watchman.

In order to avoid errors from deterioration the solutions were usually made up just before using, 5 mgs. of the base having been weighed up and dissolved in 5 c. c. of Ringer solution. The solvent was acidified with hydrochloric acid slightly in excess of the amount necessary to transform the adrenalin into the chloride. If more specific information is desired as to the age of a solution at the time of any given injection it is easily obtained by the aid of the following data in conjunction with the time of the injection given in the tables:

Mice Nos. 12, 13, 14, 15, 16, solution made up about 9 a. m. November 27, 1908.

Mice Nos. 42 to 46, inclusive, solution made up about 11 a. m. December 2, 1908.

Mice Nos. 52 to 56, inclusive, solution made up about 9 a. m. December 4, 1908, and diluted to 1:5,000.

Mice Nos. 62 to 66, inclusive, the solution was made up between 9 and 11 a. m. December 4, 1908.

Mice Nos. 72 to 76, inclusive, the solution was made up 11.50 a. m. December 5, 1908.

Mice Nos. 72, 73, 74, 75, and 76 had been injected with 0.008, 0.014, 0.017, 0.044, and 0.085 mg. of adrenalin, respectively.

Mice Nos. 82, 83, 84, 85, and 86, solution made up 11.50 a. m. December 5. These mice had been injected on December 4 with 0.021, 0.042, 0.069, 0.081, and 0.082 mg. of adrenalin, respectively.

Nos. 112 to 116, inclusive, solution made up 11.30 a. m. December 9.

Nos. 152 to 160, inclusive, solution made up about 2.30 p. m. January 4, 1909.

Nos. 215 to 232, inclusive, solution made up between 2 and 3 p. m. January 9.

TOXICITY OF SYNTHETIC DL-ADRENALIN.

If instead of natural l-adrenalin synthetic dl-adrenalin hydrochloride be used the symptoms of toxicity are practically the same as those described for adrenalin. There is, however, greater irregularity in the results. In exceptional cases 0.006 to 0.008 mg. per gram mouse may cause death. (Table XII.) By increasing the size of the initial dose the number of deaths likewise becomes larger, so that injections of 0.012 mg. may result in a death rate even as high as does one of 0.020 mg. per gram body weight. The lethal dose, therefore, may be said to be from 0.012 to 0.016 mg. per gram. This would make the natural product 1.5 to 2 times as toxic as the synthetic. It is interesting to observe that the toxicity of the two substances is in direct proportion to their vaso-constrictor action as measured by the blood-pressure method.

TABLE XII.—*Toxicity of synthetic dl-adrenalin hydrochloride.*

Number of mouse.[a]	Mg. base per gm.[b]	Body weight.	Injected.			Died.
			Total.		Time.	
		Grams.	*Mg.*	*C. c.*		
102	0. 002	15. 48	0. 030	0. 03	Dec. 9, 9.45 a. m.	
142	0. 002	26. 57	0. 053	0. 05	Dec. 10, 3.43 p. m.	
147	0. 002	25. 27	0. 05	0. 05	Dec. 10, 3.56 p. m.	
103	0. 004	14. 44	0. 06	0. 06	Dec. 9, 9.48 a. m.	
143	0. 004	23. 24	0. 092	0. 09	Dec. 10, 3.45 p. m.	
148	0. 004	17. 43	0. 069	0. 07	Dec. 10, 3.58 p. m.	
104	0. 006	15. 22	0. 091	0. 09	Dec. 9, 9.51 a. m.	
144	0. 006	23. 50	0. 141	0. 14	Dec. 10, 3.48 p. m.	
149	0. 006	23. 00	0. 138	0. 14	Dec. 10, 3.59 p. m.	
343	0. 006	13. 56	0. 081	0. 08	Mar. 15, 3.10 p. m.	Mar. 15, 3.32 p. m.
344	0. 006	11. 77	0. 070	0. 07	Mar. 15, 3.11 p. m.	
345	0. 006	12. 97	0. 077	0. 07	Mar. 15, 3.12 p. m.	
346	0. 006	13. 34	0. 080	0. 08	Mar. 15, 3.14 p. m.	
347	0. 006	14. 72	0. 088	0. 08	Mar. 15, 3.16 p. m.	
105	0. 008	17. 24	0. 138	0. 14	Dec. 9, 9.53 a. m.	Dec. 10, 12 m.
145	0. 008	24. 21	0. 194	0. 19	Dec. 10, 3.50 p. m.	
150	0. 008	24. 24	0. 194	0. 19	Dec. 10, 4.01 p. m.	
348	0. 008	20. 46	0. 163	0. 16	Mar. 15, 3.18 p. m.	
349	0. 008	16. 15	0. 130	0. 13	Mar. 15, 3.20 p. m.	Mar. 15, 5.30 p. m. (F.).
350	0. 008	15. 39	0. 123	0. 12	Mar. 15, 3.22 p. m.	
351	0. 008	13. 84	0. 110	0. 11	Mar. 15, 3.24 p. m.	
106	0. 010	15. 87	0. 159	0. 16	Dec. 9, 9.56 a. m.	Dec. 10, 12.15 a. m. (W.).
146	0. 010	22. 46	0. 225	0. 22	Dec. 10, 3.53 p. m.	
151	0. 010	19. 37	0. 193	0. 19	Dec. 10, 4.03 p. m.	
353	0. 010	15. 45	0. 154	0. 15	Mar. 15, 3.34 p. m.	
354	0. 010	11. 80	0. 118	0. 12	Mar. 15, 3.35 p. m.	
355	0. 010	17. 38	0. 173	0. 17	Mar. 15, 3.37 p. m.	Mar. 16, 8 a. m. (F.).

[a] Mice not marked "W" or "F" were observed by the author to die at the time noted.

[b] These figures are only approximate. The actual dose is in column "Total milligrams injected."

W. Time noted by watchman.

F. Found dead at time noted.

TABLE XII.—*Toxicity of synthetic dl-adrenalin hydrochloride*—Continued.

Number of mouse.	Mg. base per gm.	Body weight.	Injected.			Died.
			Total.		Time.	
		Grams.	*Mg.*	*C. c.*		
356	0.010	12.26	0.122	0.12	Mar. 15, 3.39 p. m.	
357	0.010	18.47	0.184	0.18	Mar. 15, 3.40 p. m.	Mar. 17, 1.15 a. m.
161	0.012	18.09	0.217	0.22	Jan. 4, 3.25 p. m.	
164	0.012	15.11	0.181	0.18	Jan. 4, 3.35 p. m.	Jan. 6, 2.30 p. m.
167	0.012	15.18	0.182	0.18	Jan. 4, 3.40 p. m.	Jan. 5, 12.15 a. m. (W.).
188	0.012	14.55	0.174	0.17	Jan. 8, 11.29 a. m.	
191	0.012	16.20	0.194	0.19	Jan. 8, 11.44 a. m.	Jan. 9, 1.30 a. m. (W.).
194	0.012	15.15	0.181	0.18	Jan. 8, 11.52 a. m.	Jan. 10, 10.30 a. m. (W.).
358	0.012	18.00	0.180	0.18	Mar. 15, 3.42 p. m.	
359	0.012	13.62	0.136	0.13	Mar. 15, 3.44 p. m.	
360	0.012	13.49	0.135	0.13	Mar. 15, 3.46 p. m.	
361	0.012	15.80	0.158	0.15	Mar. 15, 3.47 p. m.	
362	0.012	20.24	0.202	0.20	Mar. 15, 3.48 p. m.	
162	0.016	19.91	0.319	0.32	Jan. 4, 3.28 p. m.	Jan. 5, 1.30 a. m. (W.).
165	0.016	15.92	0.255	0.25	Jan. 4, 3.36 p. m.	Jan. 6, 10.05 a. m.
168	0.016	15.35	0.246	0.25	Jan. 4, 3.44 p. m.	
189	0.016	14.35	0.230	0.23	Jan. 8, 11.40 a. m.	Jan. 10, 10.30 a. m. (W.).
192	0.016	21.54	0.345	0.34	Jan. 8, 11.48 a. m.	Jan. 8, 4.50 p. m. (F.).
195	0.016	14.08	0.225	0.23	Jan. 8, 12.05, p. m.	Jan. 10, 10.30 a. m. (W.).
163	0.020	21.37	0.427	0.43	Jan. 4, 3.32 p. m.	
166	0.020	17.85	0.357	0.36	Jan. 4, 3.38 p. m.	Jan. 5, 12.15 a. m. (W.).
169	0.020	15.54	0.311	0.31	Jan. 4, 3.47 p. m.	
190	0.020	17.53	0.350	0.35	Jan. 8, 11.42 a. m.	Jan. 8, 4.50 p. m. (F.).
193	0.020	19.30	0.386	0.39	Jan. 8, 11.50 a. m.	Jan. 9, 2.30 a. m. (W.).
196	0.020	12.54	0.250	0.25	Jan. 8, 12.07 p. m.	

W. Time noted by watchman.
F. Found dead at time noted.

NOTE.—Concentration of the solutions made from synthetic dl-adrenalin hydrochloride crystals and th time of mixing them are as follows:
Nos. 102 to 106, inclusive, 6 mg. crystals in 5 c. c. Ringer solution about 9 a. m. December 9, 1908.
Nos. 142 to 151, inclusive, 6 mg. crystals dissolved in 5 c. c. of Ringer solution just before using.
Nos. 161 to 169, inclusive, 6 mg. crystals dissolved in 5 c. c. of Ringer solution about 3 p. m. January 4, 1909.

TOXICITY OF ARTERENOL HYDROCHLORIDE.

Arterenol (see Table XIII) differs from natural l- and synthetic dl-products in two essentials; (1) it is far less toxic, and (2) it is, as said before, more variable in its action. If the animal can survive the first tremendous strain upon the organism, it has greater chances of recovery from arterenol than from doses of adrenalin causing a corresponding degree of prostration. If the animal is again injected after recovery from sublethal doses, the resulting prostration is as a rule correspondingly less severe, and doses that are usually lethal no longer proved to be so. This is illustrated by mice in groups (a) and (b) (Table XIII). In the one case 0.040 mg. and in the other 0.080 mg. per gram mouse is with one exception in each group successfully resisted. Whether or not this is a case of acquired tolerance or whether it is a

mere accident that these groups are made up of unusually resistant mice, is difficult to say. A greater number of experiments will have to be made to decide positively. Nevertheless, the blank spaces in the last column of the table indicate a resistance to the second dose and is very suggestive of the idea that the first injections are a determining factor in making this resistance possible.

At any rate, it is highly important in experiments of this nature that only the results obtained with fresh mice be compared with each other. When this is done it will be observed (Table XIII) that as small a dose as 0.008 mg. per gram body weight may cause death; on the other hand, even three times this dose kills only 1 mouse out of 14. The dose must be 0.040 mg. per gram mouse before any appreciable number die, though not a few mice may survive a dose as large as 0.080 mg. per gram. It is then safe to say that the lethal dose for the average mouse is about 0.040 mg. per gram body weight. If the lethal dose be placed at 0.040 mg. per gram, then natural l-adrenalin is five times as toxic as arterenol.

TABLE XIII.—*Toxicity of arterenol hydrochloride.*

Number of mouse.[a]	Mg. base per gm.[b]	Body weight.	Injected.			Died.
			Total.		Time.	
		Grams.	*Mg.*	*C. c.*		
67	0.004	14.24	0.057	0.06	Dec. 5, 9.23 a. m.	
107	0.004	15.43	0.061	0.06	Dec. 9, 9.22 a. m.	
68	0.008	16.50	0.132	0.13	Dec. 5, 9.28 a. m.	
108	0.008	18.34	0.146	0.15	Dec. 9, 9.24 a. m.	
69	0.012	19.19	0.240	0.24	Dec. 5, 9.30 a. m.	Dec. 8, 12.45 p. m.
109	0.012	17.26	0.207	0.21	Dec. 9, 9.27 a. m.	
117	0.012	16.23	0.195	0.20	Dec. 9, 5.36 p. m	
179	0.012	14.47	0.173	0.17	Jan. 5	
182	0.012	19.77	0.237	0.24	Jan. 5	
185	0.012	16.87	0.200	0.20	Jan. 5	
70	0.016	17.20	0.275	0.27	Dec. 5, 9.34 a. m.	
110	0.016	19.94	0.318	0.32	Dec. 9, 9.30 a. m.	Dec. 11, 9.40 a. m. (F.).
118	0.016	17.18	0.275	0.27	Dec. 9, 5.38 p. m.	Dec. 9, 5.43 p. m.
180	0.016	18.77	0.300	0.30	Jan. 5	
183	0.016	18.90	0.302	0.30	Jan. 5	
186	0.016	14.54	0.233	0.23	Jan. 5	
111	0.020	10.56	0.211	0.21	Dec. 9, 9.37 a. m.	
119	0.020	14.80	0.296	0.30	Dec. 9, 5.40 p. m.	
181	0.020	16.30	0.326	0.33	Jan. 5	
184	0.020	15.92	0.318	0.32	Jan. 5	
187	0.020	15.92	0.318	0.32	Jan. 5	
120	0.024	19.13	0.459	0.46	Dec. 9, 5.43 p. m.	Dec. 12.
197	0.024	15.63	0.370	0.37	Jan. 8, 1.25 p. m.	
200	0.024	13.62	0.330	0.33	Jan. 8, 1.30 p. m.	
203	0.024	13.32	0.320	0.32	Jan. 8, 1.55 p. m	

[a] Mice not marked "W" or "F" were observed by the author to die at the time noted.

[b] These figures are only approximate. The actual dose is in column "Total Mgs. or C. c. injected."

F. Found dead at time noted.

TABLE XIII.—*Toxicity of arterenol hydrochloride*—Continued.

