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A STUDY OF MELTING-POINT DETERMINATIONS

WITH SPECIAL REFERENCE TO THE MELTING-POINT REQUIREMENTS OF THE U. S. PHARMACOPŒIA

BY

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A STUDY OF MELTING-POINT DETERMINATIONS WITH SPECIAL REFERENCE TO THE MELTING-POINT REQUIREMENTS OF THE UNITED STATES PHARMACOPŒIA.[a]

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INTRODUCTION.

Section 7 of the federal food and drugs act of June 30, 1906, states:

That for the purpose of this act an article shall be deemed adulterated:

In case of drugs:

First. If, when a drug is sold under or by a name recognized in the United States Pharmacopœia or National Formulary, it differs from the standard of strength, quality, or purity as determined by the tests laid down in the United States Pharmacopœia or National Formulary official at the time of investigation.

This section of the food and drugs act confers upon the Pharmacopœia, in its field of operation, the exalted dignity of a federal statute—makes it the law of the land in the vitally important matter of official drug standards—and imposes upon that body of men which constitutes the United States Pharmacopœial Convention the very great responsibility of producing a Pharmacopœia which shall, in every sense, uphold the dignity of the law, and be worthy to represent the final authority on the standardization of drugs.

That the present Pharmacopœia (eighth revision) falls short in many respects of establishing an adequate legal standard for drugs is, I believe, very generally recognized. That the forthcoming revision (ninth) of this important book will fall short of attaining the ideal is, as with most ideals, probably true. But, although the present Pharmacopœia may be generally and justly considered the best that has yet been produced, it is undoubtedly true that great improvement, in every respect, can and doubtless will be accomplished in the ninth revision.

A legal standard does not, of course—and, in the case of drugs, should not—necessarily imply the highest degree of purity attainable.

[a] Manuscript submitted for publication July 20, 1910.

A *sine qua non* for an effective legal standard, however, is a perfectly uniform, clearly defined, unmistakable procedure, whose application should be required in determining such standard. That is, if the United States Pharmacopœia, the organ through which the federal law on drugs becomes operative, requires that a drug should meet certain tests in order to be considered of pharmacopœial standard, such tests and their mode of application should be so clearly defined as to eliminate, as far as practically possible, all causes of variation in the results obtained from their application by different individuals. It is in this respect that the eighth revision of the Pharmacopœia fails to provide adequate legal requirements—especially as applied to the determination of some of the physical constants, notably the so-called melting points and boiling points.

The very general use of the melting-point determination as a means of identifying and as a test for the purity of a compound, the great confidence that is usually placed by the rank and file of chemists in the melting-point determination for the purpose to which it is applied, and the possible simplicity and convenience of method that may be used in the great majority of cases for the reasonably reliable determination of this constant would seem to place this test among the most important in the standardization of those pharmacopœial compounds to which it is capable of application.

Under present conditions, however—considering the extreme lack of conformity in methods and procedure that now prevails for melting-point determinations in different laboratories and the consequent variation in results for the same product that frequently obtain—this test, as a means of standardization, has very definite limitations.

For the individual investigator whose work does not directly concern the public health, and whose conception of the melting point and his means of determining it may be a matter of personal judgment, it is perhaps not essential that this test should be standardized, although such an attitude, especially when the work leads to publication of results, is undoubtedly conducive to a greater or less degree of chaos in the whole subject. The present legal status of the Pharmacopœia, however, makes it not only important, but imperative, that the melting-point test on official compounds should be made in accordance with a standard procedure, to the end that reasonably concordant values for the same product may result at the hands of different independent manipulators. That the melting-point requirements of the United States Pharmacopœia (eighth) are quite inadequate to any such degree of efficiency has long been recognized. The practical impossibility, under present conditions, of conforming to the standard imposed therein has repeatedly been urged upon the

committee of revision by the different manufacturers and other individuals concerned; and various recommendations for revision have been made, leading finally to definite action by the committee.

The United States Pharmacopœia is a publication which is certainly very closely related to the public health. The proper development and improvement of this book in the matter of standards offers not only a legitimate but a very worthy field for active interest on the part of the Public Health and Marine-Hospital Service. It is perfectly logical and appropriate therefore that in this instance, as in others, the Committee of Revision of the United States Pharmacopœia should solicit and obtain the cooperation of the Public Health and Marine-Hospital Service.

As a result of this cooperation the division of pharmacology of this laboratory, in which an investigation on the solubilities of pharmacopœial compounds was already well under way, undertook an investigation of the melting points for the twofold purpose of selecting or devising a method or methods for melting-point determinations which could be recommended to the committee of revision for official adoption, and by means of such methods to standardize the melting points of pharmacopœial compounds.

The work so far accomplished in this investigation is herewith reported. It represents but a small part—and that the simplest part—of the work that a careful investigation of the subject involves. The real nature of the problem, its extent and complication, seem to be very generally misunderstood and underestimated. The progress of the work has disclosed details of procedure whose effect upon the melting point may be significant, but to determine which would involve independent investigations of no small proportions. Certain of these details will doubtless be indicated and perhaps made the subject of limited discussion at the appropriate point in this bulletin. It is believed, however, that, regardless of such complications, a practical and efficient standardization of melting points that will meet the present needs of the Pharmacopœia will be accomplished by the adoption of official methods with carefully defined procedure.

In selecting and applying the method recommended in this bulletin, the specifications suggested by the committee of revision—which substantially called for a method combining the utmost degree of simplicity, general availability, and economy consistent with the desired efficiency—have been constantly kept in mind. The method evolved by consideration of these qualifications permits of no claim to originality in its design. Fundamentally it is an adaptation of the simplest method in common use. In essential details the apparatus has already been recommended to the committee of revision and has been officially adopted by at least two foreign pharmacopœias. In the

course of this work, however, the belief has firmly developed that while one form of apparatus may be superior to another for obtaining theoretical accuracy in melting-point determinations, the essential feature of any method for the purpose of standardization is uniformity of practice. The means, therefore, by which it is hoped standardization of United States Pharmacopœia melting points may be realized through the method here recommended consists in the requirement of uniform detailed procedure rather than in the form of apparatus used. The special procedure by means of which the final results herewith reported have been obtained will be clearly outlined in later discussion.

It is perhaps truismatic, if not premature, to suggest that should the committee consider favorably the method here recommended the final results herewith submitted, as in determining any acceptable standard, should be duplicated by as many investigators as possible, working independently and according to the same procedure, before being adopted as the official standard for the compounds represented.

While temporarily connected with this laboratory, Dr. B. F. Lovelace, professor of chemistry at the University of Alabama, was for a short time an able associate in this work during the period of orientation, and a good share of credit is due to his initiative for whatever value may be ascribed to the work represented by this bulletin.

PLAN OF PROCEDURE.

In undertaking this investigation it was of course necessary to gain, if possible, a clear conception of the nature of the problems involved. To that end the United States Pharmacopœia was carefully consulted for the selection of such compounds as were considered to possibly need investigation of their melting points or boiling points (including both solids and liquids). These compounds (about 300), together with all essential data, bearing (in the case of solids) not only upon melting points but also upon their general behavior toward heat, were listed upon cards, each compound upon a separate card. Similar data from all other conveniently available pharmacopœias (including the German, the French, the British Codex, the Austrian, and the Swiss pharmacopœias), and from the more important sources in the chemical and pharmaceutical literature (principally Richter, Beilstein, and Schmidt for organic compounds; Moissan and Schmidt for inorganic compounds) were subsequently added to these cards in such a manner as to make for convenient and effective comparative study. The following copy of one of the cards will serve to illustrate the plan and arrangement and its efficiency in emphasizing important facts bearing upon the problem in hand:

Compound.	Pharmacopœia.	Data.
Acetanilidum (C_8H_9NO) ..	United States.....	Melting point, 113°. Boiling point, 295° (without decomposition); upon ignition, consumed without residue.
	German	Melting point, 113°; boiling point, 295°; 0.1 gram should leave no residue on ignition.
	French	Melting point, 113° to colorless liquid; boiling point, 295°.
	British Pharmacopœial Codex.	Melting point, 113.5°.
	Austrian.....	Melting point, 113°–114°; boiling point, 295°
	Swiss	Melting point, 113–114°; boiling point 294°–295°.
	Literature:	
	Richter.....	Melting point, 115°–116°; boiling point 303.8°.
	Beilstein	Melting point, 112°; boiling point, 303.8°.
	Schmidt.....	Melting point, 113°–114°; boiling point, 295°.
	Meyer und Jacobson...	Melting point, 115°; boiling point, 304° (stable).

The result of a careful study of this fairly comprehensive compilation of data, and of other features of the literature in its application to melting points, was to emphasize the need and importance of the investigation, as well as to aid in giving direction to the work needed.

The cards thus completed (except that with some of the less important compounds data from the literature was not included) were divided into groups according to their supposed importance and in order to facilitate progress in the work.

The first group included 37 of the more important organic compounds which seemed to offer no complication—such as water of crystallization or other minor difficulty—to rapid investigation of their melting points, and constitutes the only group with which this bulletin is specifically concerned. Subsequent experimental work on the compounds of this group, however, disclosed the fact that decomposition in connection with melting may constitute a very serious complication to the standardization of the melting points, a complication which will be made the subject of a more complete discussion in another part of the bulletin. As a result of this development the list of compounds for immediate final investigation was reduced to 24. This list includes the following compounds:

Acetanilidum.
Acetphenetidinum (phenacetine).
Acidum benzoicum.
Acidum camphoricum.
Acidum salicylicum.
Antipyrina.
Atropina.
Betanaphthol.
Camphora monobromata.
Chloral formamidum.
Cocaina.
Homatropinæ hydrobromidum.
Hyoscyaminæ hydrobromidum.
Naphthalenum.
Phenylis salicylas.
Piperina.
Pyrogallol.
Resorcinol.
Salicinum.
Santoninum.
Sulphonethylmethanum.
Sulphonmethanum.
Thymol.
Vanillinum.

The other 13 compounds originally included in the first group consist of the following:

Acidum tartaricum.
Aconitina.
Ammonii benzoas.
Apomorphinæ hydrochloridum.
Benzosulphinidum.
Camphora.
Cocainæ hydrochloridum.
Hexamethylenamina.
Hydrastina.
Hydrastininæ hydrochloridum.
Iodoformum.
Saccharum lactis.
Strychnina.

Of these compounds all except camphor were considered to show a greater or less degree of decomposition on melting, and therefore to need special investigation, as suggested, by extensive experimentation with ammonium benzoate and others. Camphor was placed in another group because of its inadaptation to investigation by a capillary-tube method.

NEED FOR INVESTIGATION AND STANDARDIZATION.

As derived from the mode of attack just outlined, probably the most significant fact indicating the necessity for an investigation of melting points leading to the standardization of methods is the divergence that is frequently found between the values published in different sources as the melting point of the same product.

The comparative data on the melting point of acetanilide, already given, will serve in some measure to illustrate this fact. It is further indicated and emphasized by the following additional data as obtained from cards of several other compounds included in the original first group of 37:

Source of data.	Melting point of cocaine hydrochloride.	Melting point of pyrogallol.	Melting point of iodoform.	Melting point of resorcinal.
Pharmacopœia:	*Degrees.*	*Degrees.*	*Degrees.*	*Degrees.*
United States	189.9	132	115	109–111
German	183	131–132	120	110–111
French	186	133	119	119
British Pharmacopœial Codex	180–186	131	115	119
Austrian	183	130	.....	110–111
Swiss	182	131–133	120	110–111
Literature:				
Richter	.....	132.5–133.5	119	110 119
Beilstein	181.5 [a] 186	132.5–133.5	119	110 119
Schmidt	186	131–132	119	118 [b] 111–112
Hager	181.5–200	.....	.....	.....

[a] Decomp's. [b] Impure.

Many examples of such divergence might be cited, and though doubtless all would not show as great a degree of variation as is found in the examples here given, they would still be significant in illus-

tration of the need of a careful investigation and standardization of melting points.[a]

It might be contended with apparent logic that a consideration of such comparative data has no direct bearing upon the melting-point requirements of the United States Pharmacopœia, in that different standards are therein represented—the values found in the literature representing, presumably, perfectly pure compounds, while the different pharmacopœias might readily represent standards differing from each other. Such a contention would doubtless be a sound one providing the builders of the different pharmacopœias did actually and experimentally establish their respective standards. But the evidence is reasonably conclusive, at least as applied to many synthetic products, that the different pharmacopœias have transcribed requirements not only from each other but also from the scientific literature, both as to numerical values and text, thereby blending or ignoring the supposed differences in standards. Such a contention is further controverted by the fact that the melting-point values published in the scientific literature are frequently lower than the standards estab-

[a] While proof reading was in progress on the galley sheets of this bulletin Dr. Atherton Seidell, of this laboratory, received a sample of diaspirin from Prof. W. A. Puckner, of Chicago, with request that a report upon the melting point of the sample be submitted to him from this laboratory, and with the statement that "according to the manufacturer it melts at 178 degrees." In his communication Professor Puckner also noted the fact that different individuals in his laboratory and at the University of Chicago, working independently, obtained different results, none of which agreed with the manufacturer's claim. Determinations in this laboratory by different individuals, using the same apparatus, but differing in details of procedure, yielded results which neither agreed with each other nor with those reported by Professor Puckner nor with the manufacturer's claim.

All the results at hand obtained from this sample of diaspirin are given in the following tabulation. The determinations were all made with standardized thermometers and readings corrected for emergent stem:

Source of data.	Number of individual determination.	Melting point or melting interval.	Remarks.
		Degrees.	
Manufacturer		178	
Chemical Laboratory, American Medical Association, Chicago.	2	164	Double bath used; rate: 5 degrees per minute, and 2 degrees for last 10 degrees. M. p. = 1st sign of melting; correction according to Rimbach table (refer this Bul., p. 67).
Chicago University	2	171	Rate: 3 degrees to 4 degrees per minute.
	1	169	Rate: 2 degrees per minute.
Hygienic Laboratory	[a] 1	176.5–178.5	Rate: not regulated (rough determination).
	1	170.5–172.5	Rate: not regulated.
	[b] 2	169.2–170.5	Rate: 3 degrees per minute for 20 degrees below melting and 0.5 degrees per minute during melting.

[a] & [b]—results by different investigators.

Professor Puckner reports upon another sample for which results obtained in his laboratory differed from the melting point claimed by the manufacturer by as much as 15 degrees.

This brief report of the incident is incorporated in this bulletin, with the kind permission of Professor Puckner, because it offers a still further striking, and up to date, illustration of the urgent need of a standard method and procedure for melting-point determinations—especially of pharmacopœial compounds.

lished for the same compounds by some of the pharmacopœias. It is, moreover, highly probable that for most of the synthetic products the difference between " c. p." and " pharmacopœial " standards, from the standpoint of purity, is not of practical significance in the standardization of melting points.

And, furthermore, among those concerned with any one standard there is more or less contention as to the melting-point value which should apply to a given compound in conformity with the standard in question. The protest of the manufacturers' committee and of individuals and their plea before the committee of revision for the official allowance of a definite (and rather liberal) margin above and below the present impractical melting-point requirements in the United States Pharmacopœia are but further manifestations of the condition disclosed by the comparative data submitted. The chemical and pharmaceutical literature is replete with examples that might be cited in support of these arguments. But, be the argument what it may, in the compilation and study of such comparative data the end justifies the means, in that it is considered not only to indicate the urgent need for a careful investigation of the whole subject but also to disclose the heart of the melting-point problem as it applies to the pharmacopœia, viz., the divergence in melting-point values of the same product as conscientiously determined by different investigators.

Such a condition must inevitably induce either effective protest or disregard of any official standard which does not adequately define the procedure by which such standard is to be definitely established—a requirement which, in this instance, necessitates the determination and, as far as is practically possible, the elimination of all causes of divergence in the melting-point values of the same product as independently determined.

CAUSES OF DIVERGENCE IN MELTING-POINT VALUES.

To attempt an exhaustive discussion of this topic, in all its technical, theoretical, and mathematical details, would doubtless involve a degree of complication which would defeat the purpose here intended. For that reason it is the intention in this bulletin to avoid any deeply involved theoretical consideration of the subject of melting points, and, therefore, in this instance, to point out mainly those superficial causes of divergence which would seem to be comparatively easy of elimination, and the elimination of which would probably make possible a practical pharmacopœial standard that could be effectively established.

As a result of the conclusions derived from much experimental work in the course of this investigation, supplemented by a study of the published work of other investigators in so far as it was found to

bear upon the problem in hand, the practically significant causes of divergence in melting-point determinations may be briefly summarized as follows:

1. Varying definition of melting point.
2. The common practice of coordinating melting point and decomposition point.
3. The great variety of methods used.
4. Variable practice in the use of thermometers.
5. Varied individual manipulation.
6. Variation in physical condition and in preliminary treatment of the compound.

DISCUSSION OF INDIVIDUAL CAUSES.

I. *Varying definition of the melting point.*—The general conception of temperature constancy as a prevailing condition in the melting phenomenon of a pure substance—commonly illustrated by melting ice—would seem to preclude the possibility of varying definition of the melting point.

According to such conception, it is simply that temperature (point) at which the substance melts. If absolute purity in chemical preparations could be realized, such an ideal might become a practical reality. Purity, however, as applied to the average chemical substance is a relative and not an absolute term. But, according to the average chemical and pharmaceutical teaching, the effect of impurity upon the melting behavior of a substance is simply to lower the melting point, and therefore the general conception of melting phenomena is unaffected by the presence of impurity. The term "melting point" itself implies such a conception.

That the present melting-point requirements of the United States Pharmacopœia are based upon some such conception as that just outlined would seem to be indicated by the fact that in the great majority of cases a single unqualified value is specified as the melting point of the compound to which it is applied. That such implied accuracy, even from the single standpoint of melting-point interpretation, is not justified by ordinary experience; that variable definition of the melting point by unrestricted independent investigators, under present conditions, is practically inevitable; and that such varying definition constitutes a real cause of divergence in melting-point determinations of the same compound, will perhaps be made clear by the following citations and discussion:

A paper recently published from the Geophysical Laboratory, Washington, D. C., by W. P. White (1), probably represents the most advanced technique and theory in melting-point determination. Although the paper deals specifically with high-temperature work

(melting point of minerals), and is for the most part far too technical to be of value in this connection, the following generalizations and conclusions are of practical significance and pertinent to this discussion:

> The preeminent value of melting ice as a temperature standard has made familiar the great constancy of the ideal melting point and its independence of external temperatures. The great majority of actual melting-point determinations, however, fail to show this ideal constancy and display *a melting interval*[a] rather than *a point*.[a]

And again:

> The recognition of the part played by impurity changes radically the ordinary conception of melting-point phenomena. Instead of a constant temperature the observer has to deal with one varying continually from beginning to end.

Finally, in his summary, White states that "the *true melting point*[a] is the *high end*[a] of the oblique *melting interval*."[a] That is, for the purpose here intended, the melting point may be defined, according to White, as that temperature (point) at which under certain conditions the substance is just completely melted.

On the other hand, Reissart ([2]), in 1890, reporting the results of a careful investigation of several melting-point methods as applied to the ordinary organic compounds, states, as one of his conclusions in discussing capillary-tube methods, that the beginning of melting is always the point to be observed. The substance of Reissert's reasoning in reaching such a conclusion is that the temperature within the capillary tube containing the sample is always lower than at the walls, hence the material in direct contact with the walls of the tube records the influence of heat before the bulk of sample within the tube has apparently been affected; and, therefore, the beginning of melting, as observed under such conditions, more closely approximates the true melting point than any other point that might be selected throughout the experiment. That is, for the purpose here intended the melting point may be defined according to Reissert as that temperature (point) at which, under certain conditions, the substance first begins to melt.

The direct comparison without careful analysis of such diametrically opposed conclusions is doubtless more or less fallacious. But many investigators accept and apply them regardless of conditions that might effectively establish each, and therefore the comparison, in application to this discussion, would seem to be justified.

Further illustration of the confusion in melting-point interpretation that prevails in practice and of the divergence in melting-point values that may consequently result may be obtained from a consideration of a paper published in 1908 by J. Bishop Tingle and H. F. Roelker ([3]).

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[a] Italics Menge's.

In this work the authors attempted, with moderate success, to utilize the melting point for the purpose of determining the percentage composition of binary mixtures of the three isomeric nitranilinis. The tabulated results of the three sets of determinations contain only a single value as the melting point of any one mixture; but the authors state, with reference to some of them, that the value given represents the mean of the temperature interval (interval not given) during which the mixture was observed to be melting; and, with reference to other values, the authors state that the mixture was not completely melted at the temperature recorded as the melting point. Regarding the remaining values, not included in the two special cases mentioned, no statement is made as to whether they represent the beginning or the end of melting, which, for mixtures, would presumably involve a more or less extended range. Disregarding the latter condition, however, we have here a third and fourth possible cause for variation in melting-point values as a result of different interpretations of the melting point.

In contrast to and including all definitions so far derived, Wegschneider ([4]), in a convincing discussion of melting-point determinations in a capillary tube, makes the contention, with which White (loc. cit.) is in accord, that in melting-point determinations it is a melting interval and not a melting point that is always observed—the contention being based upon two considerations:

1. The difference in temperature between the mercury in the thermometer and the substance in the capillary tube—the magnitude of this difference depending on the rate of heating; and

2. The fact that some degree of impurity is always present, necessarily resulting in the observation of a melting interval.

Wegschneider differs with White in that he strongly recommends that the entire melting interval be recorded, rather than any selected point, for the reason that otherwise an erroneous idea of the exactness of melting-point determinations is conveyed. In this connection, however, White (loc. cit. footnote, p. 459) states, "The assignment of any lower limit is entirely arbitrary."

In citing the apparently widely divergent opinions of these several distinguished investigators relating to the question of correct interpretation of the melting point, there has, of course, been no intention of reflecting upon the work represented. On the contrary, I have proceeded upon the assumption that the names of the investigators involved give to the work a character that is beyond my power to impugn. The only purpose here has been to bring out in striking contrast the various interpretations of melting phenomena that commonly prevail. When it is considered that the different examples given represent in each case but one of many that might be cited, the general confusion in melting-point interpretation becomes apparent.

When it is further considered that many slightly impure compounds show a melting interval of several degrees, as determined by ordinary methods, the selection in different cases of the beginning or the end or the mean of that interval as the melting point becomes a very significant cause of divergence in melting-point values; and the urgent need of a standard comprehensive definition of the so-called "melting point" as applied in the standardization of pharmacopœial compounds is made clear. The only question is, What definition should be adopted?

The results of the investigation in this laboratory, as far as completed upon the melting points of pharmacopœial compounds, have led to the conclusion that the melting-point requirements of the United States Pharmacopœia should specify a melting interval, representing the beginning and the end of melting for a product of Pharmacopœial standard. The reasons for this recommendation are mainly:

1. The fact that different makes of the same standard product frequently differ slightly in their melting points makes inconsistent and illogical the requirement of a melting *point*, while, on the other hand, the *limits* of a reasonably exact melting *interval* could be so determined as to include all products that were considered of pharmacopœial standard.

2. According to White (loc. cit., p. 460) the presence of a slight amount of impurity is very much more effective in extending the low limit of the melting interval than in lowering the "true melting point" (high limit of the melting interval); therefore, as a means of pharmacopœial standardization, the observation of a melting *interval* would be far more effective for detecting slight excess of impurity than the observation of a melting *point*.

The effective operation of such a requirement, however, necessitates a clear, unmistakable, definition of the limits—more especially the low limit—of the melting interval. Experience in this laboratory has shown that the beginning of melting of the same substance under the same conditions may be variously defined, to the extent of several degrees variation, by different individuals. It is a fact of common experience in melting point determinations—at least, by capillary-tube methods—that substances frequently show some effect of the heat—variously described as "cintering," "shrinking," "first sign," etc., several degrees before any definite melting is perceptible. In some cases this "first sign" is sharp and complete, no further change being apparent until definite melting begins. In other cases the change from first sign to first melting is gradual, making extremely difficult the exact determination of the latter point.

In connection with this investigation the observations described above induced an attempt to fix upon some definite behavior in the

region of the melting point, which was a reasonably constant characteristic of the compound under investigation; and which, whether or not theoretically correct, could be defined, for the sake of uniformity, as the low limit of the melting interval.