Number of mouse.	Mg. base per gm.	Body weight.	Injected.			Died.
			Total.		Time.	
		Grams.	*Mg.*	*C. c.*		
206	0.024	15.01	0.360	0.36	Jan. 9, 1.50 p. m.	
209	0.024	16.48	0.400	0.40	Jan. 9, 1.56 p. m.	
212	0.024	17.37	0.420	0.42	Jan. 9, 2.4 p. m.	
314	0.024	14.06	0.330	0.16	Jan. 20, 6.56 p. m.	
317	0.024	15.17	0.360	0.18	Jan. 20, 7.2 p. m.	
320	0.024	18.21	0.430	0.21	Jan. 20, 7.11 p. m.	
179a	0.024	13.51	0.320	0.32	Jan. 8, 12.37 p. m.	
121	0.028	14.43	0.404	0.40	Dec. 9, 5.45 p. m.	
185a	0.024	15.72	0.380	0.38	Jan. 8, 12.54 p. m.	
182a	0.024	18.78	0.450	0.45	Jan. 8, 12.44 p. m.	Jan. 11, 1.45 a. m. (W.).
183a	0.032	17.60	0.590	0.59	Jan. 8, 12.47 p. m.	
186a	0.032	12.88	0.410	0.41	Jan. 8, 12.56 p. m.	
198	0.032	20.23	0.650	0.65	Jan. 8, 1.27 p. m.	Jan. 9, 9.40 a. m. (F.).
201	0.032	15.25	0.490	0.49	Jan. 8, 1.33 p. m.	
204	0.032	13.75	0.440	0.44	Jan. 8, 1.58 p. m.	Jan. 9, 12.50 a. m. (W.).
207	0.032	16.60	0.530	0.53	Jan. 9, 1.53 p. m.	Jan. 10, 12.15 a. m. (W.).
210	0.032	13.40	0.430	0.43	Jan. 9, 1. 58 p. m.	
180a	0.032	17.59	0.590	0.59	Jan. 8, 12.39 p. m.	
213	0.032	14.14	0.45	0.45	Jan. 9, 2.6 p. m.	
315	0.030	21.06	0.63	0.31	Jan. 20, 6.58 p. m.	Jan. 20, 7.8 p. m.
318	0.030	16.21	0.48	0.24	Jan. 20, 7.6 p. m.	Jan. 22, 4.30 p. m.
321	0.030	19.84	0.59	0.29	Jan. 20, 7.13 p. m.	Jan. 22, 9 a. m. (W.).
199	0.040	18.12	0.72	0.72	Jan. 8, 1.28 p. m.	Jan. 9 (?), 10.45 a. m.
202	0.040	19.20	0.77	0.77	Jan. 8, 1.54 p. m.	Jan. 11, 10.30 a. m. (W.).
205	0.040	13.04	0.52	0.52	Jan. 8, 1.59 p. m.	
203a	0.040	13.67	0.54	0.54	Jan. 12, 4.4 p. m.	Jan. 12, 4.15 p. m.
208	0.040	15.58	0.62	0.62	Jan. 9, 1.55 p. m.	Jan. 11, 12.10 a. m. (W.).
211	0.040	17.44	0.70	0.70	Jan. 9, 2 p. m.	Jan. 11, 3 p. m. (W.).
214	0.040	17.87	0.72	0.72	Jan. 9, 2.8 p. m.	Jan. 10, 10.30 a. m. (W.).
305	0.040	20.12	0.80	0.40	Jan. 20, 6.7 p. m.	Jan. 20, 6:12 p. m.
308	0.040	19.52	0.78	0.36	Jan. 20, 6.15 p. m.	Jan. 20, 6.31 p. m.
311	0.040	15.02	0.60	0.30	Jan. 20, 6.24 p. m.	
316	0.040	14.48	0.58	0.29	Jan. 20, 7 p. m.	Jan. 20, 7.7 p. m.
319	0.040	15.39	0.61	0.30	Jan. 20, 7.9 p. m.	
205a	0.040	12.45	0.49	0.49	Jan. 12	
197a	0.040	14.07	0.56	0.56	Jan. 12	
200a	0.040	13.60	0.54	0.54	Jan. 12	
201a	0.040	14.21	0.57	0.57	Jan. 12	
322	0.040	17.90	0.71	0.35	Jan. 20, 7.15 p. m.	Jan. 21, 7.55 p. m. (F.).
181a	0.040	16.45	0.66	0.66	Jan. 8, 12.42 p. m.	
184a	0.040	14.17	0.56	0.56	Jan. 8, 12.52 p. m.	
187a	0.040	14.78	0.59	0.59	Jan. 8, 12.58 p. m.	
206a	0.040	12.87	0.51	0.51	Jan. 12	
209a	0.040	15.38	0.61	0.61	Jan. 12	
210a	0.040	13.45	0.53	0.53	Jan. 12	
212a	0.040	14.94	0.60	0.60	Jan. 12	
213a	0.040	13.54	0.55	0.55	Jan. 12	
217a	0.040	11.48	0.46	0.46	Jan. 12	
219a	0.040	12.63	0.50	0.50	Jan. 12	
222a	0.040	12.26	0.49	0.49	Jan. 12	

F. Found dead at time noted.
W. Time noted by watchmen.

TABLE XIII.—*Toxicity of arterenol hydrochloride*—Continued.

Number of mouse.	Mg. base per gm.	Body weight.	Injected.			Died.
			Total.		Time.	
		Grams.	*Mg.*	*C. c.*		
233	0.040	14.35	0.56	0.56	Jan. 12	
234	0.040	18.16	0.72	0.72	Jan. 12	
235	0.040	16.90	0.67	0.67	Jan. 12	
237	0.040	18.39	0.73	0.73	Jan. 12	
238	0.040	15.06	0.60	0.60	Jan. 12	
239	0.040	16.35	0.65	0.65	Jan. 12, 4.50 p. m.	Jan. 16, 8 a. m.
240	0.040	13.57	0.57	0.57	Jan. 12	
241	0.040	15.21	0.61	0.61	Jan. 12	
323	0.040	15.85	0.63	0.31	Mar. 15, 12.18 p. m.	Mar. 17, 6.45 a. m. (W.).
324	0.040	22.22	0.88	0.44	Mar. 15, 12.20 p. m.	Mar. 17, 12.20 p. m. (F.).
325	0.040	19.89	0.80	0.40	Mar. 15, 12.23 p. m.	
326	0.040	15.39	0.61	0.30	Mar. 15, 12.25 p. m.	Mar. 16, 6 a. m. (W.).
327	0.040	13.92	0.55	0.27	Mar. 15, 12.27 p. m.	
197b	0.060	14.53	0.87	0.43	Jan. 16, 11.1 p. m.	
200b	0.060	13.60	0.84	0.42	Jan. 16, 11.3 p. m.	
201b	0.060	14.51	0.87	0.43	Jan. 16, 11.5 p. m.	
306	0.060	17.44	1.05	0.52	Jan. 20, 6.11 p. m.	Jan. 20, 6.21 p. m.
309	0.060	23.06	1.38	0.69	Jan. 20, 6.18 p. m.	Jan. 20, 7.14 p. m.
312	0.060	19.03	1.14	0.57	Jan. 20, 6.27 p. m.	Jan. 23, 8 p. m.
328	0.060	21.37	1.28	0.64	Mar. 15, 12.33 p. m.	Mar. 15, 3.30 p. m. (F.).
329	0.060	16.54	0.99	0.49	Mar. 15, 12.40 p. m.	
330	0.060	19.07	1.14	0.57	Mar. 15, 12.42 p. m.	Mar. 17, 1.15 a. m. (W.).
331	0.060	20.23	1.21	0.60	Mar. 15, 12.44 p. m.	Mar. 17, 1.15 a. m. (W.).
332	0.060	21.68	1.30	0.65	Mar. 15, 12.45 p. m.	Mar. 15, 1 p. m.
205b	0.080	12.85	1.02	0.54	Jan. 16, 11.7 p. m.	
206b	0.080	13.62	1.09	0.54	Jan. 16, 11.14 p. m.	Jan. 17, 11.30 a. m. (W.).
209b	0.080	15.62	1.25	0.62	Jan. 16, 11.11 p. m.	
217b	0.080	13.09	1.05	0.52	Jan. 16, 11.24 p. m.	
219b	0.080	12.09	0.96	0.48	Jan. 16, 11.27 p. m.	
222b	0.080	12.89	1.03	0.51	Jan. 16, 11.29 p. m.	
307	0.080	14.85	1.19	0.59	Jan. 20, 6.13 p. m.	Jan. 22, 4 a. m. (F.).
310	0.080	16.14	1.28	0.64	Jan. 20, 6.20 p. m.	
313	0.080	15.50	1.24	0.62	Jan. 20, 6.31 p. m.	
333	0.080	12.96	1.03	0.51	Mar. 15, 12.54 p. m.	Mar. 15, 1.12 p. m.
334	0.080	21.85	1.74	0.87	Mar. 15, 12.57 p. m.	Mar. 20, 8 a. m. (W.).
335	0.080	18.11	1.44	0.72	Mar. 15, 12.59 p. m.	Mar. 19, 9.30 a. m. (W.).
336	0.080	13.68	1.09	0.54	Mar. 15, 1.10 p. m.	Mar. 19, 9.30 a. m. (W.).
337	0.080	11.81	0.94	0.47	Mar. 15, 1.6 p. m.	Mar. 15, 1.1 p. m.
338	0.100	14.45	1.44	0.72	Mar. 15, 1.8 p. m.	Mar. 17, 9 a. m. (F.).
339	0.100	13.16	1.31	0.65	Mar. 15, 1.10 p. m.	
340	0.100	18.56	1.85	0.92	Mar. 15, 1.13 p. m.	Mar. 15, 3.30 p. m. (F.).
341	0.100	12.02	1.20	0.60	Mar. 15, 1.14 p. m.	Mar. 15, 1.30 p. m. (F.).
342	0.100	13.01	1.30	0.65	Mar. 15, 1.17 p. m.	Mar. 16, 6 a. m. (W.).

W. Time noted by watchman.
F. Found dead at time noted.

PROTOCOLS TO EXPERIMENTS OF TABLE XIII.

It is important in making comparative studies that the experimental animal be healthy and free from the effects of previous injections and that the drugs employed show no signs of deterioration. Since some of the animals in Table XIII were

injected more than once and some of the solutions were not used immediately, the following data is inserted to show this:

Mice Nos. 67 to 70, inclusive, 5 mg. arterenol hydrochloride crystals dissolved in 5 c. c. Ringer solution between 9 and 11 a. m. December 4, 1908.

Nos. 67, 68, 69, and 70 were previously injected November 30 with 0.22, 0.28, 0.42, and 0.61 mg., respectively, of homorenon hydrochloride.

Mice Nos. 107 to 111, inclusive, 6 mg. arterenol hydrochloride dissolved in 5 c. c. Ringer solution 9 a. m. December 9.

Nos. 117 to 121, inclusive, 6 mg. arterenol hydrochloride dissolved in 5 c. c. Ringer solution 11.30 a. m. December 9.

Nos. 179, 180, 181, 182, 183, 184, 185, 186, and 187, 6 mg. arterenol hydrochloride dissolved in 5 c. c. Ringer January 5 just before injecting; 179a, 180a, 181a, 182a, and 183a injected with a solution made up 3 p. m. January 7, for blood-pressure experiment on January 7, whereas 184a, 185a, and 186a were injected with a solution made up in the same way 12.49, January 8.

Nos. 197, 198, 199, 200, and 202, 6 mg. arterenol hydrochloride dissolved in 5 c. c. Ringer, 12.49 p. m. January 8, 1909; 203, 204, and 205 similar solution made up 1.50 p. m. January 8; 197b, 200b, 201b, and 205b injected with a solution containing 12 mg. arterenol crystals dissolved in 5 c. c. Ringer, 10.57 p. m. January 16.

Nos. 206, 207, 208, 209, 210, 211, 212, 213, and 214, 6 mg. arterenol hydrochloride crystals dissolved in 5 c. c. Ringer 1.30 p. m. January 9, 1909; 206a, 209a, 210a, 212a, and 213a, 6 mg. crystals per 5 c. c. dissolved 3.53 p. m. January 12.

Nos. 206b, 209b, 210b, 212b, and 213b, 12 mg. arterenol HCl dissolved in 5 c. c. Ringer 10.57 p. m. January 16.

Nos. 217b, 219b, and 222b, 12 mg. arterenol hydrochloride crystals dissolved in 5 c. c. Ringer 10.57 p. m. January 16.

Nos. 233, 234, 235, 236, 237, 238, 239, 240, and 241, respectively, injected January 5 with 0.17, 0.30, 0.33, 0.24, 0.30, 0.32, 0.20, 0.23, and 0.32 mg. of arterenol hydrochloride and with the same drug again on January 8 with 0.32, 0.59, 0.66, 0.45, 0.59, 0.56, 0.38, 0.41, and 0.59 mg., respectively.

TOXICITY OF HOMORENON HYDROCHLORIDE.

It has been shown that it requires eighty times as much homorenon to produce the same rise of blood pressure in the dog as is caused by the same amount of natural l-adrenalin. If the relative toxicity of this compound as compared with l-adrenalin were in direct proportion to the relative vaso-constrictor action, then the lethal dose of homorenon ought to be 0.640 mg. per gram mouse, and as a matter of fact the lethal dose is somewhere between 0.568 and 0.711 mg. per gram mouse. It is true that a smaller dose than this sometimes kills and very rarely a mouse may survive as large a dose as 0.994 mg. per gram. But anything above 0.568 mg. so often kills that one is safe in saying that the lethal dose is slightly more than 0.568 and a little less than 0.711 mg. per gram body weight. According to Table XIV, then, the natural l-adrenalin is from eighty to eighty-eight times as toxic as ethyl-amino-aceto-catechol, commercially known as homorenon.

TABLE XIV.—*Toxicity of homorenon hydrochloride.*

Number of mouse.[a]	Mg. base per gm.[b]	Body weight.	Injected.			Died.
			Total.		Time.	
		Grams.	*Mg.*	*C. c.*		
59	0.237	13.71	3.25	0.32	Dec. 4, 9.7 a. m.	
78	0.237	17.92	4.24	0.21	Dec. 7, 11.24 a. m.	
38	0.255	21.42	5.46	0.25	Dec. 2, 8.49 a. m.	
57	0.255	18.83	8.40	0.48	Dec. 3, 9.18 a. m.	
89	0.260	12.50	3.25	0.16	Dec. 7, 2.20 p. m.	
93	0.284	20.34	5.77	0.28	Dec. 7, 3.44 p. m.	
296	0.284	13.31	3.78	0.07	Jan. 20, 4.39 p. m.	
299	0.284	21.15	6.00	0.12	Jan. 20, 4.46 p. m.	Jan. 23, 8 a. m. (W.).
302	0.284	15.32	4.30	0.08	Jan. 20, 4.53 p. m.	
60	0.296	20.89	6.18	0.62	Dec. 4, 9.10 a. m.	
90	0.347	18.28	6.35	0.31	Dec. 7, 2.24 p. m.	
61	0.356	14.70	5.23	0.52	Dec. 4, 9.14 a. m.	
79	0.356	22.32	7.94	0.39	Dec. 7, 11.26 a. m.	Dec. 10 (F.).
94	0.426	19.32	8.23	0.41	Dec. 7, 3.46 p. m.	
122	0.426	21.91	9.33	0.46	Dec. 10, 2.11 p. m.	
127	0.426	16.97	7.23	0.36	Dec. 10, 2.24 p. m.	
132	0.426	17.24	7.35	0.36	Dec. 10, 2.40 p. m.	
137	0.426	12.33	5.50	0.27	Dec. 10, 2.55 p. m.	
297	0.426	21.94	9.34	0.18	Jan. 20, 4.41 p. m.	Jan. 22, 4 p. m. (F.).
300	0.426	18.90	8.00	0.16	Jan. 20, 4.48 p. m.	
303	0.426	18.52	7.80	0.15	Jan. 20, 4.55 p. m.	
260	0.427	14.30	6.15	0.12	Jan. 16, 3.50 p. m.	
263	0.427	17.30	7.38	0.14	Jan. 16, 3.58 p. m.	
266	0.427	22.78	9.73	0.19	Jan. 16, 4.5 p. m.	
269	0.427	18.57	7.93	0.16	Jan. 16, 9.49 p. m.	Jan. 16, 10.14 p. m.
272	0.427	19.13	8.17	0.16	Jan. 16, 10 p. m.	Jan. 16, 11.30 p. m.
275	0.427	16.94	7.23	0.14	Jan. 16, 10.6 p. m.	
287	0.427	15.05	6.40	0.13	Jan. 20, 3.8 p. m.	Jan. 20, 3.39 p. m.
290	0.427	15.63	6.70	0.13	Jan. 20, 3.23 p. m.	Jan. 24, 3.36 p. m.
293	0.427	16.36	6.98	0.14	Jan. 20, 3.29 p. m.	
278	0.430	14.92	6.40	0.13	Jan. 16, 11.46 p. m.	
279	0.430	19.02	8.17	0.16	Jan. 16, 11.49 p. m.	Jan. 16, 11.58 p. m.
280	0.430	16.79	7.18	0.14	Jan. 16, 11.50 p. m.	
281	0.430	15.04	6.45	0.13	Jan. 16, 11.52 p. m.	
282	0.430	18.52	7.95	0.16	Jan. 16, 11.54 p. m.	Jan. 17, 11.30 a. m. (W.).
283	0.430	15.02	6.45	0.13	Jan. 16, 11.56 p. m.	
284	0.430	13.43	5.76	0.11	Jan. 16, 11.58 p. m.	
285	0.430	15.83	6.79	0.13	Jan. 16, 12.1 p. m.	Jan. 17, 4 p. m. (W.).
91	0.434	15.10	6.55	0.32	Dec. 7, 2.26 p. m.	
80	0.474	19.30	9.14	0.45	Dec. 7, 11.29 a. m.	
97	0.474	14.20	6.73	0.33	Dec. 8, 9.42 a. m.	
39	0.518	17.16	8.89	0.40	Dec. 2, 9.7 a. m.	
95	0.568	14.22	8.07	0.40	Dec. 7, 3.50 p. m.	
123	0.568	21.71	12.32	0.61	Dec. 10, 2.14 p. m.	
128	0.568	13.46	7.66	0.38	Dec. 10, 2.27 p. m.	
133	0.568	13.74	7.81	0.39	Dec. 10, 2.42 p. m.	
138	0.568	12.36	7.02	0.35	Dec. 10, 2.58 p. m.	
251	0.568	20.88	11.86	0.23	Jan. 16, 3.15 p. m.	