As the result of many determinations with this object in view it was found that, if the size of the capillary tube (about 1 mm. diameter) and the column of sample (about 3 mm. high) used for each determination is approximately the same and other details of procedure are uniform, that temperature at which any point in the column of sample is observed to collapse against the side of the tube is approximately constant for the same product, and may be defined as the beginning of melting.

The high point of the melting interval is, of course, defined as that temperature at which the last trace of sample becomes liquid.

The results reported in another part of this bulletin for those compounds whose investigation has been completed were determined in accordance with the definition of "melting point," here suggested.

In addition to the melting interval, the "first sign" is also reported but only as an approximate value. The observation of this point involves one detail in which the so-called "personal factor" can not be entirely eliminated. Its determination is further considerably affected by slight variation in conditions, such for example as light. It is therefore a detail of melting phenomena which does not readily admit of practical standardization.

II. *The Common Practice of Coördinating Melting Point and Decomposition Point.*[a]—It is perhaps quite generally recognized among chemists that when melting is accompanied by decomposition the melting point is not so sharply defined nor so easily duplicated as when no decomposition occurs. In successive determinations on such a compound, however, by the same procedure, the variation in results is not generally of significant magnitude, a fact which, in the case of the individual, would doubtless lead to the coördinate use of melting and decomposition point determinations. On the other hand, if the procedure in determinations of the decomposition point is even moderately varied, especially as to rate of heating, the resulting values for the same product are often markedly divergent. Therefore, considering the wide diversity of methods and varied manipulation that now prevail in melting-point determinations, it follows that the coördination of melting point and decomposition point would generally lead to more striking divergence in the values independently obtained on a compound which decomposes at or below the melting point than on one which does not. A reference to the com-

[a] Decomposition point as used in this bulletin indicates decomposition at or below the melting point.

parative data, already submitted (p. 14), on the melting point of cocaine hydrochloride and of iodoform will serve in some measure to illustrate this fact. Comparative data on practically all similar compounds would simply emphasize it.

That the two determinations are often considered of equivalent value as a test is indicated, in greater or less degree, by the literature, especially the pharmacopœias. In the United States Pharmacopœia the term "melting point" is often applied, without qualification, to a compound which decomposes on melting. In other instances the only qualification is that the substance "melts about" the temperature quoted, and in still others the phrase "with decomposition" follows the value given as the "melting point." In all cases the data is included among the tests, and in no case is it implied that decomposition affects the procedure involved in a melting-point determination or its value as a test.

At the outset of this investigation a preliminary experiment, involving work by three different investigators, was conducted for the purpose of disclosing in some measure the effect on the melting-point values of the same compounds as a result of independent methods and procedure in the determinations. In this experiment, however, uniformity was prescribed to the extent that each manipulator should use the same thermometer for a given compound and that no preliminary treatment, such as powdering and drying, be applied to the compounds (thereby eliminating two of the common and effective causes of divergence).

A consideration of this experiment at this point is determined by the fact that one of its ultimate results was to direct especial attention to decomposition as an important phase of the melting-point problem, the compounds impartially selected for the test accidentally including several which decompose on melting.

To facilitate discussion the three investigators concerned in the experiment will be designated as A, B, and C.[a] All three investigators independently selected capillary-tube methods for the determinations. The apparatus used by A and C was identical and consisted of a simple round bottom, wide-mouth bulb of about 50 cc. capacity, concentrated sulphuric acid being used for the bath. The apparatus used by B consisted of a similar but larger bulb, filled nearly to the neck with concentrated sulphuric acid, and containing

[a] A consideration of the results obtained in this experiment should include recognition of the fact that the investigators involved are all men who have had exceptional advantages in the way of chemical training (each at a different institution) and are rated as experts in their respective fields. It seems fair to assume, therefore, that their failure to recognize the necessity and importance of uniform procedure in melting-point determinations is typical of the majority of chemists and pharmacists.

a test tube, so adjusted in the neck of the bulb that it extended to within a few millimeters of the bottom. The position of the test tube in the bulb was maintained by the weight of similar acid in the tube and by projections blown in the side of the tube so as to catch on the neck of the bulb. In this case the sample and thermometer bulb were immersed in the acid of the test tube. Standard thermometers were used—the same thermometer being used for the same compound in all cases—but no correction for emergent stem was applied. The three sets of determinations were made entirely independent of each other, and there was no discussion of results or methods until all the experiments were completed.

The compounds tested and the results obtained in each case are here tabulated:

TABLE I.—*Comparative melting points as independently determined by different investigators.*

Compound.	A.	B.	C.
	Degrees.	*Degrees.*	*Degrees*
Antipyrine	109.5–110.5	109–109.5	108.5–110
Iodoform	113 –114.5	114–116.5	115 –116
Camphoric acid	179 –183	178–181.5	176 –181
Saccharine	209 –216	211–212	200 –216
Aconitine	188 –188.5	178–180	[a] 190
Phenacetin	131 –132	131–132	130 –132
Acetanilide	110 –112.5	111–112	108.5–112.5

[a] Decomposed.

The method of heating, as applied by each experimenter, was to play a Bunsen flame directly upon the walls of the bulb containing the bath. In no case was the rate of heating definitely regulated, but subsequent discussion indicated, with practical certainty, that in this respect there was considerable variation. Each observer recorded an interval (rather than a point) as the melting point, but showed marked differences in conception of the behavior which should define that interval. A recorded the interval from the first definite formation of liquid to the point of complete fusion; C's interpretation agreed with A's as to the upper limit, but included the first definite sign of change (not necessarily involving liquid) as the beginning of melting; while B recorded the interval over which he observed the sample to be melting, but did not definitely define the beginning nor wait for complete fusion. In those cases where decomposition occurred these interpretations were more or less modified to suit the conditions.

In the light of these facts the results of the experiment offer abundant material for discussion. Their application to previous, as well as to remaining, topics of discussion is obvious. As before stated, however, the experiment is cited at this point because it illustrates

in a striking way the possible effect that may result from decomposition when associated with a melting-point determination.

Of the compounds selected for the experiment, iodoform, aconitine, and possibly to a slight extent saccharine, show decomposition on melting, but, in this instance, the results obtained for aconitine especially attracted attention. The degree of divergence between the values determined by B and those by A and C was considered far greater than could reasonably be attributed to the effect of decomposition under ordinary conditions. It was thought probable—and may have been true—that B simply made a mistake of 10° in reading the thermometer. At any rate the fact led to further investigation of the decomposition point.

In subsequent experiments with aconitine it was found that, if the rate of heating is varied or the temperature held at different points in different tests, the melting or decomposition point of this compound will vary accordingly. Although no attempt was made to determine the limits of this behavior, the results (uncorrected) obtained in a series of experiments varied from about 180° to 192.5°, indicating not only that the result reported by B may record the exact behavior observed in his experiment, but also the imperative need of uniform manipulation in determinations of this type if reasonable concordance in results is to be obtained. The evidence upon this point furnished by aconitine was corroborated by subsequent work with iodoform, cocaine hydrochloride, ammonium benzoate, and other compounds which, in greater or less degree, show decomposition at the "melting point."

From a reasonably exhaustive study of the literature it would appear that few investigators have made any special note regarding the extreme variability of the decomposition point, and none seems to have questioned its value as a test as compared with the simple melting point.

Wegscheider([4]), in the paper previously referred to, states that in the case of substances which decompose below the melting point, the temperature of melting depends upon the rate of heating. He recommends that in such cases the exact manner of heating should be given in connection with the value quoted, and concludes that even then it is difficult for independent observers to obtain concordant results.

In an abstract of a paper on "The Melting Point of Aconitine," by F. O. Taylor([5]), read before the division of pharmaceutical chemistry at the last general meeting of the American Chemical Society, it is stated that the author calls attention "to the variation in this constant as recorded by different authorities. Results of 35 determinations confirm Dunstan's results of 188.5° C. and show that

the melting point may be seriously affected by the manipulation employed."

A part of Circular No. 729 of the United States Pharmacopœia committee of revision reports recommendations offered by B. L. Murray([6]) for the correction of various United States Pharmacopœia tests. Among these recommendations is found the statement that the melting point of iodoform "is about 119° C. instead of 115° C." Nothing is said of decomposition nor of the consequent necessity for very exact procedure in order to obtain the value recommended. It is probable, however, that the form of recommendation as originally made included some recognition of decomposition as a complication in this instance, for in "Some general observations on the United States Pharmacopœia," previously made by Mr. Murray([7]), a special procedure is suggested for "preparations that can be heated only a very short time without decomposing."

In his book on Identification of Pure Organic Compounds, S. P. Mulliken ([8]), in describing his capillary-tube method for melting-point determinations, calls attention to decomposition as a factor in such determinations by the following statement:

> The melting points of a few compounds which fuse at high temperatures with decomposition and loss of water, carbon dioxide, or ammonia have been found to be sharper when observed in capillaries sealed at both ends. These melting points are, however, of very little value unless accompanied by a statement of all the dimensions of the capillary and of the quantity of substance fused, for they will be found often to vary many degrees with a change in these conditions because of differences in the gas pressure of the decomposition products.

Doubtless many other investigators have observed that decomposition constitutes some measure of complication in melting-point determinations, but have not considered it of sufficient importance to justify special attention or discussion.

The attempt in this laboratory to establish the conditions and procedure best adapted, without complicating the general method, to offset the effect of decomposition in melting-point determinations led to extended experimentation with different compounds and to many divergent values. The conclusions reached were: I. That moderate variation in manipulation will in most cases cause considerable variation in results. II. That reasonable concordance will nearly always result from the application of practically any perfectly uniform procedure.

For the purpose of pharmacopœial standardization it was considered desirable that the conditions to be prescribed for testing any special class of compounds should conform as nearly as possible to those to be required in the principal method. From this point of view a procedure for decomposition-point determinations was adopted and its efficiency tested by many determinations on several

pharmacopoeial samples of aconitine and of ammonium benzoate with results that were considered satisfactory.

Undoubtedly more detailed discussion, especially with reference to the experiments performed and the results obtained, of this phase of the melting-point problem would be considered desirable were it not for the fact that subsequent developments led to the tentative conclusion that the acceptance of the decomposition point as a constant whose determination, like that of the melting point, offers a fairly exact measure of the purity of a compound is not justified, or, at least, not without more exhaustive investigation than appears to have been given the subject. The basis for this conclusion will here be given detailed consideration.[a]

The specifications under ammonium benzoate in the Pharmacopœia contain the following statement: "The salt fuses at 193° to 194° C. (379.4° to 381.2° F), with decomposition * * *." Considered in connection with the purity rubric of 98 per cent, this statement would doubtless be generally construed to mean that if a sample of ammonium benzoate fuses, with decomposition, at 193° to 194° C., it may be considered to be 98 per cent pure, or very nearly so. It was upon this assumption that the decomposition points of four pharmacopœial samples of ammonium benzoate had already been standardized in accordance with the procedure previous referred to, the results obtained being practically concordant for the different samples and with the United States Pharmacopœia requirement, thereby indicating a purity of at least 98 per cent, which subsequent analyses confirmed. The analytical data for the different samples, however, showed a variation as great as 1 per cent, with no corresponding variation of the decomposition point. Another sample, which had been kept in a vacuum desiccator for a short time and was found upon analysis to contain only 94 per cent of ammonium benzoate, also showed a decomposition point practically identical with that of the pure material. The wide variation which frequently results in melting-point determinations of a compound of this type might reasonably be explained upon the assumption that it is due to varying degrees of impurity, introduced as a direct result of the decomposition itself, it being further assumed that decomposition is a gradually continuous, rather than a sharply defined, phenomenon; or, at least, that it begins slowly at comparatively low temperature and increases disproportionately with rising temperature until it finally becomes so rapid as to be strikingly evident. According to this reasoning, if a sample of such a compound be heated very slowly a greater degree of decomposition will result, and consequently a larger

[a] The substance of the following discussion has already been given in a paper on The Pharmacopoeial Tests for Ammonium Benzoate, by Seidell and Menge, Am. Jour. Pharm., January, 1910.

proportion of impurity will be introduced into the sample before the so-called melting point is reached than would be the case if the sample were heated rapidly, and the final observation will accordingly be lower. The results obtained with ammonium benzoate of varying purity as described above apparently controverted such an hypothesis and led to an attempt to test it.

The principal products in the partial decomposition of ammonium benzoate are doubtless the volatile compound ammonia and the solid benzoic acid. Therefore, to determine the effect of varying degrees of decomposition upon the melting point of ammonium benzoate would practically mean to determine the effect upon that point of benzoic acid in varying proportions. Upon this simple basis the problem was approached from the two extremes, ammonium benzoate and benzoic acid, two series of samples, which gradually approached each other in composition, being thus obtained.

The first series was prepared by subjecting pure ammonium benzoate to continual desiccation in the presence of sulphuric acid in a Hempel desiccator under diminished pressure (about 50 mm.), portions being removed at irregular intervals for analytical and melting-point determinations. In this way six samples were obtained, varying in composition from 98.6 per cent to 57.5 per cent ammonium benzoate. The last sample (57.5 per cent) exhausted the supply of material started with in that experiment. The second series was prepared by mechanically mixing benzoic acid and ammonium benzoate in proportions varying from 50 per cent each at the one extreme to pure benzoic acid at the other, six samples being obtained in this way.

The melting or decomposition points determined for the different samples of both series, together with the duration of desiccation required to produce the varying degrees of decomposition indicated in the first series, will be found in the following table. The samples of the first series have been designated by Roman numerals and those of the second by letters. A standard thermometer was used and the observed reading was corrected for emergent stem.

TABLE II.—*Melting points of mixtures of ammonium benzoate and benzoic acid*

Sample.	Per cent composition.		Melting or decomposition point (cor.).	Remarks.
	$C_6H_5COONH_4$.	C_6H_5COOH.		
			Degrees.	
I	99.2	Trace.	192.5–194.3	Vigorous effervescence.
I_a	99.2	Trace.	188.2–189.2	Effervescence after 5 to 7 minutes
II	99.6	Trace.	193 –194.3	Vigorous effervescence.
III	99.9	Trace.	193.1–194.8	Do.
IV	99.7	Trace.	193.8–194.8	Do.
V[a]	98.6	1.2	193.1–194.3	Do.
VI[a]	94	6.4	191.7–193.5	Do.
VII[a]	92.1	8 ±	192.9–194	Do.
VIII[a]	88.4	11.8	190.4–192.4	Do.
IX[a]	79.29	20.5±	190.7–193.3	Slight melting and effervescence.
X[a]	57.5	43	188.6–192.2	Last trace effervesces slightly.
A	49.83	50.17	187.1–192.7	Slight effervescence finally.
A_1	49.83	50.17	183.9–190.2	Heated very slowly from 175.
B	21	79	152 –177.7	Definite sign at 120–121, but n liquid until 152; no effervescenc
C	5.73	94.27	121.3–122.3–148.9	Most melts 121–122; no efferve cence.
C_1	5.73	94.27	121.3–131.5–149	C, remelted after cooling.
D	3.85	96.15	120.8–121.3–146.9	Behavior similar to C.
E	2.2	97.8	121.3–121.8	Melts without decomposition.
F		100.0	121.4–122.4	Do.

[a] Samples VII and IX represent degree of decomposition of two samples of ammoniu benzoate as a result of standing in an ordinary desiccator over sulphuric acid for abou two and one-half months. Samples V, VII, VIII, and X were obtained by subjecting sample of pure ammonium benzoate to vacuum desiccation (about 50 mm. Hg) in th presence of sulphuric acid for 6, 42, 112, and 184 hours, respectively. I_a and A_1: Th experiments I and I_a, also A and A_1, illustrate effects of different manipulations in deter mining the melting point of the same sample.

A clearer interpretation of the melting points of the mixtures a given in Table II is perhaps obtained by a consideration of th

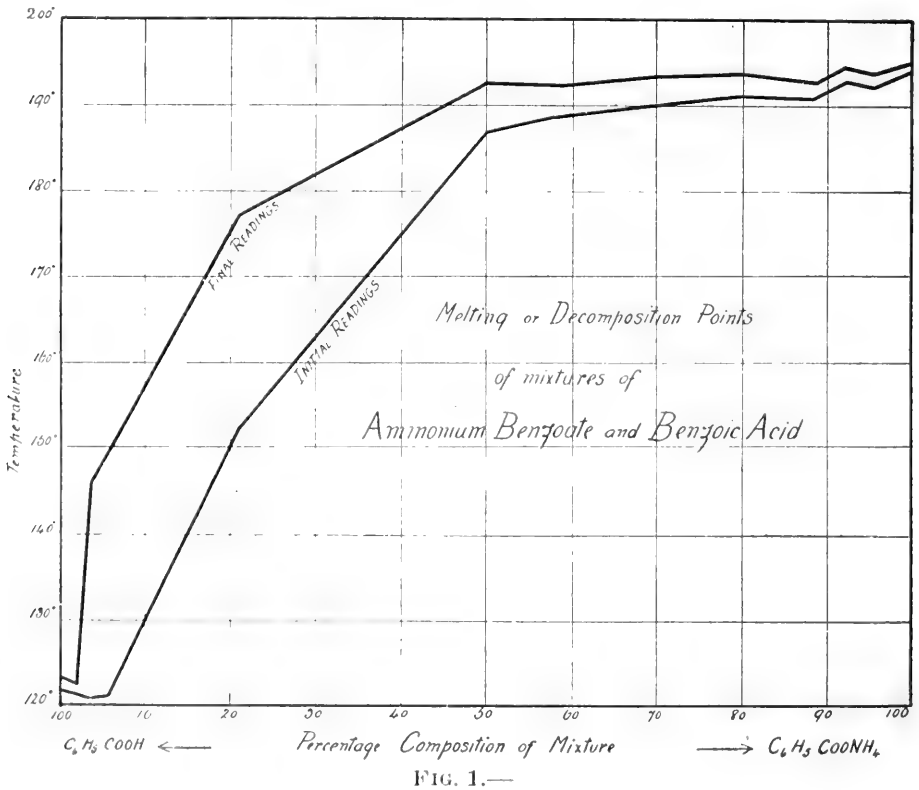

FIG. 1.—

graphic representation (fig. 1). The lower curve represents the temperatures at which the various mixtures began to melt, the

beginning of melting being determined as nearly as possible in accordance with the definition previously suggested in this bulletin. The upper curve represents the final reading, not necessarily the point of complete liquefaction, for in all cases where 80 per cent or more ammonium benzoate was present the effervescence was sufficiently vigorous to drive the material up the tube, and in those cases the final reading represents the point of vigorous effervescence. The distance between the two curves at any point represents the range over which the sample was melting or decomposing.

The curves show clearly that the variation in melting point or decomposing point between a sample of pure ammonium benzoate and a sample containing 50 per cent of benzoic acid is barely significant.

It would seem to require only casual consideration of the data submitted to lead to the very definite conclusion that a determination of the "melting point" of ammonium benzoate is quite useless as a practical test of purity—at least in the presence of benzoic acid, even to the extent of nearly 50 per cent. A further tentative conclusion is logically induced, to the effect that a melting-point determination by any of the usual methods of any compound which behaves similarly is entirely unreliable and should therefore not be required as a test in the standardization of this class of pharmacopœial compounds. The only justifiable use, if any, of the decomposition point would seem to be as a crude means of identification.

It is presumably possible that the behavior of ammonium benzoate and benzoic-acid mixtures is not typical of mixtures of other compounds with their respective decomposition products. Further investigation along these lines, however, was not considered feasible at this time. Therefore, upon the assumption that the results obtained are of general application, those compounds which show decomposition on melting were eliminated from the list selected for immediate standardization, as previously indicated (p. 14).

If the United States Pharmacopœia is to establish and maintain with dignity an effective legal standard for drugs, it would seem undesirable and unwise to include or retain among its official tests a test the utility or efficiency of which, for the purpose intended, even though not conclusively proven to be entirely inadequate, is open to serious question. From this point of view and as a result of the work with ammonium benzoate described above, it is recommended that the melting-point requirement for those compounds which show definite decomposition at or below the melting point be omitted from the pharmacopœia, or, if retained, that it be definitely described as nonofficial and of use only as a rough means of identification for that class of compounds.

III. *The great variety of methods used.*—The term "method" as applied to a melting-point determination may, perhaps, be logically

considered to include all details of operation involved in such a determination. As used in this discussion, however, it is intended to refer primarily to the form of apparatus and includes other details only when they are considered essential features of the method or are essential to a comprehensive discussion.

A brief review of some of the methods now in use may induce a clearer conception of the significance and importance of this phase of the melting-point problem. The very general practice of publishing the results of melting-point determinations without reference to the method used makes impossible the selection, for the purpose of comparative study, of only those methods which are really most commonly applied. Any method, however, which possesses any advantages whatever, doubtless finds application in greater or less degree, and therefore, in so far as diversity of methods may be responsible, contributes its share to the existing divergence in melting-point values.

Although the methods described and, whenever possible, illustrated in the following paragraphs, probably do not include all that are in common use, they are considered to fully serve the purpose of illustrating a rather remarkable diversity of means to a common end; and they include most of the methods that could be found described in the conveniently available literature.

For the simple purpose of observing some degree of order in the consideration of the methods here included, they have been roughly divided into three groups, as follows: 1, Capillary-tube methods; 2, electrical or acoustical methods; and 3, miscellaneous methods.

1. Capillary-tube methods.—Undoubtedly some form of a capillary-tube method is the means most commonly employed for melting-point determinations. The type, however, admits of great diversity of detail in form of apparatus and in manner of application—as, for example, whether metal or glass, single or double, container for a liquid or an air bath, or for an air bath within a liquid bath, etc. These differences suggest a further natural subdivision of capillary-tube methods into two classes, as follows:

Class *a*. Methods in which the capillary tube is immersed in an air bath.

Class *b*. Methods in which the capillary tube is immersed in a liquid bath.

The methods included in class *a* will first be considered:

The method of Anschutz and Schultz (9), described and illustrated by the authors in 1877, probably represents one of the oldest special forms of capillary-tube methods now in common use. It consists of a round-bottom distilling flask (A) with a long neck (a flat-bottom flask may be used). Fused to the lip of the neck of the flask is a long moderately wide test tube (B), the bottom of which reaches very nearly to the bottom of the flask. The bottom of the test tube may be

lined with a layer of asbestos or not, as desired. At the side of the neck in the top of the bulb is a tubulure (C), through which the bath is introduced (concentrated sulphuric acid or molten paraffin) in sufficient quantity to half fill the flask. The apparatus is heated over a wire gauze. When it is in use the tubulure carries a tube (D), ground to fit, which contains a very small bore connecting the inner part of the flask with the air. At other times the tube D is replaced by a calcium-chloride tube (E) to prevent gradual dilution of the acid by absorption of atmospheric moisture. The capillary tube containing the sample is attached to a thermometer and lowered to position in the test tube, which serves as an air bath. The advantages claimed for the method are that excessive fuming at high temperature is practically eliminated; the bath gives no trouble from contamination and lasts without change for a long time; no stirring is needed; if the thermometer is not too long a correction for emergent stem need not be applied, since the air bath in which the thermometer is immersed is practically entirely surrounded by the vapor of the acid or oil bath; and, finally, it does not need constant watching. The accompanying illustration (fig. 2) is a copy of that published with the original description. Different investigators claim that the results obtained by this method are too high, a claim that is commonly made of methods which involve the use of an air bath as the ultimate means of heating the sample. Objection to it as a method for official adoption by the United States Pharmacopœia may be based, aside from other considerations, on the ground that the apparatus is practically impossible of construction to the average individual, starting with a flask and test tube, and if purchased as such would probably be too costly.

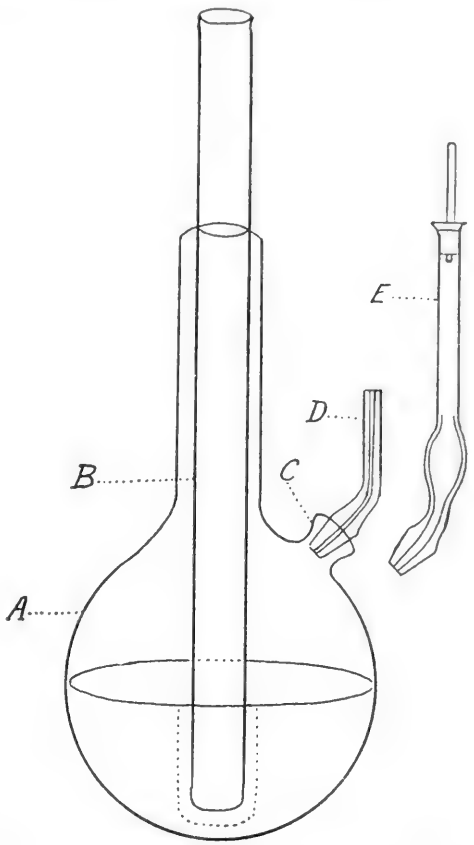

FIG. 2.—Apparatus of Anschutz and Schultz.