[a] Mice not marked "W" or "F" were observed by the author to die at the time noted.
[b] This column approximate dose only; actual dose in "total mg. and c. c. columns."

W. Time noted by watchman.
F. Found dead at time noted.

TABLE XIV.—*Toxicity of homorenon hydrochloride*—Continued.

Number of mouse.	Mg. base per gm.	Body weight.	Injected.			Died.
			Total.		Time.	
		Grams.	*Mg.*	*C. c.*		
254	0.568	18.20	10.37	0.30	Jan. 16, 3.36 p. m.	Jan. 16, 3.55 p. m.
257	0.568	20.66	11.74	0.23	Jan. 16, 3.40 p. m.	Jan. 16, 3.47 p. m.
261	0.568	20.06	11.40	0.22	Jan. 16, 3.51 p. m.	
264	0.568	21.64	12.27	0.24	Jan. 16, 3.59 p. m.	Jan. 16, 4.15 p. m.
267	0.568	19.76	11.22	0.22	Jan. 16, 4.8 p. m.	
270	0.568	19.60	11.13	0.22	Jan. 16, 9.56 p. m.	
273	0.568	17.84	10.47	0.21	Jan. 16, 10.12 p. m.	
276	0.568	19.22	10.91	0.22	Jan. 16, 10.8 p. m.	Jan. 16, 10.21 p. m.
291	0.568	17.83	10.1	0.20	Jan. 20, 3.25 p. m.	Jan. 20, 3.49 p. m.
294	0.568	18.35	10.4	0.21	Jan. 20, 3.31 p. m.	Jan. 20, 3.57 p. m.
298	0.568	19.08	10.78	0.21	Jan. 20, 4.44 p. m.	Jan. 20, 5 p. m.
301	0.568	14.20	8.00	0.16	Jan. 20, 4.49 p. m.	Jan. 20, 5.20 p. m.
304	0.568	17.13	9.7	0.19	Jan. 20, 4.57 p. m.	
288	0.583	14.73	8.6	0.17	Jan. 20, 3.12 p. m.	Jan. 20, 3.57 p. m.
81	0.593	17.62	10.44	0.52	Dec. 7, 11.32 a. m.	Dec. 7, 12.5 p. m.
98	0.593	14.12	8.37	0.41	Dec. 8, 9.45 a. m.	
99	0.711	17.05	12.12	0.60	Dec. 8, 9.47 a. m.	
124	0.710	17.07	12.11	0.60	Dec. 10, 2.17 p. m.	
129	0.710	12.97	9.21	0.46	Dec. 10, 2.31 p. m.	
134	0.710	20.10	14.27	0.70	Dec. 10, 2.45 p. m.	
139	0.710	18.11	12.86	0.64	Dec. 10, 3 p. m.	
215	0.711	19.05	13.53	0.27	Jan. 16, 3.17 p. m.	Jan. 16, 3.52 p. m. (F.).
255	0.711	23.65	16.79	0.33	Jan. 16, 3.38 p. m.	Jan. 16, 4.10 p. m. (F.).
258	0.711	19.09	13.56	0.27	Jan. 16, 3.44 p. m.	
262	0.711	19.80	14.05	0.28	Jan. 16, 3.54 p. m.	
265	0.711	25.00	17.75	0.35	Jan. 16, 4.3 p. m.	Jan. 16, 4.7 p. m.
268	0.711	22.58	16.03	0.32	Jan. 16, 4.10 p. m.	Jan. 16, 4.20 p. m.
271	0.711	19.47	13.84	0.27	Jan. 16, 9.58 p. m.	Jan. 16, 11.30 p. m.
274	0.711	15.59	11.08	0.22	Jan. 16, 10.4 p. m.	Jan. 16, 10.16 p. m.
277	0.711	19.80	14.07	0.28	Jan. 16, 10.10 p. m.	Jan. 16, 10.21 p. m.
289	0.711	15.13	10.7	0.21	Jan. 20, 3.21 p. m.	
292	0.711	18.56	13.20	0.26	Jan. 20, 3.26 p. m.	Jan. 20, 3.47 p. m.
295	0.711	16.86	12.00	0.24	Jan. 20, 3.35 p. m.	Jan. 20, 3.51 p. m.
40	0.777	19.47	15.13	0.69	Dec. 2, 9.5 a. m.	Dec. 2, 10.25 a. m.
100	0.830	13.26	11.01	0.55	Dec. 8, 9.50 a. m.	
125	0.852	16.37	13.95	0.69	Dec. 10, 2.19 p. m.	
130	0.852	18.28	15.57	0.78	Dec. 10, 2.33 p. m.	
135	0.852	19.37	16.50	0.81	Dec. 10, 2.48 p. m.	
140	0.852	17.55	14.95	0.74	Dec. 10, 3.3 p. m.	Dec. 10, 3.35 p. m.
170	0.853	12.89	10.99	0.20	Jan. 5, 3 p. m.	
173	0.853	12.57	12.38	0.24	Jan. 5, 3.5 p. m.	Jan. 5, 3.29 p. m.
176	0.853	14.37	12.26	0.24	Jan. 5, 3.14 p. m.	Jan. 5, 3.31 p. m.
245	0.853	20.05	17.04	0.34	Jan. 16, 1.25 p. m.	Jan. 16, 1.45 p. m.
248	0.853	22.60	19.21	0.38	Jan. 16, 1.34 p. m.	Jan. 16, 1.57 p. m.
253	0.853	18.05	15.34	0.30	Jan. 16, 3.19 p. m.	Jan. 16, 4 p. m.
256	0.853	19.17	16.29	0.32	Jan. 16, 3.39 p. m.	Jan. 16, 4.12 p. m.
259	0.853	14.71	12.50	0.25	Jan. 16, 3.46 p. m.	Jan. 16, 4 p. m.
101	0.949	14.14	13.42	0.53	Dec. 8, 9.53 a. m.	Dec. 8, 11 a. m.
126	0.994	12.15	12.08	0.60	Dec. 10, 2.22 p. m.	
131	0.994	15.73	15.64	0.78	Dec. 10, 2.36 p. m.	Dec. 10, 3.35 p. m.
136	0.994	14.11	14.02	0.70	Dec. 10, 2.50 p. m.	Dec. 10, 3.15 p. m.

F. Found dead at time noted.

TABLE XIV.—*Toxicity of homorenon hydrochloride*—Continued.

Number of mouse.	Mg. base per gm.	Body weight.	Injected.			Died.
			Total.		Time.	
		Grams.	*Mg.*	*C. c.*		
141	0.994	19.43	19.31	0.96	Dec. 10, 3.6 p. m.	Dec. 10, 3.30 p. m.
171	0.994	14.50	14.41	0.28	Jan. 5, 3.2 p. m.	Jan. 5, 3.20 p. m.
174	0.994	13.20	13.12	0.26	Jan. 5, 3.9 p. m.	Jan. 5, 3.31 p. m.
177	0.944	13.76	13.68	0.28	Jan. 5, 3.15 p. m.	Jan. 5, 3.38 p. m.
243	0.994	20.64	20.52	0.41	Jan. 16, 1.21 p. m.	Jan. 16, 1.28 p. m.
246	0.994	22.59	22.33	0.44	Jan. 16, 1.27 p. m.	Jan. 16, 1.39 p. m.
249	0.994	18.31	18.12	0.36	Jan. 16, 1.36 p. m.	Jan. 16, 1.55 p. m.
41	1.038	19.47	20.21	0.92	Dec. 2, 9 a. m.	Dec. 2, 9.53 a. m.
172	1.137	20.93	23.80	0.48	Jan. 5, 3.4 p. m.	Jan. 5, 3.15 p. m.
175	1.137	17.00	19.53	0.38	Jan. 5, 3.10 p. m.	Jan. 5, 3.29 p. m.
178	1.137	13.42	14.78	0.30	Jan. 5, 3.20 p. m.	Jan. 5, 3.47 p. m.
244	1.137	15.22	17.20	0.34	Jan. 16, 1.22 p. m.	Jan. 16, 2 p. m.
247	1.137	17.78	20.11	0.40	Jan. 16, 1.29 p. m.	Jan. 16, 1.39 p. m.
250	1.137	22.37	25.31	0.50	Jan. 16, 1.37 p. m.	Jan. 16, 1.50 p. m.

PROTOCOLS TO EXPERIMENTS OF TABLE XIV.

The solutions of homorenon hydrochloride used in the different toxicity experiments were made up as follows:

Nos. 37 to 41, inclusive, 66 mg. homorenon hydrochloride crystals dissolved in 3 c. c. of Ringer solution about 8.30 a. m. December 2.

Nos. 47 to 51, inclusive, 20 mg. of crystals dissolved in 2 c. c. of Ringer solution about 9 a. m. December 3.

Nos. 57 to 61, inclusive, 25 mg. crystals dissolved in 2.5 c. c. of Ringer solution 11 a. m. December 7.

Nos. 87, 88, 89, 90, and 91 injected December 4 with 0.89, 1.90, 3.25, 6.18, and 5.23 mg., respectively, of homorenon, and 92, 93, 94, 95, and 96 were each, respectively, injected on December 2 with 0.038 mg. of adrenalin, 0.125, 0.225, 0.518, and 1.45 mg. homorenon.

Nos. 97 to 101, inclusive, 20 mg. crystals dissolved in 1 c. c. of Ringer solution.

Nos. 122 to 141, inclusive, 20 mg. per 1 c. c. of Ringer solution made up 2 p. m. December 10.

Nos. 122, 123, 124, 125, and 126 had been injected with 0.021, 0.042, 0.069, 0.081, and 0.082 mg., respectively, of adrenalin base December 4, and again on December 7 with 0.040, 0.080, 0.103, 0.128, and 0.130 mg., respectively, of the same drug.

Nos. 127, 128, 129, 130, and 131 were injected December 4 with 0.890, 1.90, 3.25, 6.18, and 5.23 mg., respectively, of homorenon, and again on December 7 with 1.33, 2.25, 3.25, 6.35, and 6.55 mg., respectively, of the same drug.

No. 132 was injected December 2 with 0.038 mg. of adrenalin base and on December 7 with 2.48 mg. homorenon, No. 133 on December 4 and 7, respectively, with 0.890 and 2.48 mg. of homorenon, while Nos. 134, 135, and 136 were each injected on December 2 with 0.125, 0.225, and 0.518 mg., respectively, of homorenon, and again on December 7 with 5.77, 8.23, and 8.07 mg., respectively, of the same drug.

Finally mice Nos. 139, 140, and 141, respectively, had been injected on December 3 with 0.554, 0.876, and 2.688 mg., and again on the 7th of December with 2.08, 4.24, and 9.14 mg. of homorenon.

Nos. 170 to 178, inclusive, 250 mg. of homorenon hydrochloride crystals dissolved in 5 c. c. of Ringer, about 2.45 p. m. January 5, 1909.

Nos. 242 to 250, inclusive, and 267 and 268, 250 mg. of crystals dissolved in 5 c. c. Ringer solution about 1 p. m. January 16.

Nos. 251 to 266 and 269 to 286, inclusive, 250 mg. of crystals dissolved in 5 c. c. Ringer solution 3 p. m. January 16 for each set of nine.

Nos. 287 to 304, inclusive, 250 mg. of crystals dissolved in 5 c. c. Ringer solution 2.30 p. m. January 20, 1909.

MEASUREMENT OF MYDRIASIS IN THE FROG'S EXCISED BULBUS.[a]

As is well known the dilator mechanism of the frog's eye is quite sensitive to adrenalin and not a little emphasis has been laid upon the delicacy with which it reacts to minute traces of this and certain other derivatives of the catechol group. Because of this mydriatic action, the apparent simplicity of the technique involved, and the availability of experimental animals, the enucleated eye of the frog seems to furnish a suitable method for standardizing adrenalin solutions.

Meltzer was the first to call attention to this method and nearly a year later Ehrmann, acting upon Meltzer's suggestion, published some very interesting experiments with the excised frog's eye. In Ehrmann's suggestive paper it is not only shown that adrenalin solutions of different concentration call forth varying degrees of mydriasis, but it leads one to suppose that the enucleated frog's eye is sensitive to such minute quantities of adrenalin as may occur in the blood drawn from the vena cava. Taking advantage of the sensitiveness of the frog's eye he also uses this method as a means for demonstrating the activity of adrenalin still in the blood of injected animals bled immediately after the fall of blood pressure that invariably follows the initial effect of this drug.[b] Just how reliable these conclusions are remains to be decided by future experiments. Cameron, in his paper on methods of standardizing suprarenal preparations, states that the method is not only tedious but very unreliable, since frogs vary in their response to adrenalin.

Various factors do influence the reaction of the iris to adrenalin. Perhaps the most important ones are: (1) Conditions that interfere with the frog's normal metabolism and nutrition, (2) the varying intensity of light to which the eye is exposed, (3) the temperature of the medium surrounding the enucleated bulbus, (4) injuries to the coats of the bulbus that may result in lowering the intra-ocular tension and alter the rate of diffusion, and, finally, (5) mechanical

[a] The section on the pupil was completed November 15, 1908, but publication was delayed so that the comparative study herein contained might be made.

[b] Dryer as early as 1897–1899 showed that adrenalin is secreted into the circulation after stimulation of the splanchnic nerve. This excellent piece of work has not received the attention of German writers that it should, especially since Dryer is one of the first to prove conclusively that stimulation of the splanchnic causes an increased secretion by the adrenal glands.

stimuli. One of the main problems of this method is to devise a technique that minimizes the chief sources of error.