The Roth apparatus ([10]), as proposed by C. F. Roth in 1886, and as shown in the accompanying copy of his illustration (fig. 3), is a slight modification of the Anschutz and Schultz apparatus. It was devised primarily to eliminate the necessity for emergent stem correction and thus to insure some degree of uniformity in the melting-point values independently obtained for the same compound.

In contrast to Anschutz and Schultz, Roth carefully specifies the dimensions of his apparatus, the bulb (a), the neck (b), and the inner tube (c) being respectively 65, 28, and 15 millimeters in diameter, and the total length 200 millimeters. The tubulure (11 millimeters wide), grooved part way at d, is fitted with a glass stopper which is perforated so that one opening is at the top and the other at the side as shown (d and e), the bath being connected with the air only when the stopper is so adjusted that the opening in the side communicates with the groove in the tubulure. The inner tube, of course, serves as an air bath. Pure concentrated sulphuric acid is used as the liquid bath and fills the outer vessel to a point about halfway up the neck (f), thereby insuring a greater uniformity of temperature in all parts of the system than is the case with the Anschutz and Schultz method.

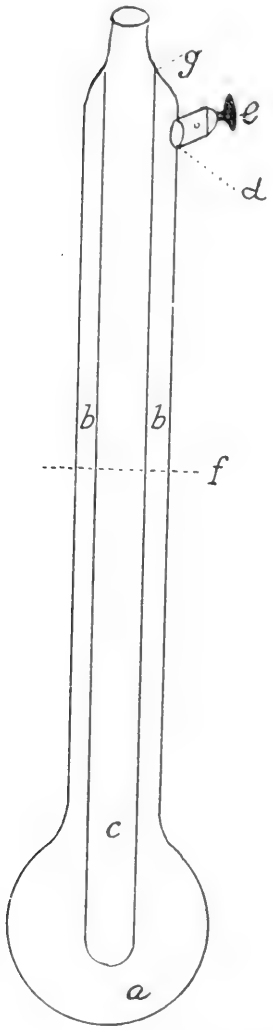

FIG. 3.—The Roth apparatus.

The method was tested on six substances whose melting points ranged from 123.5° to 234.5°. The results directly obtained were compared with results as correctly determined in the usual way (thermometer immersed to –10° mark directly in acid bath and corrected for emergent stem). The divergence ranged from 0° to 0.6°.

The objections to this method are practically the same as apply to that of Anschutz and Schultz.

A dissertation by G. Roster in 1879, as reported by H. Schiff ([11]), contains a brief and inadequate description of a special method used by the former for melting-point determinations. The Roster apparatus, as described by Schiff, is a small double air bath, comprising two concentric brass tubes fastened together, and containing glass windows in such relation as to permit of the necessary observation during an experiment. The substance for investigation is suitably attached to the thermometer bulb and placed in position in the bath, the whole system being finally arranged in a horizontal position. The report contains no further discussion of the method and no more detailed description could be found.

In 1890 D. B. Dott ([12]) described a method which he claims gives good results with compounds of pharmacopœial standard, especially those melting at high temperature. The Dott apparatus is a small simple air bath, consisting of a copper vessel closed at one end and fitted with glass windows front and back. Two brass wires

through the sides support a piece of sheet asbestos. The sample to be investigated, placed in a "thin glass tube" (presumably capillary), is attached to a thermometer which carries a cork fitting the open end of the copper vessel, and lowered to position well above the asbestos sheet. The original description included no illustration of the apparatus.

The device of G. M. Beringer([13]), described in 1891, is essentially the same as the Dott apparatus, but has, at least, the great advantage of being more readily available and of easier construction. The windowed, copper vessel recommended by Dott is displaced in the Beringer method by a "tall, plain beaker," "a crystallizing beaker with ground edge" preferred. The beaker is covered by a circular piece of glass of somewhat larger diameter than the beaker and through the center of which a hole three-fourths to 1 inch in diameter has been drilled. The hole in the center of the cover glass is fitted with a cork, perforated for carrying the thermometer. A "small glass tube," drawn out and cut off at the fine end at an angle of 45°, is used for carrying the sample, and is tied by means of a thread to the thermometer so that the small end does not extend below the lower limit of the thermometer bulb. The thermometer is then adjusted to such a position in the beaker as to bring the bulb and sample in about the center of the bath. Beringer claims that his method yields uniform and correct results, and also claims for his apparatus the following advantages over that proposed by Dott: that "being entirely of glass there is no unequal absorption of heat by certain parts; there is an entire absence of currents of air, and there is an unobstructed view of all sides of the tube, so that observations as to change of color, shriveling of the mass, charring, etc., which are especially desirable in certain organic bodies, as, for example, alkaloids, can be easily made." No illustration of the apparatus accompanies the description.

Class *b*, Methods in which the capillary tube is immersed in a liquid bath: A method of this type, in very general use is the so-called "Græbe method." In connection with a paper on tetrachlorphtalic acid, C. Græbe([14]) briefly describes the method as consisting of a bulb into which a test tube is inserted, both bulb and test tube being filled with sulphuric acid. The principal feature of the method as applied by Græbe was the complete immersion, in the acid bath, of the mercury thread of the thermometer used. For the convenient application of this feature at high temperatures, Græbe made use of several thermometers, the graduations on which began at different temperatures ranging from about 100° C. to about 300° C.

As applied in different laboratories and by different individuals, this method is variously modified—most generally by ignoring the "principal feature" described above. One modification is illustrated

in the method used by the investigator B in the preliminary experiment described on p. 22. The cut (fig. 4) is an illustration of the Græbe method as applied by Tyrer and Levy.[25]

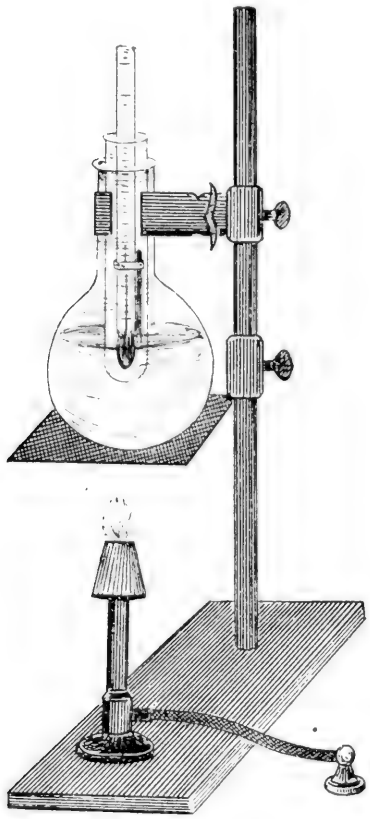

FIG. 4.—A form of the Græbe apparatus (after Tyrer and Levy).

This method is undoubtedly a good one for general use, but there does not seem to be any special and practical advantage in the double bath.

A method that may be considered a special modification of the Græbe method is described and illustrated (fig. 5) by S. P. Mulliken[15]. The modification consists essentially in the use of a cork for the inner tube and in the use of a special bath at high temperatures (above 200° C.). The special bath will be given further consideration in later discussion.

The Mulliken apparatus is described as a round-bottomed flask, with a bulb 65 mm. in diameter and a neck 75 mm. long and 20 mm. in diameter. The capacity of the flask is about 200 cc. The inner tube (A) is a test tube 15 mm. in diameter and is freely suspended in the flask, the lip of the tube resting upon the mouth of the flask. As in the Græbe method, both the inner tube and flask are filled with the bath, in this instance to the level B. With reference to the inner bath the author states that it is "a very bad practice to use the test tube as an air bath." Specific dimensions are given for the capillary tubes to be used and a method for making and loading them described. The capillary tube is fastened to the thermometer by means of a rubber band (clipped from a piece of small rubber tubing) or, at high temperature, by a spiral of platinum wire, in such position that the sample is centrally located at the side of the thermometer bulb.

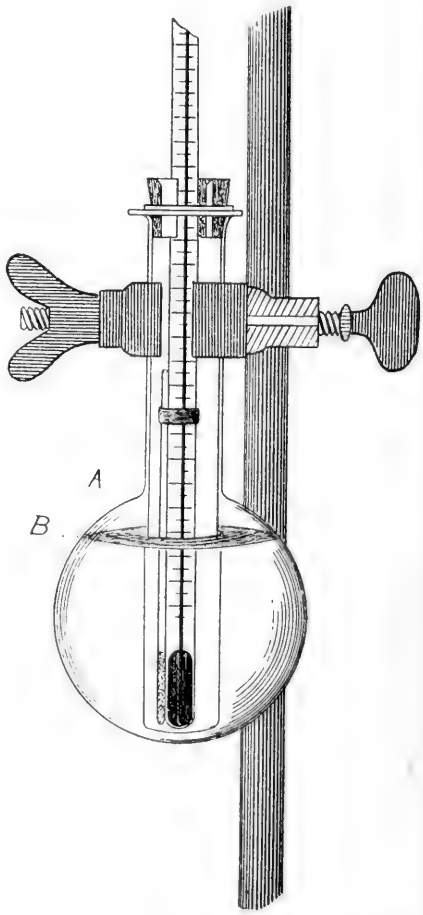

FIG. 5.—The Mulliken apparatus.

This method appears to be an excellent one, but is not considered to go far enough in refinement of detail to insure the highest degree of efficiency for the purpose of standardization of pharmacopœial compounds.

In a contribution "On melting point," by Edmund J. Mills ([16]), published in 1881, the author describes and illustrates a method for melting-point determinations, which he devised for the purpose of insuring a steady rise in temperature and very exact results in a comparatively simple apparatus. The accompanying illustration (fig. 6) is a copy of that published by the author. The apparatus is described by the author as follows:

> The apparatus, of which an engraving, on a scale of one-fourth,[a] is given herewith consists of a bath nearly filled with oil of vitriol. In this is inserted a glass funnel, having on its lower edge six equidistant semicircular cuts of about 5 mm. radius, and, at the end of the neck, four of the same. A thin test tube, resting freely on the funnel, contains a bath of paraffin oil in which the thermometer's bulb is centrally placed; against the bulb, in a little tube separately represented, is fixed the substance whose melting point is to be determined. When the large bath is heated, constrained and regular convection takes place in the liquid; the effect upon the thermometer is such as to cause the mercury to rise with very great steadiness.

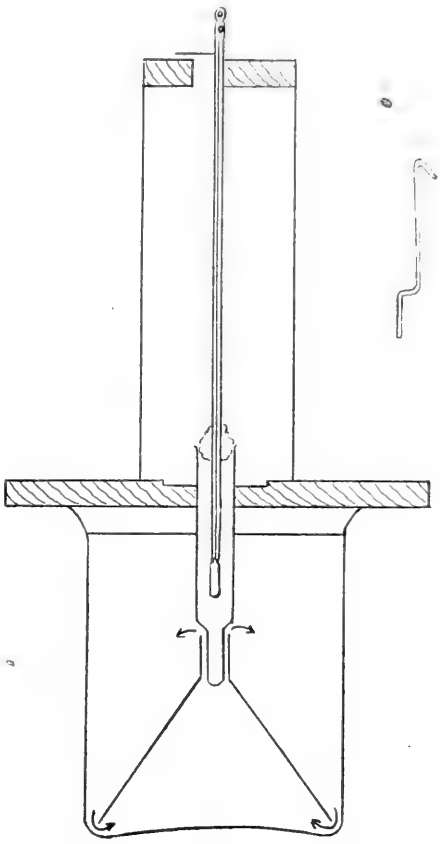

FIG. 6.—Mill's apparatus.