As is well known, the frog's pupil is an elliptical-like opening the long axis of which is almost parallel to the long axis of the body. On the lower margin of the pupil, almost in line with the short axis of the ellipse, there is a small notch, whereas the upper margin, though usually continuous, is sometimes indented by a less conspicuous notch. In measuring the eye, especially when widely dilated, these notches furnish convenient points of reference in locating the short axis. The long axis in the undilated eye is of course easily located by reason of the shape of the anterior and posterior margins of the pupil.

In orienting the bulbus previous to measuring the size of the pupil it is necessary to have a suitable container. Glass vessels of about 3 c. c. capacity were used, made from hard bacteriological test tubes 17 mm. in diameter, the edges being ground smooth so that it might be sealed with a cover slip to avoid evaporation.

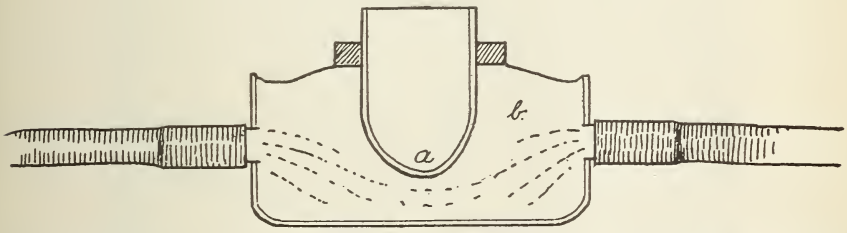


FIG. 1.—Water bath. *a*, Glass container, described in text; *b*, water bath through which a steady stream of water from the temperature regulator is kept flowing.

Except in a few experiments described later, special care was taken to avoid the changes in temperature and light values that occur in a laboratory lighted by direct or diffuse sunlight. To this end a dark room was fitted up with a 16-candlepower incandescent light so arranged that the excised bulbi could be lighted from a given distance, thus making it possible to maintain a light of constant intensity. Of course at a distance of 10 inches such a light generates enough heat to warm 2 c. c. of solution to 30° or more, depending upon the room temperature. In order to avoid not only such high temperatures, but more especially fluctuations in the same, the water bath, indicated in figure 1, was devised. It is constructed in such a way that the glass container rests in a current of water, the temperature of which is so regulated that the temperature of the solution in the container can also be kept constant.

Preliminary experiments demonstrated that a mere naked eye observation of the changes in size of the pupil is of little value, and so an instrument of precision was sought that would enable the

observer to express in millimeters the exact length of the long and short axes of the pupil. The device which proved to be most satisfactory was made for me by Messrs. Gaertner & Co., of Chicago. Their "simple comparator" was modified by the author to meet the needs

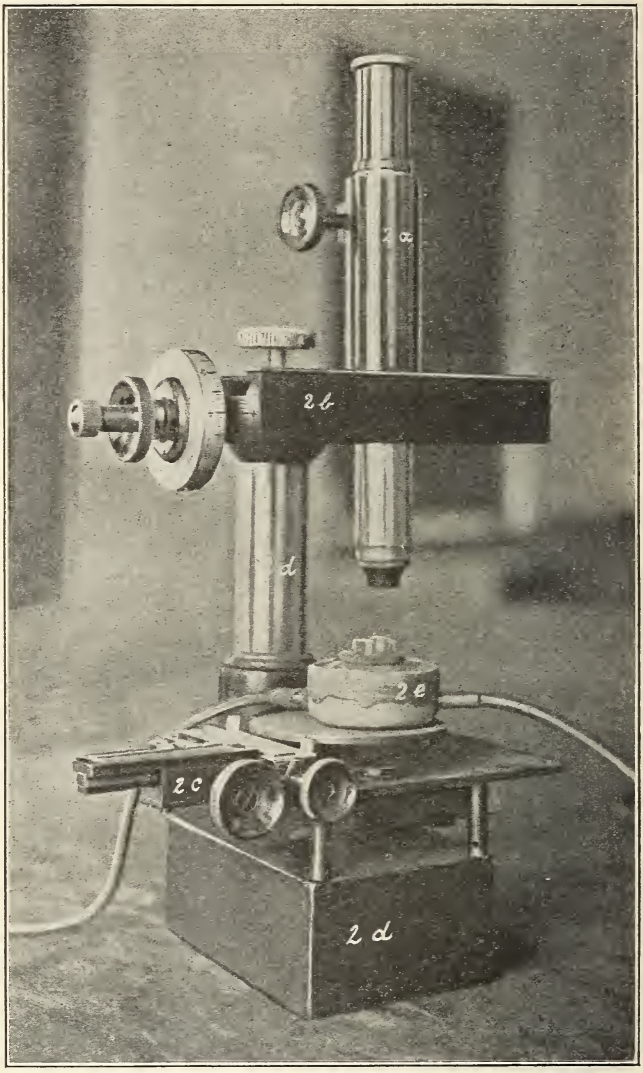


FIG. 2.—Pupilometer. 2*a*, Reading microscope with cross wires; 2*b*, micrometer slide (see fig. 3); 2*c*, adjustable substage (see fig. 4); 2*d*, support; 2*e*, water bath (see fig. 1). Temperature regulators and light omitted for sake of clearness.

of the present series of experiments. (See fig. 2.) The essential parts of the apparatus are, (1) a reading microscope with cross wires in the eyepiece (fig. 2a), (2) a micrometer slide (fig. 2b and fig. 3), (3) an

adjustable stage (fig. 2c and fig. 4), and, finally, (4) a support (fig. 2d). The cross wire in the eyepiece furnishes a point of reference, and in making a reading the eye is first oriented so as to bring its optical

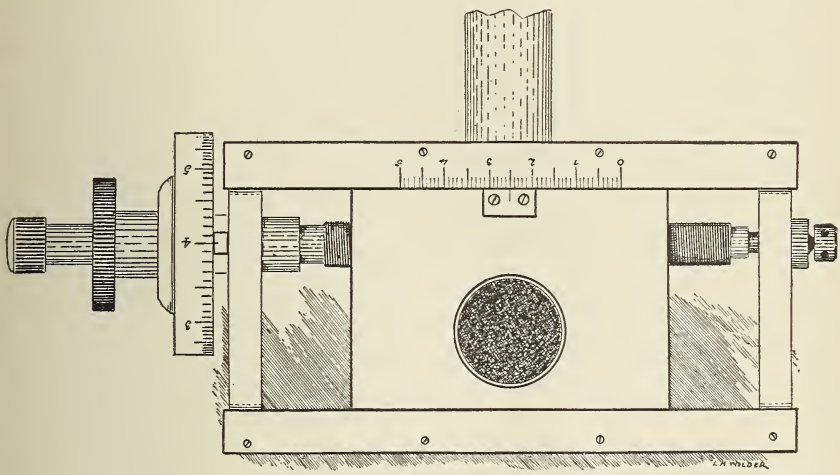


FIG. 3.—Micrometer slide. Micrometer screw has a diameter of 10 mm., pitch of 0.5 mm. The micrometer head has a diameter of 50 mm. and is divided into 100 parts, each division reading to 0.005 mm.

axis parallel with the axis of the microscope. Then by means of a coarse adjustment the desired edge of the pupil is brought into line

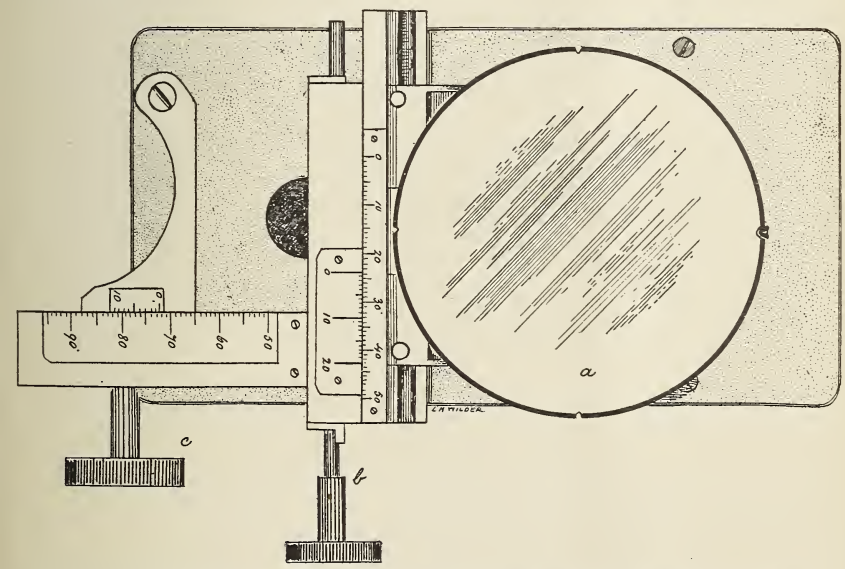


FIG. 4.—Adjustable substage. *a*, Revolving support, upon which the water bath 2*e*, fig. 2, rests; *b*, *c*, rack and pinion providing quick adjustment for centering edge of pupil against the cross wires.

with the cross wire and the long axis of the pupil measured; the object stage is rotated to 90° and the short axis measured. In this way a given eye can be measured many times without disturbing it

by jars or other forms of mechanical stimulation except when it is necessary to change the solution in the container.

Since the unit that most concerns us in this paper is the time required by the pupil to dilate to a maximum it becomes necessary to explain a few points in this connection. (1) In some eyes the long axis is the first to reach a maximum length; at other times it is the short axis. (2) The axis first to reach a maximum length usually shortens while the other axis is in the process of elongating. It is only occasionally that both axes reach their maximum length simultaneously, or that the one waits upon the other. Hence it often becomes difficult to determine the exact moment at which the eye is most widely dilated unless the absolute area of the pupil be calculated. This is too tedious a process, so as a rule one of the following methods is used: (1) The mean of the time required for the two axes to reach their greatest length; (2) the moment of maximum dilation determined by the first axis reaching its greatest length or turning point. Unless otherwise stated, the last method, though less accurate, is the one used throughout the paper.

I always endeavored to choose frogs of the same variety, that weighed not less than 17 grams nor more than 30. The head was cut off just back of the eyes and the bulbi removed from their sockets at once. In removing the eyeball undue pressure was avoided and care taken not to cut the sclerotic coat. The bulbi were immediately dropped into a container of Ringer solution, observed and measured. When necessary to change the solution, it was carefully pipetted off and a fresh solution of nearly the same temperature substituted.

In spite of all precautions certain eyes will dilate and remain dilated in the Ringer solution for an unusual length of time. When this occurs they should be rejected. Furthermore, eyes from frogs of low vitality seem to be more susceptible to adrenalin than those of healthy, vigorous animals. This source of variation is the most difficult to overcome, and perhaps accounts for many of the variations that occur in experiments of this nature.

TABLE XV.—*Mydriatic action of adrenalin upon the frog's eye when exposed to bright sunlight.*

Experiment 17, July 22, 1908. Rana pipiens. Weight about 17 grams. Killed 10.13 a. m., enucleated 10.18. The solutions made from P., D. and Co.'s 1:1,000 adrealin solution. The temperature varied from 29.8° to 32.5° C., and the brightness of the light varied from the bright noonday sunlight to that which obtains at 4.30 p. m. of a clear July day. (−) always indicates decrease in size, i. e., constriction of the pupil.

Ringer. A.			Adrenalin solution + Ringer 1:5,000,000 B.			Adrenalin solution + Ringer 1:2,500,000 C.			Adrenalin solution + Ringer 1:625,000 D.			Ringer. E.			°
Time.	Long axis.	Short axis.	Time.	Long axis.	Short axis.	Time.	Long axis.	Short axis.	Time.	Long axis.	Short axis.	Time.	Long axis.	Short axis.	C.
A. m.	*M. m.*	*M. m.*	*A. m.*	*M. m.*	*M. m.*	*A. m.*	*M. m.*	*M. m.*	*A. m.*	*M. m.*	*M. m.*	*A. m.*	*M. m.*	*M. m.*	
10.20	1.4	2.6	10.22	1.5	2.5	10.24	1.5	2.5	10.02	1.5	2.8	10.30	1.5	2.6	29.8
	.....	.....	10.33	In adr.		10.33	In adr.		10.33	In adr.					
10.33	[a] 1.1	2.4	10.30	1.1	2.4	10.40	1.3	2.4	10.45	1.4	2.6	10.47	1.5	2.8	30.0
	[b] −.3	− .2		− .4	− .1		− .2	− .1		− .1	− .2		.0	+ .2	
10.50	1.1	2.4	10.52	1.4	2.4	10.54	1.3	2.0	10.50	1.1	2.5	11	1.1	2.5	30.5
	− .3	− .2		− .1	− .1		− .2	− .5		− .4	− .3		− .4	− .1	
11.00	1.2	2.3	11.02	1.2	2.2	11.04	1.2	2.0	11	1.0	2.4	11.20	1.0	2.1	31.3
	− .2	− .3		− .3	− .3		− .3	−.5		− .5	− .4		− .5	− .5	
11.40	1.1	2.1	11.42	1.1	2.1	11.44	1.5	2.1	11.04	1.1	2.4	11.50	1.1	2.4	32.5
	− .3	− .5		− .4	− .4		.0	− .4		− .4	− .4		− .4	− .2	
P. m.			*P. m.*			*P. m.*			*P. m.*			*P. m.*			
12.20	1.2	2.2	12.32	1.2	2.3	12.24	1.5	2.3	12.28	1.2	2.4	12.30	1.2	2.4	31.8
	− .2	− .4		− .3	− .2		.0	− .2		− .3	− .4		− .3	− .4	
12.53	1.3	2.3	12.55	1.4	2.4	12.56	2.0	2.5	12.59	1.1	2.4	1.1	1.1	2.4	31.0
	− .1	− .3		− .1	− .1		+ .5	.0		− .4	− .4		− .4	− .2	
2.04	1.4	2.7	2.06	1.5	2.3	2.08	2.4	2.6	2.12	1.4	2.5	2.14	1.5	2.6	31.0
	.0	+ .1		.0	− .2		+ .9	+ .1		− .1	− .3		.0	.0	
3.14	1.6	2.6	3.16	1.5	2.8	3.18	2.6	2.8	3.22	1.6	2.5	3.24	1.7	2.5	31.5
	+ .2	.0		.0	+ .3		+1.1	+ .3		+ .1	− .3		+ .2	− .1	
4.14	1.9	2.6	4.16	1.6	2.5	4.18	2.7	2.9	4.22	1.6	2.6	4.24	1.9	2.6	30.6
	+ .5	.0		+ .1	.0		+1.2	+ .4		+ .1	− .2		+ .4	.0	

[a] First line in each group indicates actual length of axes in millimeters.
[b] Second line in each group shows change in length of axes.

Other investigators in their experiments with adrenalin and the excised bulbus used sunlight, which as is well known varies greatly in intensity. Since the stimulating action of adrenalin is supposed to counteract the stimulating action of light it would seem of the greatest importance to use light stimuli of a constant value and of optimum intensity. This, I believe, I have accomplished by the use of electric light which, so far as I know, has never been used before in experiments of this nature.

In the first place the constricting effect of very bright sunlight is usually greater than can be overcome by the dilating effects of the

adrenalin present in a 1:5,000,000 or even in a 1:625,000 solution. Therefore, the readings from the start have a negative sign, that is, the pupil constricts. It is only after remaining in the solution two or three hours that signs of dilation appear, and even then there is no positive evidence in favor of the adrenalin causing this dilation.

The readings in columns A and E of Table XV are from eyes in Ringer solution. The maximum constriction is −.3 for the long axis and −.5 mm. for the short axis of A; −.5 by −.5 mm. for E. Later, as the light grows dimmer and the temperature lower, the same eyes dilate making the difference in their sizes a little after 4 p. m. from that when first measured for A +.5 by .0 mm.; and for E +.4 by .0 mm., the pupils at the end being slightly larger than when first measured. It is true that C shows greater dilation than this, even as much as +1.2 by +.4, but D in a much stronger solution of adrenalin than C dilates less. In the light of simliar sets of experiments, I am inclined not to stress the importance of adrenalin in bringing about the dilation in B, C, or D, for if they had been in Ringer solution alone they would without doubt have reacted in much the same way. On the whole I found sunlight unsatisfactory. With few exceptions, eyes under its influence in weak solution constricted, and if there were dilation later, it was only after exposure to Ringer solution and at a temperature which my previous experience with salt solutions has shown to be positively injurious to all muscle tissue.

Mechanical stimuli are likewise an important factor in bringing about dilation of the pupil. Due care must be taken in changing the solution and in orienting the pupil, especially at such temperatures as render smooth muscle unusually irritable.

TABLE XVI.—*The effect of mechanical stimuli upon the pupil of the excised bulbus, kept at a constant temperature and lighted by artificial light.*

Time.	Long axis.	Short axis.	Time.	Long axis.	Short axis.	Time.	Long axis.	Short axis.
A. m.	*M. m.*	*M. m.*	*A. m.*	*M. m.*	*M. m.*	*A. m.*	*M. m.*	*M. m.*
10. 12	2. 235	2. 055	11. 51	1. 975	0. 945	1. 12	1. 670	1. 170
10. 25	2. 220	1. 860	11. 58	1. 670	1. 165	1. 15	1. 710	1. 200
10. 48	1. 835	1. 440	*P. m.*			1. 20	1. 825	1. 400
11. 18	1. 535	0. 970	12. 2	1. 735	1. 290	1. 26	1. 930	1. 645
11. 29	1. 515	0. 960	12. 11	1. 695	1. 240	1. 36	1. 935	1. 610
11. 46	1. 490	0. 900	12. 37	1. 635	1. 045	1. 48	1. 850	1. 405
11. 48	By means of a capillary pipette the eye is gently pipetted about in its bath and then oriented as usual.		12. 48	1. 650	1. 150	2. 8	1. 815	1. 310
			1. 2	1. 635	1. 080	2. 27	1. 800	1. 370
			1. 9	Ringer pipetted off and 2 c. c. of a 1:3,125,000 solution of adrenalin added.				

Table XVI illustrates in a general way the reaction of the pupil to mechanical stimuli. Soon after being placed in the container the eye dilates to a maximum (10.48), then constricts to a minimum, in which condition it remains for a long time if all influences be kept constant. But by gently pipetting the eye about as is done (11.48 a. m.) it dilates, sometimes slowly, but in this case rapidly, after which it again constricts. The experiment was continued (1.9) by replacing the Ringer solution with a very dilute solution of adrenalin of the same temperature, disturbing the eye to about the same extent as before. This again caused dilation similar to that following the mechanical stimuli just described. At first sight this last dilation might easily be attributed to the adrenalin since the eye not only dilates more slowly but the dilation persists. Other observations, however, lead me to conclude that the adrenalin has but little action and that which it has is exerted in supplementing the mechanical stimuli and perhaps in retarding the after constriction.

Since ordinary sunlight is unsatisfactory for quantitative work of this nature, artificial light was substituted. If the proper intensity be used and the eyes observed at 22 to 23° C. then, instead of constriction, dilation may occur with very dilute solutions. The dilation sometimes present with a concentration of 1 of adrenalin to 625,000 of Ringer or with weaker solutions is, however, not at all suited for quantitative determinations.

TABLE XVII.—*Mydriatic action of natural l-adrenalin.*

EXCISED FROG'S EYE.

Experiment 39a. 1: 2,000.			Experiment 41a. 1: 5,000.			Experiment 55b. 1: 25,000.		
Time.	Long axis.	Short axis.	Time.	Long axis.	Short axis.	Time.	Long axis.	Short axis.
A. m.	*M. m.*	*M. m.*	*P. m.*	*M. m.*	*M. m.*	*P. m.*	*M. m.*	*M. m.*
10. 12	[a] 2. 135	[a] 1. 700	3. 56	[a] 2. 010	[a] 1. 430	2. 10	[a] 1. 760	[a] 1. 660
10. 15	[a] 2. 115	[a] 1. 690	4. 5	[a] 2. 055	[a] 1. 650	2. 32	[a] 2. 030	[a] 1. 840
10. 20	[a] 2. 070	[a] 1. 720	.....	.....	.....	3. 25	[a] 1. 765	[a] 1. 435
10. 21	[a] 2. 125	[a] 1. 730	.....	.....	.....	3. 30	[a] 1. 730	[a] 1. 510
10. 26	[a] 2. 135	[a] 1. 740	4. 8	[a] 2. 100	[a] 1. 650	3. 42	[a] 1. 790	[a] 1. 595
10. 27	In adrenalin.		4. 9	In adrenalin.		3. 44	In adrenalin.	
10. 29	[b] 2. 185	[b] 1. 750	4. 11	[b] 2. 105	[b] 1. 805	3. 46	[b] 1. 900	[b] 1. 795
2	[c] 0. 050	[c] 0. 010	2	[c] 0. 005	[c] 0. 155	2	[c] 0. 110	[c] 0. 200
10. 31	[b] 2. 250	[b] 1. 935	4. 13	[b] 2. 510	[b] 2. 305	3. 48	[b] 1. 990	[b] 1. 940
4	[c] 0. 115	[c] 0. 195	4	[c] 0. 410	[c] 0. 655	4	[c] 0. 200	[c] 0. 345

[a] Actual length of axis measured in 2 c. c. of Ringer solution at 23° C.

[b] Actual length of axis at time measured.

[c] Change in length of axis after the eye is exposed to 2 c. c. of adrenalin solution for the number of minutes indicated to left in time column.

TABLE XVII.—*Mydriatic action of natural l-adrenalin*—Continued.

EXCISED FROG'S EYE—Continued.

Experiment 39a. 1:2,000.				Experiment 41a. 1:5,000.				Experiment 55b. 1:25,000.		
Time.	Long axis.		Short axis.	Time.	Long axis.		Short axis.	Time.	Long axis.	Short axis.
10.27	In adrenalin.			4.9	In adrenalin.			3.44	In adrenalin.	
A. m.	*M. m.*		*M. m.*	*P. m.*	*M. m.*		*M. m.*	*P. m.*	*M. m.*	*M. m.*
10.34	*a* 2.515		*a* 2.440	4.15	*a* 2.700		*a* 2.510	3.54	*a* 2.100	*a* 2.065
7	*b* 0.380	23.0°	*b* 0.700	6	*b* 0.600	23.8°	*b* 0.860	6	*b* 0.310	*b* 0.470
10.36	*a* 2.830	C.	*a* 2.685	4.18	*a* 2.750	C.	*a* 2.640	3.52	*a* 2.235	*a* 2.150
9	*b* 0.695		*b* 0.945	9	*b* 0.650		*b* 0.990	8	*b* 0.445	*b* 0.555
10.38	*a* 2.950		*a* 2.785	4.21	*a* 2.850		*a* 2.760	3.54	*a* 2.360	*a* 2.245
11	*b* 0.815		*b* 1.045	12	*b* 0.750		*b* 1.110	10	*b* 0.570	*b* 0.650
10.40	*a* 2.950		*a* 2.830	4.23	*a* 2.860		*a* 2.800	3.58	*a* 2.520	*a* 2.350
13	*b* 0.815		*b* 1.090	14	*b* 0.760		*b* 1.150	14	*b* 0.730	*b* 0.755
10.42	*a* 2.950		*a* 2.830	4.25	*a* 2.840		*a* 2.780	4.00	*a* 2.555	*a* 2.400
15	*b* 0.815		*b* 1.090	16	*b* 0.740		*b* 1.130	16	*b* 0.765	*b* 0.805
10.44	*a* 2.950	23.1°	*a* 2.830	4.27	*a* 2.840		*a* 2.800	4.3	*a* 2.610	*a* 2.430
17	*b* 0.815	C.	*b* 1.090	18	*b* 0.710		*b* 1.110	19	*b* 0.820	*b* 0.835
10.47	*a* 2.970		*a* 2.830	4.35	*a* 2.775		*a* 2.820	4.6	*a* 2.665	*a* 2.410
20	*b* 0.835		*b* 1.090	26	*b* 0.675		*b* 1.170	22	*b* 0.875	*b* 0.815
10.50	*a* 2.935		*a* 2.830	4.40	*a* 2.640		*a* 2.840	4.8	*a* 2.695	*a* 2.425
23	*b* 0.800		*b* 1.090	31	*b* 0.540		*b* 1.190	24	*b* 0.905	*b* 0.830
10.52	*a* 2.900		*a* 2.815	4.45	*a* 2.600		*a* 2.800	4.11	*a* 2.705	*a* 2.425
25	*b* 0.765		*b* 1.075	36	*b* 0.500		*b* 1.150	27	*b* 0.915	*b* 0.830
10.54	*a* 2.890		*a* 2.815	4.51	*a* 2.615		*a* 2.775	4.13	*a* 2.700	*a* 2.395
27	*b* 0.655		*b* 1.075	41	*b* 0.515		*b* 1.125	29	*b* 0.910	*b* 0.800
10.56	*a* 2.900		*a* 2.770	4.56	*a* 2.610		*a* 2.750	4.15	*a* 2.715	*a* 2.400
29	*b* 0.765		*b* 1.030	46	*b* 0.510		*b* 1.100	31	*b* 0.925	*b* 0.805
11.00	*a* 2.880		*a* 2.770	5.2	*a* 2.600	23.8°	*a* 2.690	4.17	*a* 2.700	*a* 2.390
33	*b* 0.745		*b* 1.030	52	*b* 0.500	C.	*b* 1.040	33	*b* 0.910	*b* 0.795
11.3	*a* 2.845	23.4°	*a* 2.775	5.10	*a* 2.590		*a* 2.675	4.20	*a* 2.670	*a* 2.380
36	*b* 0.700	C.	*b* 1.035	60	*b* 0.510		*b* 1.025	36	*b* 0.880	*b* 0.785
11.14	*a* 2.850		*a* 2.690	5.16	*a* 2.600		*a* 2.675	4.25	*a* 2.645	*a* 2.335
47	*b* 0.705		*b* 0.950	66	*b* 0.500		*b* 1.025	41	*b* 0.845	*b* 0.740
								4.27	*a* 2.625	*a* 2.320
								43	*b* 0.835	*b* 0.725
								4.37	*a* 2.560	*a* 2.190
								53	*b* 0.770	*b* 0.595
	c 20 min.		*c* 13 min.		*c* 14 min.		*c* 31 min.		*c* 31 min.	*c* 24 min.

a Actual length of axis at time measured.

b Change in length of axis after the eye is exposed to 2 c. c. of adrenalin solution for the number of minutes indicated to left in time column.

c Time required for each axis to reach a maximum length.

TABLE XVIII.—*Mydriatic action of natural l-adrenalin.*

EXCISED FROG'S EYE.

Experiment 44a. 1 : 125,000.			Experiment 46a. 1 : 625,000.			Experiment 47a. 1 : 3,125,000.		
Time.	Long axis.	Short axis.	Time.	Long axis.	Short axis.	Time.	Long axis.	Short axis.
A. m.	*M. m.*	*M. m.*	*A. m.*	*M. m.*	*M. m.*	*A. m.*	*M. m.*	*M. m.*
9.35	*a* 1.810	*a* 1.240	10.09	*a* 2.010	*a* 1.710	10.12	*a* 2.235	*a* 2.055
10.7	*a* 1.745	*a* 1.180	10.11	*a* 2.025	*a* 1.720	11.46	*a* 1.490	23.2° *a* 0.900
11.13	.....	.....	10.16	*a* 2.015	*a* 1.690	11.58	*a* 1.670	*a* 1.165
.....	.....	.....	10.42	*a* 1.925	*a* 1.560	*P. m.*		
11.13	*a* 1.690	*a* 1.020	11.26	*a* 1.895	*a* 1.435	12.2	*a* 1.735	*a* 1.290
						1.2	*a* 1.635	*a* 1.080
11.16	In adrenalin.		11.29	In adrenalin.		1.9	In adrenalin.	
11.18	*b* 1.735	*b* 1.175	11.34	*b* 2.075	*b* 1.615	1.12	*b* 1.670	*b* 1.170
2	*c* 0.045	*c* 0.155	5	*c* 0.180	*c* 0.180	3	*c* 0.035	*c* 0.090
11.20	*b* 1.855	*b* 1.265	11.37	*b* 2.135	*b* 1.640	1.15	*b* 1.710	*b* 1.200
4	*c* 0.165	*c* 0.245	8	*c* 0.240	23.0° *c* 0.205	6	*c* 0.075	*c* 0.120
11.21	*b* 1.880	*b* 1.375	11.40	*b* 2.140	C. *b* 1.645	1.20	*b* 1.825	*b* 1.400
5	*c* 0.190	22.0° *c* 0.355	11	*c* 0.245	*c* 0.210	11	*c* 0.190	*c* 0.320
11.26	*b* 1.905	C. *b* 1.530	*P. m.*			1.26	*b* 1.950	24.2° *b* 1.645
10	*c* 0.215	*c* 0.510	12.11	*b* 2.195	*b* 1.660	17	*c* 0.315	C. *c* 0.565
11.29	*b* 2.000	*b* 1.710	42	*c* 0.300	*c* 0.225	1.36	*b* 1.935	*b* 1.610
13	*c* 0.310	*c* 0.690	12.17	*b* 2.195	22.2° *b* 1.700	27	*c* 0.300	*c* 0.530
11.31	*b* 2.050	*b* 1.775	48	*c* 0.300	C. *c* 0.265	1.48	*b* 1.850	*b* 1.405
15	*c* 0.360	*c* 0.755	12.41	*b* 2.225	*b* 1.710	39	*c* 0.215	23.2° *c* 0.325
11.40	*b* 2.240	*b* 1.940	72	*c* 0.330	22.4° *c* 0.275	2.08	*b* 1.815	C. *b* 1.310
24	*c* 0.550	*c* 0.920	1.6	*b* 2.310	C. *b* 1.790	59	*c* 0.180	*c* 0.230
11.42	*b* 2.280	*b* 1.960	97	*c* 0.415	*c* 0.355	2.27	*b* 1.800	*b* 1.370
26	*c* 0.590	*c* 0.940	1.23	*b* 2.340	*b* 1.800	78	*c* 0.165	*c* 0.290
11.44	*b* 2.360	*b* 2.000	114	*c* 0.445	*c* 0.365	2.32	*b* 1.790	*b* 1.430
28	*c* 0.670	*c* 0.980	1.34	*b* 2.325	*b* 1.775	83	*c* 0.155	23.2° *c* 0.350
11.48	*b* 2.430	*b* 2.040	125	*c* 0.430	*c* 0.340	2.37	*b* 1.765	C. *b* 1.380
32	*c* 0.740	22.0° *c* 1.020	1.45	*b* 2.335	22.4° *b* 1.800	88	*c* 0.130	*c* 0.300
11.56	*b* 2.500	C. *b* 2.105	136	*c* 0.440	C. *c* 0.365	2.54	*b* 1.780	*b* 1.355
40	*c* 0.810	*c* 1.085	2.12	*b* 2.370	*b* 1.800	105	*c* 0.145	*c* 0.275
11.59	*b* 2.570	*b* 2.190	163	*c* 0.475	*c* 0.365	3.14	*b* 1.790	*b* 1.420
43	*c* 0.880	*c* 1.170	2.30	*b* 2.385	*b* 1.825	125	*c* 0.155	*c* 0.340
P. m.			181	*c* 0.490	*c* 0.390	3.34	*b* 1.760	*b* 1.360
12.1	*b* 2.575	*b* 2.225	2.51	*b* 2.405	*b* 1.830	145	*c* 0.125	*c* 0.280
45	*c* 0.885	*c* 1.205	202	*c* 0.510	22.8° *c* 0.395	3.54	*b* 1.795	*b* 1.295
12.9	*b* 2.635	*b* 2.345	3.11	*b* 2.420	C. *b* 1.865	165	*c* 0.160	*c* 0.215
53	*c* 1.045	*c* 1.325	222	*c* 0.525	*c* 0.430	4.31	*b* 1.720	*b* 1.245
12.13	*b* 2.670	*b* 2.395	3.42	*b* 2.445	*b* 1.875	202	*c* 0.085	24.6° *c* 0.165
57	*c* 1.080	22.2° *c* 1.375	253	*c* 0.550	*c* 0.440	6.41	*b* 1.545	C. *b* 1.000
12.21	*b* 2.670	C. *b* 2.495	4.6	*b* 2.475	*b* 1.855	332	*c* 0.090	*c* 0.080
65	*c* 1.080	*c* 1.475	277	*c* 0.580	*c* 0.420			