In an article on "An improved melting-point apparatus," by F. W. Streatfeild and J. Davies, ([17]) published in 1901, the authors assume that the customary method for melting-point determinations above 100° C. involves the use of "the well-known apparatus consisting of a thermometer suspended in a beaker of concentrated sulphuric acid and provided with a glass stirrer." For such a method they propose an additional detail of apparatus designed to eliminate the following drawbacks to the original methods: "1. Annoyance from acid fumes when working at a high temperature. 2. Danger of projection of hot acid, especially if the acid should accidentally boil. 3. Absorption of atmospheric moisture and consequent weakening of the acid when the apparatus is not in use." As shown in the accompanying copy of the

[a] The portion above the cover of the bath is not to scale.

author's illustration (fig. 7), the proposed device consists simply of a "light dome-shaped glass cover" which rests freely on the rim of the beaker, and is provided with two narrow openings, one centrally located, through which hangs the thermometer, and the other at the side, to permit manipulation of the stirrer. When attaching the capillary tube to the thermometer—accomplished by the capillarity resulting from contact of the acid between the capillary tube and thermometer—the glass cover "is raised by sliding it up the stem of the thermometer."

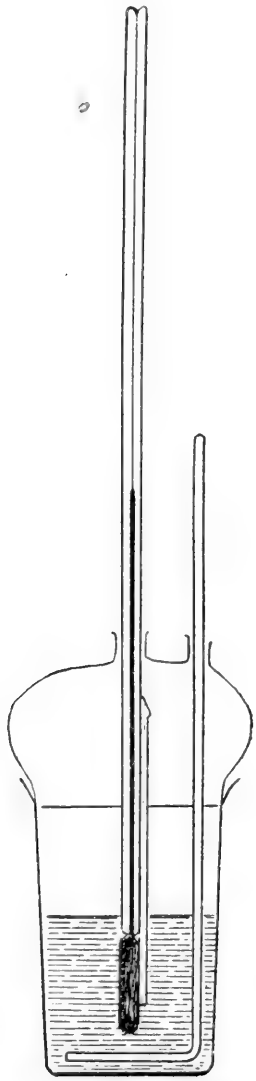

FIG. 7. The Streatfeild and Davies dome cover.

A simpler form of perforated glass cover for a melting-point apparatus has been tried in this laboratory for the purpose of condensing acid fumes from the bath at high temperature, but was considered unsatisfactory.

In a method for pharmacopœial compounds, proposed by S. J. Lewis ([18]) in 1904, much more attention is given to details of procedure than to the form of apparatus used. The method involves the use of a tall glass beaker of 400 or 500 cc. capacity, two-thirds filled with the bath (which may be water, castor oil, paraffin, or other suitable substance). The use of a standard thermometer is recommended and specific dimensions for the capillary tube are given. The thermometer and capillary are lowered into the bath until the lower end is about 3 cm. above the bottom of the beaker. Approximate rates of heating for different stages of an experiment and continuous stirring are prescribed. More or less definite directions for the preliminary treatment of compounds are also given. The method is not illustrated. Although his method is believed to permit of practical improvement in many respects, Lewis's contribution to the subject of the melting points of pharmacopœial compounds is considered to be far more rational and practical for the purpose intended than is commonly found in the literature.

A special form of apparatus for melting-point determinations by a capillary-tube method was proposed in 1907 by J. Theile([19]). The special feature of the apparatus involves only the bath container, which is designed to insure a better circulation of the acid, and therefore a more uniform rise in temperature, than obtains in the forms of apparatus commonly employed. It consists of a glass tube 2 cm. in diameter and 12 cm. long, containing at the side a glass loop 1 cm.

in diameter connecting the bottom of the main tube with the center, as indicated by the accompanying illustration (fig. 8). When in use the tube contains a sufficient quantity of the bath to just completely cover the upper opening of the loop, and the thermometer and capillary are so adjusted in the main tube that the bulb and sample are located midway between the upper and lower openings of the loop. The bath is heated by direct application of the flame to the part of the loop farthest from the main tube, thereby inducing in the latter a circulation of the acid bath from above downward and consequently causing the first sign of melting to appear at the top of the sample, a reversal of the usual process, resulting, it is claimed, in more satisfactory observation of the end point in the experiment.

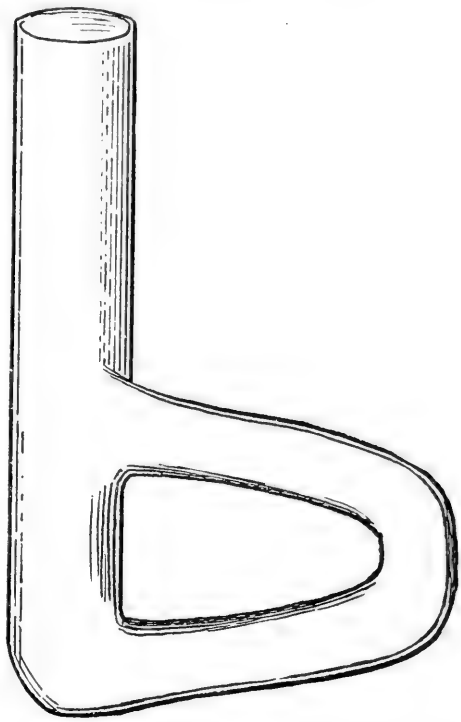

FIG. 8.—The Theile apparatus.

An abstract consideration of the advantages claimed for this tube would not seem to justify the additional cost of a very special form of apparatus for pharmacopœial purposes.

A very extended elaboration of the principle involved in the Theile method is found in a method recently described and illustrated (as in fig. 9) by H. Stoltzenberg([20]). The Stoltzenberg apparatus—"in principle a small Mammut pump"—is of two forms (A and B in the figure), one (A) being adapted for ordinary melting-point determinations involving the addition of heat, and the other (B) being so arranged that the coil only may be immersed in a suitable freezing mixture for melting-point determinations at low temperature (extending to −60° C.). In the latter case the "observation tube," containing the thermometer and capillary sample, must be frequently moistened with alcohol to prevent the tube becoming frost coated from atmospheric moisture. The arrow points to connection with a Kipp generator, which delivers a current of dry gas (carbon dioxide) into the bath at the base of the coil. The gas flow should be regular and sufficiently rapid to force some of the liquid before it. The liquid thus displaced flows back into the "observation tube" through the sloping tube which connects the top of the coil with the main tube, while the gas escapes through the bulb. A rapid circulation of the bath and consequently a rapid and uniform change in temperature

is thus induced. As indicated in the figure, the form A is heated by means of a Bunsen flame centrally located under the "heating coil," the size of the flame being regulated by a Hoffman clamp. The adjustment of the thermometer and capillary tube are clearly shown. The apparatus can also be used for solubility determinations. A more complete description will be found in the original article.

For melting-point determinations the method is doubtless an excellent one, but is obviously too highly specialized for adaptation to present pharmacopœial needs.

The so-called "Piccard method" is simply a detail of apparatus devised by Piccard ([21]) in 1875, not as a part of any particular

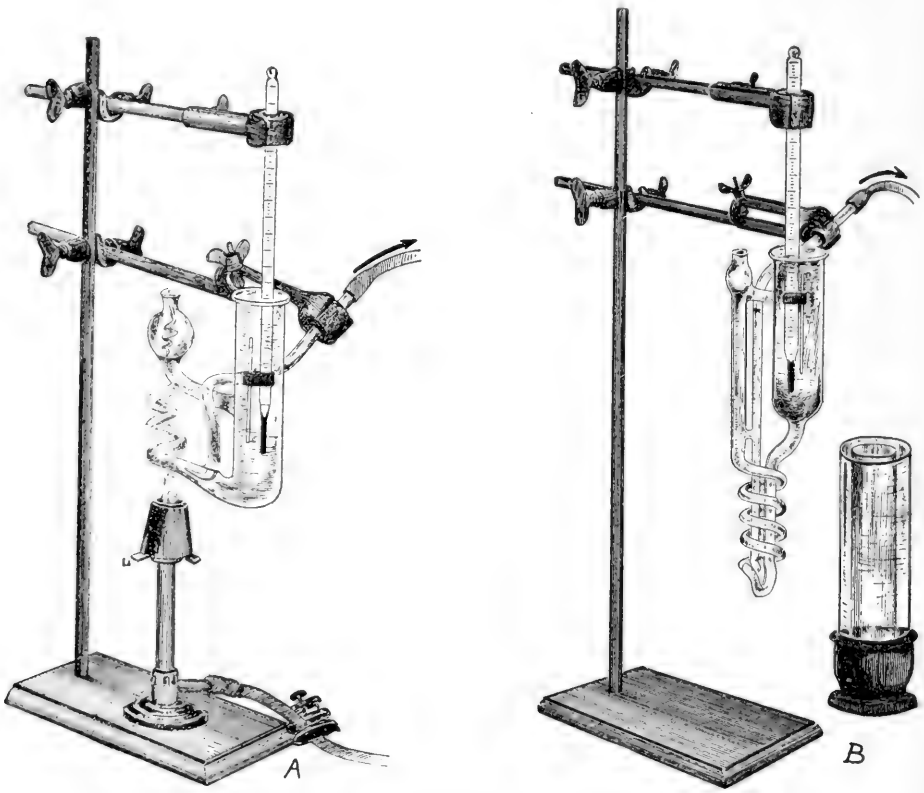

FIG. 9.—The Stoltzenberg apparatus.

method but for application to any capillary-tube method. It consists of a special form of capillary tube, illustrated by figure 10 (after Piccard), so constructed that one end of the tube constitutes an air chamber (b), sealed at the upper end. The substance to be melted is introduced into the capillary in sufficient quantity to seal the lower end of the air chamber where it tapers toward the capillary (a). On application of heat, the confined air in b expands, causing pressure on the sample at a, which, being in a conical part of the tube, thereby becomes only the more firmly impacted until complete fusion occurs, when it is suddenly forced up the tube. In certain special cases it is desirable to introduce a drop of mercury (c) below the sample. The advantage claimed for the tube consists in the conspicu-

ous behavior at the melting point, rendering observation easy even through a more or less contaminated bath, thereby eliminating the necessity of frequently renewing the bath and also relieving the strain upon the eyes which results from many consecutive determinations with ordinary capillary tubes. The devise is claimed by different investigators to consistently cause high and variable results.

Of the various capillary-tube methods so far considered, by no means including all, it is highly probable that none—and still more probable that no other type of method—finds more general application than the simplest form, in greater or less degree of modification. Such a method is perhaps illustrated, as nearly as may be, in the description of the method applied by the investigators A and C in the preliminary experiment described on page 22; that is, it involves the use of an ordinary bulb (or flask, or beaker, according to the fancy of the individual investigator), partly filled with sulphuric acid, into which a thermometer, carrying a capillary tube containing the sample, is immersed to varying depths, and to which heat is applied, generally by direct application of a Bunsen flame, to the acid container.

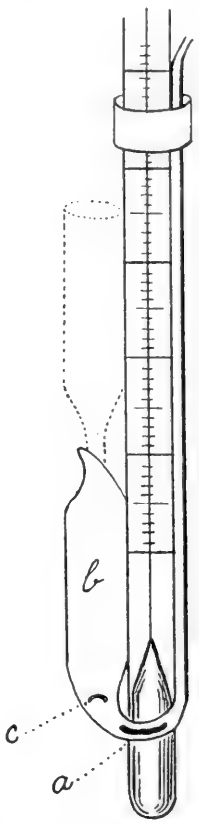

FIG. 10.—The Piccard capillary tube.

In the unregulated application of such a method—as, in fact, of most of the capillary-tube methods—by different investigators, very wide variation in the details of apparatus and manipulation is practically inevitable. The thermometer used in different cases may vary greatly in construction and in accuracy (the latter quality being often unknown); a correction to the observed melting-point values for that portion of the thermometer which extends above the bath ("emergent stem") may or may not be applied (usually is not); the capillary tube may have any dimensions that could be variously interpreted as included within the limits of such a specification; in accordance with or independent of the dimensions of the capillary tube, the amount of the sample may vary, as may also its physical condition, the factor of rate of heating, of stirring, of melting-point interpretation, etc. All are conditions which apply to most capillary-tube methods in their present degree of refinement, and which must seriously affect the values resulting from the use of such methods. Doubtless certain accidental permutations and combinations of these conditions among different investigators would result in concordant values. Other accidental combinations would probably be more or

less compensating in their effect. Still others, however, might be cumulative. And yet the published melting-point values, as a general rule, include no reference whatever to any of the conditions that prevailed in their determination. It is not surprising, therefore, that different investigators—appreciating some or all of the defects pertaining to established methods—have attempted to devise a method for melting-point determinations which would insure more reliable and concordant values than the "ordinary capillary-tube method."