a Actual length of axis. Measured in 2 c. c. of Ringer solution at 23° C.

b Actual length of axis at time measured.

c Change in length of axis after the eye is exposed to 2 c. c. of adrenalin solution for the number of minutes indicated to left in time column.

TABLE XVIII.—*Mydriatic action of natural l-adrenalin*—Continued.

EXCISED FROG'S EYE—Continued.

Experiment 44a. 1 : 125,000.			Experiment 46a. 1 : 625,000.			Experiment 47a. 1 : 3,125,000.		
Time.	Long axis.	Short axis.	Time.	Long axis.	Short axis.	Time.	Long axis.	Short axis.
11.16	In adrenalin.		11.29	In adrenalin.		1.9	In adrenalin.	
P. m.	*M. m.*	*M. m.*	*P. m.*	*M. m.*	*M. m.*	*A. m.*	*M. m.*	*M. m.*
12.26	[a] 2.670	[a] 2.465	4.36	[a] 2.485	[a] 1.825			
70	[b] 1.080	[b] 1.445	307	[b] 0.590 C.	[b] 0.390			
12.35	[a] 2.645	[a] 2.465	4.54	[a] 2.480	[a] 1.890			
79	[b] 1.055	[b] 1.445	325	[b] 0.585	[b] 0.455			
12.44	[a] 2.650	[a] 2.500	6.51	[a] 2.390	[a] 1.855			
88	[b] 0.960	[b] 1.480	442	[b] 0.495	[b] 0.420			
12.47	[a] 2.650	[a] 2.500	6.54	[a] 2.390	[a] 1.870			
91	[b] 0.960	[b] 1.480	445	[b] 0.495 22.4°	[b] 0.435			
1.12	[a] 2.640	[a] 2.525	7.32	[a] 2.325 C.	[a] 1.840			
116	[b] 0.950	[b] 1.550	483	[b] 0.430	[b] 0.405			
1.20	[a] 2.655	[a] 2.480	7.56	[a] 2.255	[a] 1.845			
124	[b] 0.965	[b] 1.460	517	[b] 0.360	[b] 0.410			
1.35	[a] 2.580	[a] 2.435						
137	[b] 0.890	[b] 1.415						
	[c] 55 min.	[c] 114 min.		[c] 307 min.	[c] 325 min.		[c] 17 min.	[c] 145 min.

[a] Actual length of axis at time measured.

[b] Change in length of axis after the eye is exposed to 2 c. c. of adrenalin solution for the number of minutes indicated to left in time column.

[c] Time required for each axis to reach a maximum length.

The readings recorded in Tables XVII and XVIII are taken from eyes immersed in 1:2,000, 1:5,000, 1:25,000, 1:125,000, 1:625,000, and 1:3,125,000 solutions of natural l-adrenalin kept at nearly constant temperatures and lighted by electric light. It will be noticed that with an increased concentration of adrenalin there is a shortening of the time required to cause maximum dilation. If we take the mean of the times in Tables XVII and XVIII required by the long and short axes to reach a maximum length it will be found that—

1:2,000 caused maximum dilation in 16 min.
1:5,000 caused maximum dilation in 22 min.
1:25,000 caused maximum dilation in 70 min.
1:125,000 caused maximum dilation in 84 min.
1:625,000 caused maximum dilation in 316 min.
1:3,125,000 caused maximum dilation in (81)? min.

On the whole the results are not very satisfactory, and if the minimum time necessary for one of the axes be taken to represent the time of dilation the results are even less concordant. The solutions in the order named above cause maximum dilation in 13, 14, 66, 55, 307, and 17 minutes, respectively.

The most reliable results are obtained by comparing two solutions upon eyes from the same frog, thus giving test objects more nearly alike, the one solution being used on the right and the other upon the left eye. Although the eyes from other frogs may show for given sets a longer or shorter "dilation time," as a rule the higher readings will be approximate multiples of the lower, so that the relative values of the coefficients of strengths thus obtained will remain about the same throughout.

As indicated in Table XIX the eyes of frogs Nos. 71, 72, and 73 yield very consistent results. Not only do the dilation times of the weaker solutions agree with each other, but those of the stronger agree and the lengths of these times are roughly proportional to the concentration. At 18° to 19° C.:

1:20,000 adrenalin solution causes maximum dilation in 28 to 29 minutes.
1:40,000 adrenalin solution causes maximum dilation in 27 to 39 minutes.
1:80,000 adrenalin solution causes maximum dilation in 55 minutes.

TABLE XIX.

	Frog No. 71.			Frog No. 72.			Frog No. 73.		
	Time.	Long axis.	Short axis.	Time.	Long axis.	Short axis.	Time.	Long axis.	Short axis.
	P. m	*M. m.*	*M. m.*	*P. m.*	*M. m.*	*M. m.*	*P. m.*	*M. m.*	*M. m.*
Right eye in 1:40,000	2.52	3.015	2.425	11.27	3.250	2.550	1.49	3.470	2.985
	2.15	1.916	1.305	10.48	1.950	1.140	1.12	2.160	1.215
	37 min.	1.055	1.120	39 min.	1.300	1.410	37 min.	1.310	1.770
Left eye in 1:20,000	2.45	3.245	2.985	11.46	3.165	2.715	.....	.....	.....
	2.17	2.205	1.480	11.17	1.720	0.810	.....	.....	.....
	28 min.	1.040	1.505	29 min.	1.445	1.905	.....	.....	.....
Left eye in 1:80,000	.....	.....	.....	.....	.....	.....	2.4	3.265	2.810
	.....	.....	.....	.....	.....	.....	1.9	2.175	1.135
							55 min.	1.090	1.675

Weight of frogs, No. 71=30 grams; No. 72=26 grams, No. 73=26 grams.

All the eyes were measured in a Ringer-adrenalin solution kept at 18° to 19° C., lighted by a 16-candlepower incandescent light under a metallic reflector. The maximum dilation is determined by the time required by first axis to reach its greatest length.

RELATIVE MYDRIATIC ACTION OF NATURAL L- AND OF SYNTHETIC DL-ADRENALIN UPON THE EXCISED FROG'S EYE.

A comparison of the degree of mydriasis excited by natural l- and synthetic dl-adrenalin seems to indicate that the former is the more active. This, however, is not brought out very sharply by comparing a large number of eyes promiscuously. For example, if the results of Tables XVII, XVIII, XXI, XXII, XXIII, and XXIV be compared the following relations seem to obtain.

TABLE XX.—*Comparison of time required to cause maximum mydriasis.*

Concentration of solution.	Synthetic dl-.		Natural l-.		Synthetic dl-.	
	Long axis.	Short axis.	Long axis.	Short axis.	Long axis.	Short axis.
	Min.	*Min.*	*Min.*	*Min.*	*Min.*	*Min.*
1:2,000	21	21	20	13	15	15
1:5,000	23	16	14	31	17	26
1:25,000	56	38	31	24	61	54
1:125,000	153	203	55	114	332	359
1:625,000	74	74	307	325	367	367
1:3,125,000	67	79	17	145	243	275

It will be noticed that one of the axes of the eyes in natural l-adrenalin nearly always reaches a maximum length before those in synthetic dl-adrenalin solution of like concentration. Furthermore, solutions less dilute than 1:125,000 are uncertain in their action, the dilation time often becoming less than that normal for solutions of greater concentration. This is brought out more clearly in Tables XVIII and XXII, in which it will be seen that the dilation time is very irregular for dilute solutions.

TABLE XXI.—*Mydriatic action of synthetic dl-adrenalin.*

EXCISED FROG'S EYE.

Experiment 50b. 1:2,000.			Experiment 52a. 1:5,000.			Experiment 55a. 1:25,000.		
Time.	Long axis.	Short axis.	Time.	Long axis.	Short axis.	Time.	Long axis.	Short axis.
P. m.	*M. m.*	*M. m.*	*P. m.*	*M. m.*	*M. m.*	*P. m.*	*M. m.*	*M. m.*
1.44	[a] 1.720	[a] 1.310	1.46	[a] 1.810	[a] 1.435	2.12	[a] 1.885	[a] 1.815
2.50	[a] 1.690	[a] 1.275	.....	.....	.....	.....	.....	.....
.....	.....	.....	.....	.....	.....	.....	.....	.....
.....	.....	.....	.....	.....	.....	.....	.....	.....
3.1	[a] 1.690	[a] 1.360	1.55	[a] 1.876	[a] 1.585	2.21	[a] 1.980	[a] 1.920
3.3	In adrenalin.		1.57	In adrenalin.		2.23	In adrenalin.	
3.6	[b] 1.745	[b] 1.440	1.59	[b] 1.900	[b] 1.880	2.26	[b] 2.190	[b] 2.125
3	[c] 0.055	[c] 0.080	2	[c] 0.030	[c] 0.295	3	[c] 0.210	[c] 0.205
3.9	[b] 1.885	[b] 1.750	2.2	[b] 2.140	[b] 2.235	2.28	[b] 2.205	[b] 2.095
6	[c] 0.195	[c] 0.390	5	[c] 0.270	[c] 0.650	5	[c] 0.215	[c] 0.175
3.12	[b] 2.095	[b] 2.010	2.4	[b] 2.485	[b] 2.460	2.30	[b] 2.225	[b] 2.135
9	[c] 0.405	[c] 0.655	7	[c] 0.615	[c] 0.875	7	[c] 0.245	[c] 0.215

[a] Actual length of axis. Measured in 2 c. c. of Ringer solution at 23 °C.

[b] Actual length of axis at time measured.

[c] Change in length of axis after the eye is exposed to 2 c. c. of adrenalin solution for the number of minutes indicated to left in time column.

TABLE XXI.—*Mydriatic action of synthetic dl-adrenalin*—Continued.

EXCISED FROG'S EYE—Continued.

Experiment 50b. 1:2,000.			Experiment 52a. 1:5,000.			Experiment 55a. 1:25,000.		
Time.	Long axis.	Short axis.	Time.	Long axis.	Short axis.	Time.	Long axis.	Short axis.
3.3	In adrenalin.		1.57	In adrenalin.		2.23	In adrenalin.	
P. m.	*M. m.*	*M. m.*	*P. m.*	*M. m.*	*M. m.*	*P. m.*	*M. m.*	*M. m.*
3.14	[a] 2.290	[a] 2.220	2.6	[a] 2.510	[a] 2.530	2.35	[a] 2.355	[a] 2.215
11	[b] 0.600	[b] 0.860	9	[b] 0.640	[b] 0.945	12	[b] 0.375	[b] 0.295
3.16	[a] 2.485	[a] 2.390	2.9	[a] 2.695	[a] 2.620	2.37	[a] 2.400	[a] 2.310
13	[b] 0.795	[b] 1.030	12	[b] 0.825	[b] 1.035	14	[b] 0.420	[b] 0.390
3.18	[a] 2.650	[a] 2.600	2.11	[a] 2.775	[a] 2.640	2.39	[a] 2.460	[a] 2.375
15	[b] 0.960	[b] 1.240	14	[b] 0.905	[b] 1.055	16	[b] 0.480	[b] 0.455
3.20	[a] 2.730	[a] 2.680	2.13	[a] 2.875	[a] 2.650	2.42	[a] 2.500	[a] 2.400
17	[b] 1.040	[b] 1.320	16	[b] 1.005	[b] 1.065	19	[b] 0.520	[b] 0.480
3.22	[a] 2.730	[a] 2.760	2.15	[a] 2.885	[a] 2.600	2.45	[a] 2.330	[a] 2.420
19	[b] 1.040	[b] 1.400	18	[b] 1.015	[b] 1.015	22	[b] 0.550	[b] 0.500
3.24	[a] 2.820	[a] 2.800	2.18	[a] 2.960	[a] 2.575	2.47	[a] 2.580	[a] 2.455
21	[b] 1.130	[b] 1.440	21	[b] 1.090	[b] 0.990	24	[b] 0.600	[b] 0.535
3.26	[a] 2.810	[a] 2.775	2.20	[a] 2.970	[a] 2.520	2.50	[a] 2.590	[a] 2.450
23	[b] 1.120	[b] 1.415	23	[b] 1.100	[b] 0.935	27	[b] 0.610	[b] 0.530
3.30	[a] 2.810	[a] 2.750	2.22	[a] 2.955	[a] 2.510	2.52	[a] 2.590	[a] 2.465
27	[b] 1.120	[b] 1.390	25	[b] 1.085	[b] 0.925	29	[b] 0.610	[b] 0.545
3.35	[a] 2.805	[a] 2.760	2.27	[a] 2.960	[a] 2.490	2.54	[a] 2.600	[a] 2.470
32	[b] 1.115	[b] 1.400	30	[b] 1.090	[b] 0.905	31	[b] 0.620	[b] 0.550
3.37	[a] 2.815	[a] 2.725	2.36	[a] 2.955	[a] 2.475	2.57	[a] 2.610	[a] 2.465
34	[b] 1.125	[b] 1.435	39	[b] 1.085	[b] 0.890	34	[b] 0.630	[b] 0.545
3.42	[a] 2.800	[a] 2.725	2.44	[a] 2.945	[a] 2.515	2.59	[a] 2.635	[a] 2.435
39	[b] 1.110	[b] 1.435	47	[b] 1.075	[b] 0.930	36	[b] 0.655	[b] 0.515
3.58	[a] 2.765	[a] 2.620	4.4	[a] 2.850	[a] 2.325	3.1	[a] 2.670	[a] 2.435
55	[b] 1.075	[b] 1.260	127	[b] 0.980	[b] 0.740	38	[b] 0.690	[b] 0.575
4.05	[a] 2.765	[a] 2.610				3.4	[a] 2.675	[a] 2.410
62	[b] 1.075	[b] 1.250				41	[b] 0.695	[b] 0.490
						3.6	[a] 2.710	[a] 2.435
						43	[b] 0.730	[b] 0.515
						3.8	[a] 2.715	[a] 2.410
						45	[b] 0.735	[b] 0.490
						3.10	[a] 2.730	[a] 2.405
						47	[b] 0.750	[b] 0.495
						3.19	[a] 2.740	[a] 2.400
						56	[b] 0.760	[b] 0.480
						3.21	[a] 2.725	[a] 2.270
						58	[b] 0.745	[b] 0.350
						3.23	[a] 2.690	[a] 2.195
						60	[b] 0.710	[b] 0.275
						4.30	[a] 2.485	[a] 2.050
						67	[b] 0.505	[b] 0.130
	[c] 21 min.	[c] 21 min.		[c] 23 min.	[c] 16 min.		[c] 56 min.	[c] 38 min.

[a] Actual length of axis at time measured.

[b] Change in length of axis after the eye is exposed to 2 c. c. of adrenalin solution for the number of minutes indicated to left in time column.

[c] Time required for each axis to reach a maximum length.

TABLE XXII.—*Mydriatic action of synthetic dl-adrenalin.*

EXCISED FROG'S EYE.

Experiment 57a. 1:125,000.			Experiment 60a. 1:625,000.			Experiment 62a. 1:3,125,000.			
Time.	Long axis.	Short axis.	Time.	Long axis.	Short axis.	Time.	Long axis.		Short axis.
A. m.	*M. m.*	*M. m.*	*A. m.*	*M. m.*	*M. m.*	*A. m.*	*M. m.*		*M. m.*
9.57	a 2.215	a 2.020	10.43	a 1.930	a 1.870	11.5	a 2.515		a 2.350
10.4	a 2.255	a 2.035	10.45	a 1.995	a 1.915	11.28	a 2.285		a 2.190
.....	a 2.175	.....	11.11	a 1.950	a 1.650	11.35	a 2.240		a 2.140
10.42	a 2.175	a 1.920	.....	.....	.....	11.55	a 2.030		a 1.765
11.29	a 2.110	a 1.750	11.53	a 1.905	a 1.640	*P. m.*			
						12.32	a 2.160		a 1.975
11.31	In adrenalin.		11.55	In adrenalin.		12.37	In adrenalin.		
11.33	b 2.230	b 1.900	11.57	b 2.010	b 1.745	12.41	b 2.350		b 2.160
2	c 0.120	c 0.150	2	c 0.105	c 0.105	4	c 0.190		c 0.185
11.35	b 2.305	b 1.915	*P. m.*			12.43	b 2.410		b 2.285
4	c 0.195	c 0.165	12.3	b 2.075	b 1.860	6	c 0.250		c 0.310
11.39	b 2.405	b 2.045	8	c 0.170	c 0.220	12.52	b 2.520		b 2.420
8	c 0.295	c 0.295	12.10	b 2.090	b 1.910	15	c 0.360		c 0.445
11.43	b 2.435	b 2.180	15	c 0.185	c 0.270	12.54	b 2.535		b 2.390
12	c 0.325	c 0.430	12.25	b 2.140	b 1.890	17	c 0.375		c 0.415
11.53	b 2.485	b 2.240	30	c 0.235	c 0.250	1.6	b 2.495		b 2.335
22	c 0.375	c 0.490	12.29	b 2.190	b 1.870	29	c 0.335		c 0.360
12.00	b 2.475	b 2.245	34	c 0.285	c 0.230	1.12	b 2.490		b 2.310
29	c 0.365	c 0.495	12.40	b 2.225	b 1.885	35	c 0.330		c 0.335
P. m.			45	c 0.320	c 0.245	1.27	b 2.465		b 2.280
12.3	b 2.515	b 2.235	12.46	b 2.230	b 1.920	50	c 0.305		c 0.305
32	c 0.405	c 0.485	57	c 0.325	c 0.280	1.44	b 2.520		b 2.335
12.7	b 2.470	b 2.185	12.58	b 2.250	b 1.870	67	c 0.360		c 0.360
36	c 0.360	c 0.435	63	c 0.345	c 0.230	1.56	b 2.515		b 2.375
12.21	b 2.505	b 2.175	1.1	b 2.240	b 1.935	79	c 0.355		c 0.400
50	c 0.395	c 0.425	66	c 0.335	c 0.295	2.19	b 2.485		b 2.240
12.24	b 2.605	b 2.215	1.6	b 2.265	b 1.990	102	c 0.325		c 0.265
53	c 0.495	c 0.465	71	c 0.360	c 0.350	2.30	b 2.570	22.8°	b 2.300
12.30	b 2.610	b 2.240	1.9	b 2.295	b 2.025	113	c 0.350	C.	c 0.325
59	c 0.500	c 0.490	74	c 0.390	c 0.385	2.39	b 2.510		b 2.305
12.34	b 2.655	b 2.230	1.18	b 2.225	b 1.940	122	c 0.350		c 0.330
63	c 0.545	c 0.480	83	c 0.320	c 0.300	2.56	b 2.410		b 2.300
12.36	b 2.690	b 2.245	1.23	b 2.005	b 1.940	139	c 0.250		c 0.325
65	c 0.580	c 0.495	88	c 0.300	c 0.300	3.18	b 2.510		b 2.340
12.40	b 2.740	b 2.240	1.39	b 2.145	b 1.750	161	c 0.350		c 0.365
69	c 0.630	c 0.490	108	c 0.240	c 0.110	3.31	b 2.385		b 2.185
12.50	b 2.830	b 2.290	1.48	b 2.145	b 1.845	174	c 0.225		c 0.210
79	c 0.720	c 0.540	113	c 0.240	c 0.205	3.45	b 2.400		b 2.255
12.54	b 2.845	b 2.345				188	c 0.240		c 0.280
83	c 0.735	c 0.595				4.18	b 2.445		b 2.255

a Actual length of axis measured in 2 c. c. of Ringer solution at 23° C.

b Actual length of axis at time measured.

c Change in length of axis after the eye is exposed to 2 c. c. of adrenalin solution for the number of minutes indicated to left in time column.

TABLE XXII.—*Mydriatic action of synthetic dl-adrenalin*—Continued.

EXCISED FROG'S EYE—Continued.

Experiment 57a. 1: 125,000.			Experiment 60a. 1: 625,000.			Experiment 62a. 1: 3,125,000.		
Time.	Long axis.	Short axis.	Time.	Long axis.	Short axis.	Time.	Long axis.	Short axis.
11.31	In adrenalin.		11.55	In adrenalin.		12.37	In adrenalin.	
P. m.	*M. m.*	*M. m.*	*P. m.*	*M. m.*	*M. m.*	*Y. m.*	*M. m.*	*M. m.*
1.6	[a] 2.850	[a] 2.355				221	[b] 0.285	[b] 0.280
95	[b] 0.740	[b] 0.605						
1.8	[a] 2.860	[a] 2.375						
97	[b] 0.750	[b] 0.625						
1.22	[a] 2.950	[a] 2.465						
111	[b] 0.840	[b] 0.715						
1.27	[a] 2.945	[a] 2.570						
116	[b] 0.835	[b] 0.760						
1.33	[a] 2.925	[a] 2.540						
122	[b] 0.815	[b] 0.790						
1.53	[a] 2.930	[a] 2.530						
142	[b] 0.820	[b] 0.780						
2.1	[a] 2.970	[a] 2.540						
150	[b] 0.860	[b] 0.790						
2.4	[a] 2.980	[a] 2.545						
153	[b] 0.870	[b] 0.795						
2.17	[a] 2.905	[a] 2.495						
166	[b] 0.795	[b] 0.745						
22.23	[a] 2.950	[a] 2.500						
172	[b] 0.840	[b] 0.750						
2.26	[a] 2.965	[a] 2.540						
175	[b] 0.855	[b] 0.790						
2.32	[a] 2.970	[a] 2.520						
181	[b] 0.860	[b] 0.770						
2.43	[a] 2.920	[a] 2.500						
192	[b] 0.810	[b] 0.750						
2.52	[a] 2.935	[a] 2.500						
201	[b] 0.825	[b] 0.800						
2.54	[a] 2.945	[a] 2.575						
203	[b] 0.835	[b] 0.825						
2.57	[a] 2.955	[a] 2.540						
206	[b] 0.845	[b] 0.790						
3.9	[a] 2.795	[a] 2.540						
218	[b] 0.685	[b] 0.790						
	[c] 153 min.	[c] 203 min.		[c] 74 min.	[c] 74 min.		[c] 67 min.	[c] 79 min.

[a] Actual length of axis at time measured.

[b] Change in length of axis after the eye is exposed to 2 c. c. of adrenalin solution for the number of minutes indicated to left in this column.

[c] Time required for each axis to reach a maximum length.

TABLE XXIII.—*Mydriatic action of synthetic dl-adrenalin.*

EXCISED FROG'S EYE.

Experiment 39b. 1: 2,000.			Experiment 41b. 1: 5,000.			Experiment 43b. 1: 25,000.		
Time.	Long axis.	Short axis.	Time.	Long axis.	Short axis.	Time.	Long axis.	Short axis.
A. m.	*M. m.*	*M. m.*	*P. m.*	*M. m.*	*M. m.*	*A. m.*	*M. m.*	*M. m.*
10.24	[a] 2.165	[a] 1.650	2.51	[a] 2.000	[a] 1.690	10.16	[a] 1.950	[a] 1.660
11.24	[a] 1.665	[a] 1.260	2.54	[a] 2.050	[a] 1.755	10.30	[a] 1.950	[a] 1.225
11.25	In adrenalin.		2.55	In adrenalin.		10.31	In adrenalin.	
Time in adrenalin solution.	Increase in length of axes.		Time in adrenalin solution.	Increase in length of axes.		Time in adrenalin solution.	Increase in length of axes.	
Minutes.	Long axis.	Short axis.	Minutes.	Long axis.	Short axis.	Minutes.	Long axis.	Short axis.
	M. m.	*M. m.*		*M. m.*	*M. m.*		*M. m.*	*M. m.*
2	[b] 0.230	[b] 0.140	2	[b] 0.105	[b] 0.390	3	[b] 0.000	[b] 0.165
4	[b] 0.285	[b] 0.315	4	[b] 0.400	[b] 0.795	5	[b] 0.050 21.2°	[b] 0.275
6	[b] 0.390 23.8°	[b] 0.530	6	[b] 0.620	[b] 0.945	7	[b] 0.090 C.	[b] 0.490
8	[b] 0.650 C.	[b] 0.815	8	[b] 0.700	[b] 1.025	9	[b] 0.160	[b] 0.490
10	[b] 0.885 23.0°	[b] 1.115	10	[b] 0.785	[b] 4.965	11	[b] 0.250	[b] 1.175
12	[b] 1.015 C.	[b] 1.280	14	[b] 0.975	[b] 1.080	13	[b] 0.310	[b] 0.185
15	[b] 1.060	[b] 1.325	17	[b] 1.010 23.9°	[b] 1.080	15	[b] 0.385	[b] 0.915
18	[b] 0.990 23.1°	[b] 1.305	20	[b] 0.980 C.	[b] 1.130	17	[b] 0.450	[b] 1.045
20	[b] 0.950 C.	[b] 1.275	22	[b] 0.970	[b] 1.180	20	[b] 0.615	[b] 1.175
23	[b] 0.935	[b] 1.270	24	[b] 0.965	[b] 1.195	25	[b] 0.740	[b] 1.310
			26	[b] 0.910	[b] 1.205	27	[b] 0.780	[b] 1.330
			30	[b] 0.865	[b] 1.170	29	[b] 0.825 21.2°	[b] 1.365
			33	[b] 0.815 23.8° C.	[b] 1.140	32	[b] 0.890 C.	[b] 1.400
						35	[b] 0.915	[b] 1.455
						37	[b] 0.935	[b] 1.515
						39	[b] 0.970	[b] 1.515
						41	[b] 0.990	[b] 1.555
						43	[b] 1.045	[b] 1.605
						46	[b] 1.050	[b] 1.585
						48	[b] 1.085	[b] 1.610
						50	[b] 1.110	[b] 1.640
						52	[b] 1.125	[b] 1.660
						54	[b] 1.145	[b] 1.685
						56	[b] 1.175 21.6°	[b] 1.665
						61	[b] 1.225 C.	[b] 1.685
						66	[b] 1.210	[b] 1.685
						69	[b] 1.205	[b] 1.665
	[c] 15 min.	[c] 15 min.		[c] 17 min.	[c] 26 min.		[c] 61 min.	[c] 54 min.

[a] The actual length of the axes are given only for the first and last reading while in Ringer at 23.°

[b] Represent the increase in length of the axis after exposure to adrenalin solution for the number of minutes indicated to the left in the time column.

[c] Time required for each axis to reach a maximum length.

TABLE XXIV.—*Mydriatic action of synthetic dl- adrenalin.*

EXCISED FROG'S EYE.