2. Electrical or acoustical methods.—It is quite probable that some form of electrical method for ordinary melting-point determinations could, with less inconvenience, be made more independent of external conditions and of the so-called "personal factor" than would be possible for the usual capillary-tube method. For example, with a well-controlled electric current as the source of heat, within the field of which the thermometer and charge are completely contained, a more perfectly regulated and uniform rise in temperature could doubtless be insured than would probably be possible or practical by any arrangement involving a flame. This and other features of the excellent "high-temperature work" now being done is suggestive of the refinement of method that may be practically possible for the more common melting-point work.

The increased efficiency of electrical methods, compared with others, as at present applied to melting-point determinations of ordinary organic compounds, seems to be limited to the elimination of the "personal factor" in the actual observation of the melting phenomenon. In all cases the melting point is mechanically indicated. The apparatus is so arranged that the substance to be melted closes the circuit of a battery when melting occurs. For that purpose the material is adjusted between terminals of the battery in a mercury bath, which also contains the thermometer bulb. At some convenient point the battery circuit includes a bell or other sounding device. When heat is applied the melting of the sample causes direct metallic contact between the terminals of the battery by means of the mercury, thus closing the circuit and causing the bell to ring. At the first sound of the bell the temperature indicated by the thermometer dipping into the mercury bath is recorded as the melting point of the substance under investigation.

Four such methods are here considered. The first was devised by J. Löwe ([22]) in 1871. It is described and illustrated (fig. 11) by the originator as consisting of a four-sided cast-iron vessel (A) 17 centimeters long, 11 centimeters wide, and 7½ centimenters high, which serves as a water or an oil bath according as the temperature desired is below or above 100° C. A cover of the same material contains in the center a well, 4½ centimeters deep and 2½ centimenters in diameter. The cover also contains a small opening near the edge for the escape

of vapors from the bath. The well in the cover serves as a container for a mercury bath, into which is immersed the bulb of an accurate thermometer (a), which is surrounded by a glass tube, open at both ends, to prevent cooling. A moderately thick, pointed, platinum wire (b) is sealed into a glass holder with sealing wax so that the ends project. This wire thus arranged is adjusted in the apparatus so that the lower end may dip into the mercury bath while the upper end is connected by a copper wire to the zinc pole of a battery cell. A second platinum wire (c) dips into the mercury bath and is connected by means of a copper wire with an electro-magnetic bell, which in turn is likewise connected with the other pole of the battery. Thus

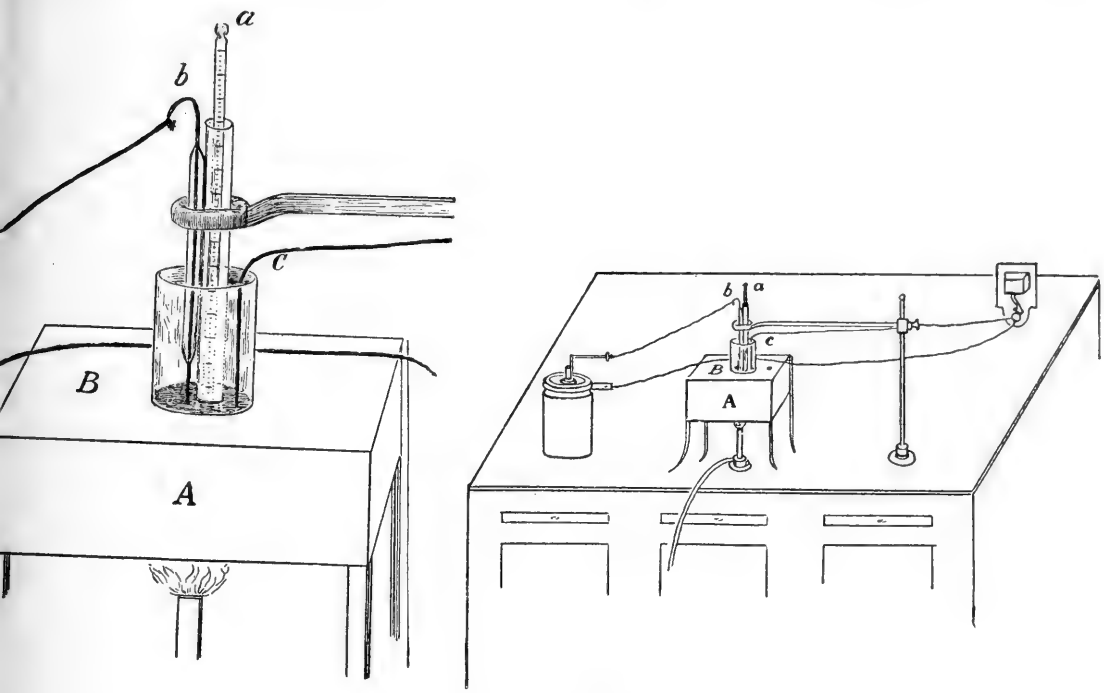

FIG. 11.—Löwe's method.

arranged, if both bare platinum wires come in simultaneous contact with the mercury bath, the circuit is closed and the bell rings.

To make a melting-point determination by this method, the apparatus is adjusted as described above. The point of the platinum wire (b) is then cleaned by ignition and coated with the substance to be investigated by dipping it two or three times into a molten sample. It is then so adjusted in the apparatus that the coated point dips into the mercury bath. The circuit remains broken, however, because of the insulating surface of substance covering the wire. The bath is then heated until the substance melts from the wire, thereby establishing connection between the wire and the mercury, closing the circuit, and causing the bell to ring. The temperature of the mercury bath when the bell rings is taken as the melting point of the compound under investigation.

In 1875 a modification of Löwe's method was proposed by C. H. Wolff([23]). He made 24 successive melting-point determinations on the same substance by the Löwe method and obtained seriously discordant results, some of which were divergent to the extent of 4.2° C. The degree of divergence was considered by Wolff to be, in some measure, jointly dependent upon the thickness of the pointed platinum wire used and of the coating of compound covering the point of the wire. The improvements suggested, therefore, were the use of a thinner platinum wire of uniform thickness and some means of insuring a uniform coating thereon of the substance to be tested. For the one Wolff replaced Löwe's "moderately thick, pointed" wire by one of the thickness commonly used in qualitative and blowpipe analysis. The new wire had a length of 8 mm. and was bent into a bow so that the ends were 5 mm. apart. The other platinum wire of the Löwe apparatus was also replaced by a similar thin wire. For the other improvement Wolff fused the substance to be tested in a suitable vessel and allowed the fused mass to cool until it began to congeal at the edges, and at this point the bow of the new bent wire was dipped once or twice into the fused substance, thereby insuring a uniform coating on the wire. The coated wire is adjusted in the apparatus by means of a metallic holder, which connects with one pole of the battery, and is lowered until the bow (coated part) of the wire dips 4 or 5 mm. into the mercury bath. The well in the cover of the Löwe apparatus is replaced in the Wolff apparatus by a small porcelain crucible, which is three-fourths filled with mercury (about 50 grams) and lowered into the water bath (Wolff worked only with compounds melting below 100° C.) until the level of the water extended at least to the level of the mercury. Twenty-two successive determinations upon the same compound, which with the Löwe apparatus showed such wide divergence in melting point, gave results with the modified apparatus between which the widest variation did not exceed 0.5° C., while in most cases the values were exactly the same. No illustration accompanies the original description of this method.

In a paper entitled "A new apparatus for the determination of the melting point," published in 1890 by A. C. Christomanos ([24]), the author recounts the disadvantages attending the use of the ordinary capillary-tube methods and proceeds to describe and illustrate (illustration reproduced in fig. 12) the new method implied in the title. Although not so stated by the author, the Christomanos method may be considered a modification of the methods of Löwe and Wolff. In principle it is identical, but differs slightly in detail. Whether or not the efficiency of the method is greater than that of Löwe or of Wolff seems not to have been specifically tested. Referring to the figure, the author describes his method somewhat as follows: A is a

cylindrical glass vessel, 12 cm. high and 6 cm. in diameter, which may be heated upon a sand or air bath. It contains two neck-like openings, one of which is fitted with a cork carrying the thermometer (C) and a thick platinum wire (f), while the other is a small fluted neck (e), made conical in order to support a test tube (B), tapering at its lower end to a capillary (c, a, p). For use the glass vessel A

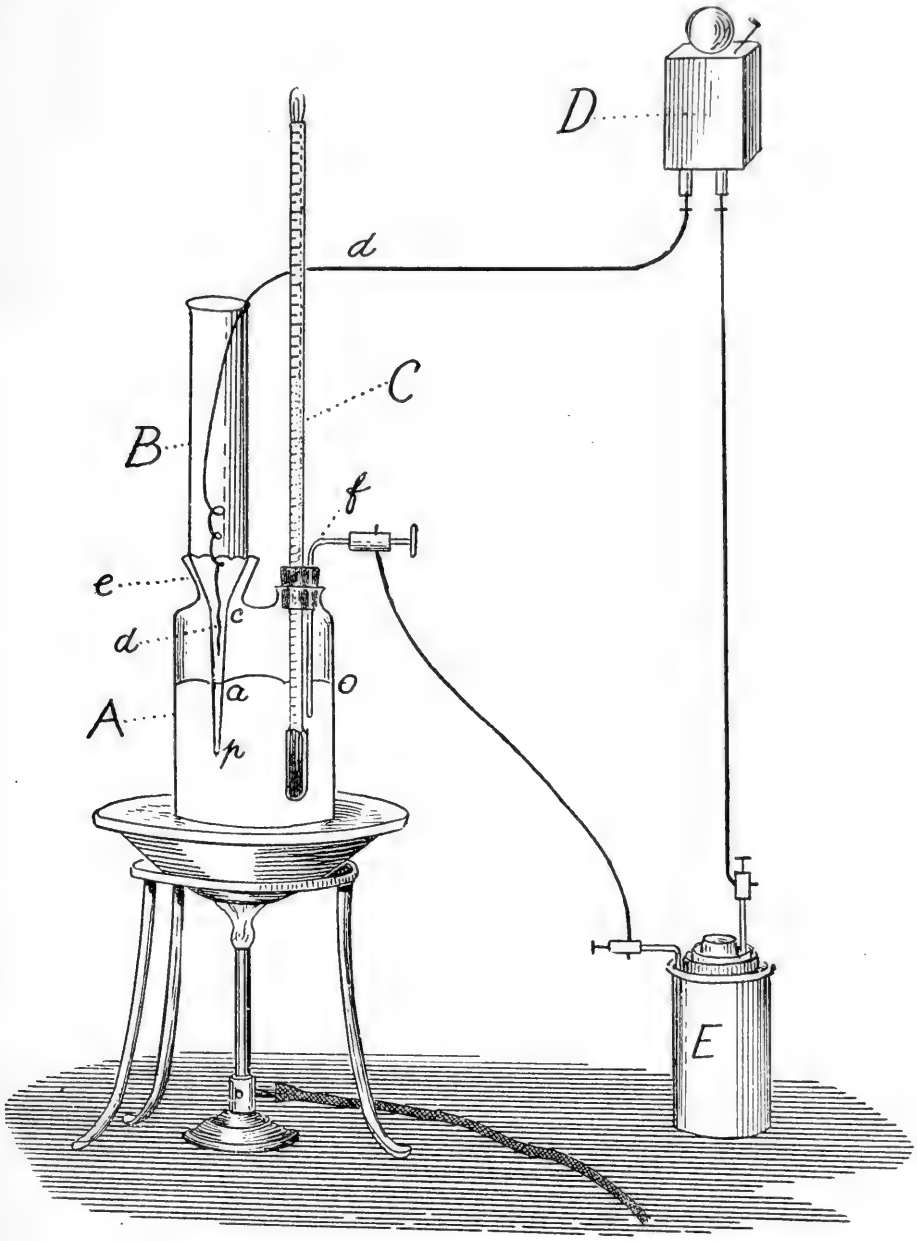

FIG. 12.—The Christomanos apparatus.

is filled with pure mercury to such a level (a, o) that the capillary end (p) of the test tube (B) dips about 2 cm. (a, p) below the surface.