Experiment 44b. 1:125,000.			Experiment 46b. 1:625,000.			Experiment 47b. 1:3,125,000.		
Time.	Long axis.	Short axis.	Time.	Long axis.	Short axis.	Time.	Long axis.	Short axis.
A. m.	*M. m.*	*M. m.*	*A. m.*	*M. m.*	*M. m.*	*A. m.*	*M. m.*	*M. m.*
9.30	[a] 1.820	[a] 1.230	10.4	[a] 1.940	[a] 1.630	10.4	[a] 2.200	[a] 2.250
9.47	[a] 1.805	20.8° C [a] 1.165	10.48	[a] 1.875	[a] 1.475	11.20	[a] 1.670	[a] 1.125
9.48	In adrenalin.		10.50	In adrenalin.		11.22	In adrenalin.	
Time in adrenalin solution.	Increase in length of axes.		Time in adrenalin solution.	Increase in length of axes.		Time in adrenalin solution.	Increase in length of axes.	
Minutes.	Long axis.	Short axis.	Minutes.	Long axis.	Short axis.	Minutes.	Long axis.	Short axis.
	mm.	*mm.*		*mm.*	*mm.*		*mm.*	*mm.*
4	[b] 0.105	[b] 0.090	4	[b] 0.035	[b] 0.105	4	[b] 0.015	23.2° [b] 0.000
7	[b] 0.085	[b] 0.095	6	[b] 0.165	[b] 0.210	10	[b] 0.010	C [b] 0.145
9	[b] 0.045	[b] 0.150	14	[b] 0.245	[b] 0.310	21	[b] 0.040	.....
11	[b] 0.090	[b] 0.190	27	[b] 0.110	[b] 0.110	32	[b] 0.300	[b] 0.400
14	[b] 0.090	[b] 0.325	31	[b] 0.095	23.0° [b] 0.055	41	[b] 0.190	[b] 0.155
16	[b] 0.130	20.8° [b] 0.395	53	[b] 0.060	C [b] 0.035	49	[b] 0.130	[b] 0.130
22	[b] 0.160	C [b] 0.470	73	[b] 0.125	[b] 0.070	70	[b] 0.185	[b] 0.210
25	[b] 0.175	21.0° [b] 0.495	77	[b] 0.130	[b] 0.070	89	[b] 0.170	[b] 0.115
28	[b] 0.270	C [b] 0.560	91	[b] 0.140	[b] 0.060	103	[b] 0.200	[b] 0.145
30	[b] 0.270	[b] 0.620	106	[b] 0.175	[b] 0.065	115	[b] 0.235	[b] 0.240
32	[b] 0.320	[b] 0.650	113	[b] 0.160	[b] 0.040	127	[b] 0.190	[b] 0.115
35	[b] 0.350	[b] 0.670	133	[b] 0.200	22.4° [b] 0.075	130	[b] 0.195	[b] 0.120
37	[b] 0.350	[b] 0.700	156	[b] 0.330	C [b] 0.090	149	[b] 0.130	22.3° [b] 0.030
41	[b] 0.395	21.4° [b] 0.720	160	[b] 0.340	[b] 0.135	163	[b] 0.035	C [b] 0.055
47	[b] 0.485	C [b] 0.800	179	[b] 0.325	[b] 0.120	169	[b] 0.050	[b] 0.005
52	[b] 0.490	[b] 0.800	182	[b] 0.325	22.4° [b] 0.120	183	[b] 0.130	[b] 0.080
59	[b] 0.460	[b] 0.785	199	[b] 0.350	C [b] 0.125	192	[b] 0.175	[b] 0.140
62	[b] 0.470	[b] 0.805	224	[b] 0.430	[b] 0.130	209	[b] 0.195	[b] 0.155
64	[b] 0.485	[b] 0.815	238	[b] 0.425	[b] 0.140	234	[b] 0.215	[b] 0.230
67	[b] 0.510	[b] 0.830	264	[b] 0.495	22.8° [b] 0.190	249	[b] 0.280	[b] 0.285
72	[b] 0.510	[b] 0.845	289	[b] 0.500	C [b] 0.195	275	[b] 0.225	[b] 0.365
82	[b] 0.555	[b] 0.875	390	[b] 0.535	[b] 0.260	312	[b] 0.180	24.6° [b] 0.265
95	[b] 0.520	22.0° [b] 0.740	344	[b] 0.505	[b] 0.290	427	[b] 0.115	C [b] 0.175
107	[b] 0.565	C [b] 0.805	367	[b] 0.555	[b] 0.315			
109	[b] 0.580	[b] 0.860	477	[b] 0.470	[b] 0.305			
123	[b] 0.585	[b] 0.775	491	[b] 0.250	22.4° [b] 0.295			
125	[b] 0.620	[b] 0.795	520	[b] 0.230	C [b] 0.285			
136	[b] 0.650	[b] 0.785	548	[b] 0.175	[b] 0.215			
138	[b] 0.660	[b] 0.825						
161	[b] 0.685	[b] 0.900						

[a] The actual length of the axis is given only for the first and last reading while in Ringer at 23.23°.

[b] Represent the increase in length of the axis after exposure to adrenalin solution for the number of minutes indicated to the left of the time column.

TABLE XXIV.—*Mydriatic action of synthetic dl-adrenalin*—Continued.

EXCISED FROG'S EYE—Continued.

Experiment 44b. 1:125,000.			Experiment 46b. 1:625,000.			Experiment 47b. 1:3,125,000.		
9.48	In adrenalin.		10.50	In adrenalin.		11.22	In adrenalin.	
Time in adrenalin solution.	Increase in length of axes.		Time in adrenalin solution.	Increase in length of axes.		Time in adrenalin solution.	Increase in length of axes.	
Minutes.	Long axis.	Short axis.	Minutes.	Long axis.	Short axis.	Minutes.	Long axis.	Short axis.
	mm.	*mm.*		*mm.*	*mm.*		*mm.*	*mm.*
164	[a] 0.720	[a] 0.955						
181	[a] 0.695	[a] 0.955						
183	[a] 0.705	[a] 1.040						
197	[a] 0.750	[a] 1.140						
199	[a] 0.750	[a] 1.140						
219	[a] 0.740	[a] 1.180						
222	[a] 0.740	[a] 1.200						
258	[a] 0.795	[a] 1.275						
260	[a] 0.815	[a] 1.275						
281	[a] 0.915	[a] 1.285						
286	[a] 0.930	[a] 1.310						
318	[a] 0.980	[a] 1.335						
325	[a] 0.980	[a] 1.355						
332	[a] 0.980	[a] 1.375						
335	[a] 0.980	[a] 1.375						
359	[a] 0.985	22.6° [a] 1.335						
362	[a] 0.985	C [a] 1.335						
388	[a] 0.955	[a] 1.325						
	[b] 332 min.	[b] 359 min.		[b] 307 min.	[b] 307 min.		[b] 243 min.	[b] 275 min.

[a] Represents the increase in length of the axis after exposure to adrenalin solution for the number of minutes indicated to the left in the time column.

[b] Time required for each axis to reach a maximum length.

It is difficult to determine the relative mydriatic activity of these two products by simply comparing the results obtained with eyes from different frogs, for when this is done the individual variations are so great that the general average of the dilation times is about the same for like concentrations as is shown by taking an equal number of readings from groups of eyes.

	Synthetic.	Natural.
	Min.	*Min.*
1:1,000	21	16
1:2,000	20	20
1:5,000	24	26
1:25,000	50	51
1:125,000	232	173

If one were to use these figures to decide, the indication would be that the natural l- and synthetic dl- products have an equal mydriatic action.

As is shown in a study of large numbers of eyes a 1:2,000 solution may in certain cases bring about maximum dilation in as short a time as a 1:1,000 solution, and in rare instances a 1:5,000 solution may compare very favorably with a 1:1,000 solution, and thus throw more or less doubt upon such determinations as agree with results obtained by the blood-pressure method. This criticism holds for eyes of different frogs; but it does not hold when comparing a 1:1,000 solution with a 1:5,000 solution upon the right eye and left eye of the same frog. In this case a series of experiments will quickly determine which is the stronger of the two solutions, and by proper checking against a solution of known strength the approximate strength of the unknown can be determined. All later experiments with the pupil indicate that the relative activity for natural l- and synthetic dl- adrenalin is about the same as determined by blood-pressure experiments. In experiment 75 the right eye was placed in a 1:40,000 solution of natural l- adrenalin, the left in a 1:40,000 solution of synthetic dl- adrenalin. The former dilated to a maximum in twenty-seven minutes; the latter in forty-six minutes. The pupils were then placed in several changes of fresh Ringer, whereupon they gradually constricted. Upon placing them again in fresh 1:40,000 solutions the right eye dilated in the natural l- in sixty-eight minutes and the left eye in synthetic dl- in eighty minutes. This and similar experiments now in progress illustrate in a general way the relative mydriatic action of these two compounds much more truly than is illustrated in a summary of my older experiments.

In conclusion it may be said that a method for the standardizing of adrenalin preparations which involves the idea of minimum doses is unreliable. The pupil method is no exception to this rule, for minimum doses are even more uncertain in their action upon the frog's eye than they are in blood-pressure experiments. It may be assumed that adrenalin acts upon muscle much as does a tetanic stimulus, in which case to secure comparative results upon a given muscle the stimulus, preferably an optimal one, must be of like intensity and duration. In any case the pupil reacts at different rates with different concentration of adrenalin. And the time required for dilation seems to be a better index to the strength of chemical stimulus than the amount of dilation. When the time intervening between the moment of stimulation and that of maximum dilation is chosen as the unit results may be obtained that compare very favorably with those obtained in blood-pressure experiments. The synthetic dl- adrenalin has about the same activity as is indicated by the blood-pressure and toxicity experiments already discussed.

THEORETICAL.

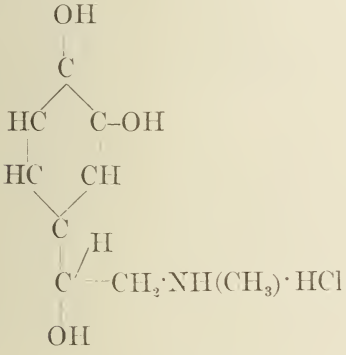
In reading the literature on the active substance of the adrenal gland one is impressed with the difference of the doses said to be lethal and of the amount of substance cited as the minimum dose necessary to cause a rise of blood pressure. These variations, I believe, are to be explained in part by differences of technique but more especially by varying amounts of adrenalin in the preparations used by the different observers. Certainly in experiments antedating 1900 the various workers must have been using at best only mixtures of adrenalin and other substances or compounds of adrenalin itself. Even the epinephrin used by Amberg seems to have had a relatively low degree of toxicity. However, the experiments with epinephrin are worth repeating; perhaps there was some error in determining the lethal dose. If epinephrin proved to have all of the properties of the pure base now known as adrenalin it would be possible to adopt this name for the active substance of the adrenal gland, which from many standpoints is to be preferred above all others. As stated earlier in this paper, Battelli worked with an unusually pure product, and while it is not altogether safe to compare results obtained with animals so widely different as mice and rabbits still it is interesting that the lethal doses given in this paper and those cited by Battelli are very nearly alike, viz., 8 to 10 milligrams per kilogram for subcutaneous injections.

It is also interesting that the relative pharmacological activity of the four compounds, as determined by any given method—say blood pressure, toxicity, or pupil experiments—should bear to each other a rather definite ratio. And when the difference in relative degree of toxicity or of vaso-constrictor activity seems to be closely associated with certain groups in the molecule or to contain arrangements of these groups in space it becomes very suggestive from a theoretical standpoint.

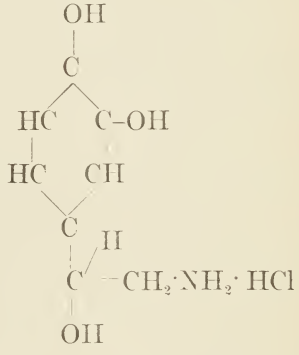
As already pointed out the natural l-adrenalin has a vaso-constrictor action one and a half times as powerful as synthetic dl-adrenalin, and nearly the same action as ortho-dioxy-phenyl-ethanol-amin (arterenol), but eighty times as powerful as ortho-ethyl-amino-dioxy-aceto-phenon (homorenon), thus making the relative activity of these substances to each other as the inverse ratio of 1:15:1:80. If, on the other hand, the relative toxicity of the substances just named be compared it will be found that they are to each other as the inverse ratio of 1:(1.5–2):5:(71–80). In comparing these ratios it will be seen that they are nearly alike, the divergence being in that part of the toxicity ratio pertaining to arterenol. This product, though causing nearly the same rises of blood pressure as the natural

l-base, is much less toxic. It would seem that the substitution of hydrogen of the amino group by a methyl or ethyl group increases the toxicity; certainly the ethyl group does this, as is shown by the increased toxicity of l- and dl- adrenalin. It would be interesting to test the effect of using different alkyl groups in displacing the hydrogen of the amino group for I am quite certain that if other radicles or more of them were introduced the toxicity would be greatly altered.

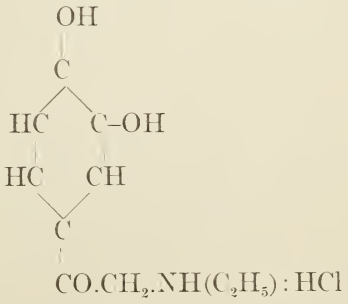
The four compounds, all catechol derivatives, possess pharmacological qualitative properties that are very nearly identical, but their quantitative properties differ widely. What is the key to this quantitative difference? Dakin and others have attributed the similarity in action to the catechol radicle, which is essential to produce a rise of blood pressure. Dakin would divide the adrenalin molecule into two parts, the catechol nucleus and the side chain, oxyethylmethylamine. It was first shown that catechol itself is quite active and that methyl-amino-acetyl-catechol is still more like adrenalin. Furthermore, it was observed that if the hydrogen of the hydroxyl groups were displaced, this compound became inactive. Then, again, the methyl ether of catechol is inactive. Now, in the three compounds—



Ortho-dioxy-phenyl-ethanol-methyl-amin hydrochloride.
(1) (Dl-adrenalin hydrochloride.)



Ortho-dioxy-phenyl-ethanol-amin hydrochloride.
(2) (Arterenol hydrochloride.)



Ethyl-amino-aceto-catechol hydrochloride.
(3) (Homorenon hydrochloride.)

we have a good illustration of the effects of changing the composition of the side chains. In (1) the composition is the same as (2), with the exception that one hydrogen is displaced by the methyl group while in (3) one hydrogen of the amino group is displaced by an ethyl group and the side chain joined to the catechol nucleus through a carboxyl group instead of a secondary alcohol group. Evidently, it is the nature of this side chain that determines the degree of activity as well as its relation. I agree with Dakin in his contention that the hydrogen atoms of the hydroxyl groups must be unsubstituted, that alkyl groups of low molecular weight tend to increase the activity much more efficiently than when larger molecules are introduced into the side chain. Indeed, several factors seem to be of prime importance in determining the activity of catechol compounds. (1) Whether the substance partakes of the nature of a ketone or of a secondary alcohol, the latter being the more active; (2) the nature of the groups displacing the H of the amino groups, as well as the number of displacements; and (3) the arrangement of the asymmetric carbon atom in space, the lævo arrangement usually being an index of the greater activity. At least this is borne out by my own results and by the results of Cushny and others.

With the theory that these are the determining factors in modifying the vaso-constrictor action of such compounds it is hoped that as a working basis it will aid in a more extended study of other compounds possessing physiological activity.

CONCLUSIONS.

1. The blood-pressure method with dogs under morphine-ether anæsthesia, the vagi cut, and very small doses of curare, is the most accurate pharmacological assay for catechol derivatives.

2. The pupil method as modified by the author is a reliable assay for adrenalin but less delicate and more tedious than the blood-pressure method.

3. Synthetic dl-adrenalin is less active as a vaso-constrictor and as a mydriatic than natural l-adrenalin, the ratio being 2:3.

4. The relative vaso-constrictor activity of the catechol derivatives in the order named, l- and dl-, ortho-dioxyphenylethanolmethylamin, ortho-dioxyphenylethanolamin and ortho-ethylaminodioxyacetophenon are to each other as the inverse ratio of 1:1.5:1:80.

5. The toxicity of the substances in the order named in (4) are to each other as the inverse ratio of 1: (1.5 to 2):5:(71 to 80).

6. The relative physiological activity of these catechol derivatives seems to depend upon the substance partaking of the properties of a secondary alcohol or of a ketone, upon the nature and number of groups displacing the hydrogen of the amino group, and upon the arrangement of the asymmetric carbon in space.

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LIST OF HYGIENIC LABORATORY BULLETINS OF THE PUBLIC HEALTH AND MARINE-HOSPITAL 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 formaldehyd 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.

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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 horse-hair 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 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 *Tænia 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 echidninus*). By W. W. Miller.

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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.

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