The substance to be tested is introduced into the capillary part of the test tube in the following manner: A portion of it is carefully melted in another tube; the molten substance is drawn into the

capillary by suction through the end p to a height of 0.5 to 1.5 cm. After cooling, the material adhering to the outer surface is removed and the tube is placed in position in the neck e. The space in the test tube above the sample is filled with mercury, drop by drop, to a height of 1.5 to 2 cm. (a, c) above the surface of the mercury in the vessel A. A thin platinum wire (d), connected with the bell (D), extends through the mercury in the test tube (c, a) to the sample a. One pole of the battery cell (E) is connected by a copper wire to the bell (D) and the other pole is similarly connected with the platinum wire f. The mercury bath is carefully heated so as to maintain a uniform rise in temperature. When the sample (a, p) melts it is forced out of the tube by the weight of the column of mercury (a, c),

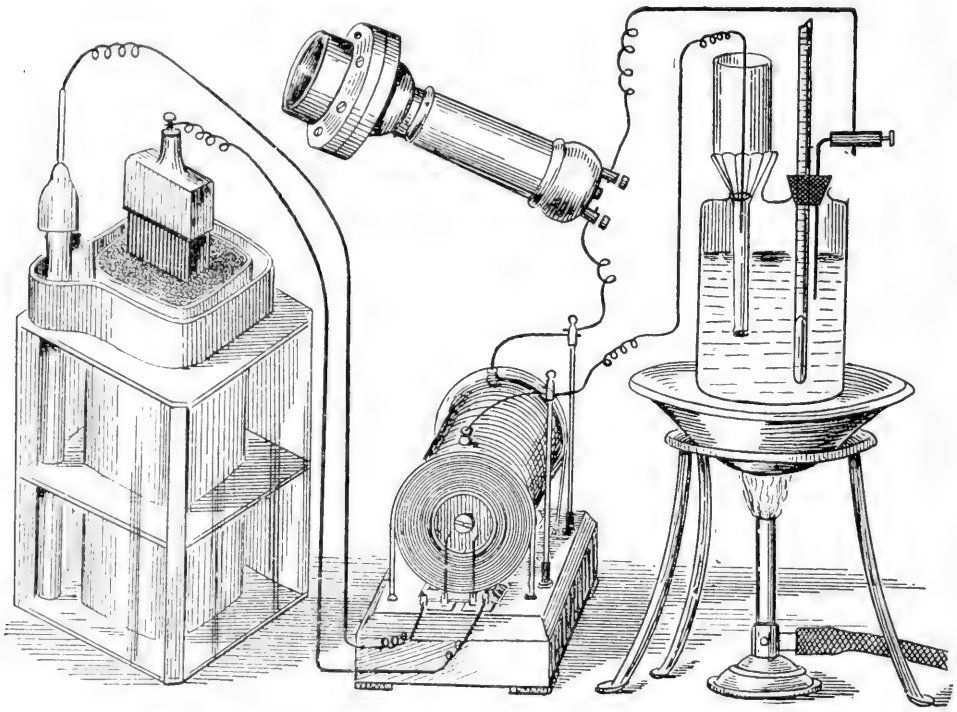

FIG. 13.--Levy's acoustical method.

which brings the latter in contact with the mercury bath, thus closing the circuit and causing the bell to ring.

As with the Löwe and the Wolff methods, the temperature of the mercury bath when the bell first rings is taken as the melting point of the substance under investigation. For determinations at temperatures above 250° C. the whole apparatus is placed under a hood with a good draft. The author considers that his apparatus entirely eliminates most of the objections which he attaches to capillary-tube methods. His melting-point determinations by this method have included, among others, the melting point of ice and that of saltpeter (338° C.).

A still further modification in the type of method under consideration is that described and illustrated (as in fig. 13) by Tyrer and

Levy ([25]) in 1900. These authors cite the objections made by different investigators to electrical methods and attempt to overcome them in their modification. The accompanying figure clearly illustrates the details in which the apparatus differs from that of the Christomanos method. An alternating current was employed (by means of the Ruhmkorff coil) with the idea of preventing increased resistance between the poles that may possibly be caused "by a secondary current formed by the electrolysis of the substance under examination." The part of the test tube which contains the sample is made wider (about 4 mm. internal diameter) in order to facilitate contact when melting occurs. The sample itself is not previously melted for introducing into the melting tube, but is firmly packed into the end of the tube to a height of about 1.5 cm. by means of a metal rod of about 3½ mm. diameter. As an indicator of contact after melting the bell of previous methods is replaced by a telephone device, through which the noise of the commutator in the Ruhmkorff coil is distinctly heard as soon as contact is made.

Objections to electrical methods are generally based upon the high results obtained in their application. These high results are variously explained: According to Landolt ([26])—using the method of Löwe and Wolff—they are due to a lagging of the sample in leaving the wire after melting, while the temperature meantime continues to rise. When the sample is contained in a tube, Landolt's reasoning would involve capillarity as a cause of delayed contact. A. Ferreil ([27]) considers the high results to be due to the preliminary melting and reheating involved. The explanation of Reissert ([2]) is that with such methods the melting must be complete before the melting point is indicated and, since the true melting point is the beginning of melting, the results obtained are, therefore, necessarily high. Tyrer and Levy (see above) suggest the further complication of "a secondary current" as an additional factor in the Landolt explanation.

The results obtained by Tyrer and Levy with their electrical method (fig. 13) would seem to indicate that all of these objections have been overcome, but at the cost of considerable complication in the apparatus. At any rate, the apparatus required for an electrical method is probably beyond the range of the present average pharmaceutical equipment, and such methods are therefore not adapted to the present needs of the United States Pharmacopœia.

3. Miscellaneous methods.—The method of Cross and Bevan ([28]), as described and illustrated by the authors in 1882, consists of a small platform (A, fig. 14), made of very thin sheet-iron or platinized silver, having an opening (a) provided for inserting the thermometer bulb and a small depression (b), about 1.5 mm. deep and 2 mm. in diameter, for carrying the sample of substance under investigation,

B is a small glass float, having a thin piece of platinum wire, flattened and bent to an L-shape, sealed into the bulb end of the float

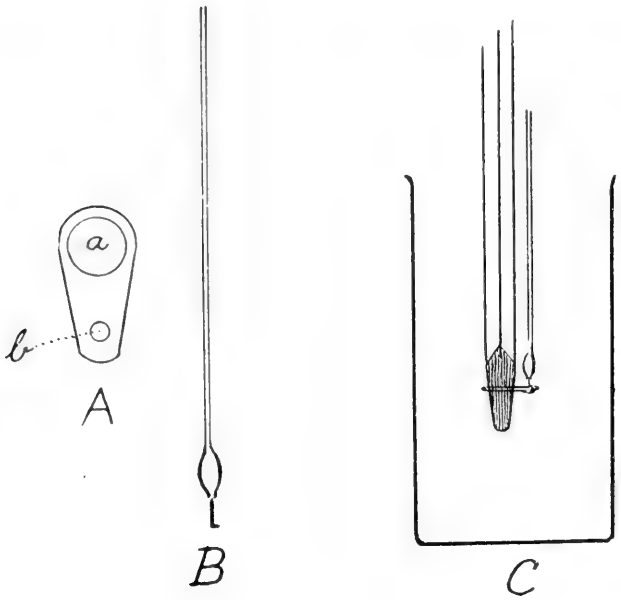

Fig. 14.—The method of Cross and Bevan.

To apply the method a small portion of the substance under investigation is melted in the depression (b) of the platform. The bend in the wire of the float is then submerged in the molten material and held until it becomes fixed by the cooling and hardening of the mass. A thermometer is adjusted in the opening (a) of the platform and the whole submerged in a beaker, as illustrated by C in the figure. When the mercury bath has been heated sufficiently to melt the sample the float (B) is released and suddenly rises to the surface. The temperature of the bath when the float rises is recorded as the melting point.

The method seems not to have met with general favor.

A method the application of which insures an interpretation of the melting point in accordance with Reissert's definition (refer., p. 18) involves the rather complicated device described and illustrated by M. L. N. Vandevyver ([29]) in 1898. In Vandevyver's apparatus a small mirror (M, fig. 15A) is fixed at an angle of 135° to the end of a wire rod (a, b). Attached to the rod above the mirror are two rings, the lower one (c) fixed and the upper one (d) movable and having an overlapping rim. A circular piece of white filter paper is clamped to the ring c by means of the ring d, and upon this paper a small piece of the substance to be tested is placed. The

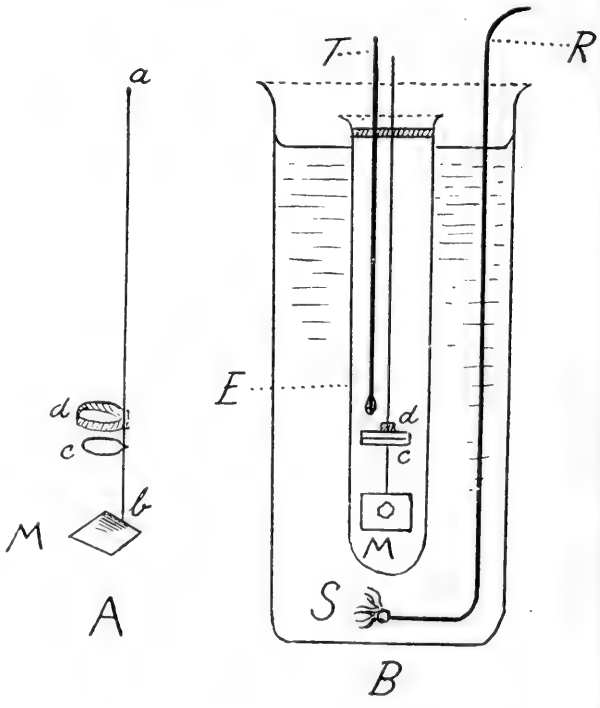

Fig. 15.—The method of Vandevyver.

upper part of the rod is fixed in a cork, which fits the tube E (fig. 15B) and which also carries a sensitive thermometer (T), so adjusted as to place the bulb in close proximity to the sample. The tube E serves as an air bath and, as shown at B in the figure, is nearly submerged in a beaker of water, glycerin, or paraffin, as the case may require. The outer bath is provided with a stirrer (R), which, when water is used as a bath, terminates in a brush (S), by which to remove air bubbles that may accumulate on the sides of the tube and interfere with the observation. The bath is slowly and carefully heated. When the sample begins to melt, it stains the paper, and the reflection of the stain in the mirror indicates the melting point, which is, of course, recorded by the thermometer. At high temperature the glass mirror is replaced by a metallic mirror. Other modifications are made according to the nature of the substance tested.

This method, as applied by Tyrer and Levy ([25]), appears not to have been very satisfactory.

The method described and illustrated (as in fig. 16) in 1899 by M. Kuhara and M. Chikashigé([30]) seems to have been designed primarily to eliminate the capillary tube. For the latter these authors substituted two cover glasses (used in microscopic work), cut in halves, "between which the substance to be tested is introduced, either in powder, in crystals, or in thin slices." The layer of substance, if powder, can be made very thin by "pressing and sliding the two pieces between the fingers." The authors further describe their method as follows:

> The surface exposed is very large compared with the quantity of the substance taken, and, consequently, its behavior toward heat may be distinctly observed. Before the substance is melted the glass appears opaque, while it becomes transparent when fusion occurs. The thinner the layer the more distinct is the demarcation; but with volatile substances a quantity somewhat in excess of what is apparently essential should be taken in order to make allowance for loss by volatilization.
>
> The pair of glass pieces is then fastened to a holder made of platinum foil and tied, if necessary, with a piece of fine wire of the same material. The holder, which can easily be made by folding the foil and cutting it with scissors, as shown in the annexed figure, is suspended in a wide test tube into which is inserted a thermometer close to the holder. The test tube, serving as an air bath, is immersed in the sulphuric-acid bath almost to its mouth. The further steps of the process require no modification of the old methods.
>
> The glass pieces can be used any number of times unless they are broken; this is considered another superiority over the tube method.

L. Dobbin([31]) in 1904 recommended a method involving the use of a thermostat, previously heated so as to register 1° or 2° above the melting point of the substance to be tested. Fifteen or twenty grams of the substance, previously dried by some prescribed method, is then

introduced and stirred with a thermometer, from which frequent readings are taken. When an intimate mixture of molten and solid material is obtained the temperature remains constant for some minutes ("as with ice and water"). This temperature is recorded as the melting point. The author admits that while this method seems "to constitute a theoretically satisfactory mode," it is imprac-

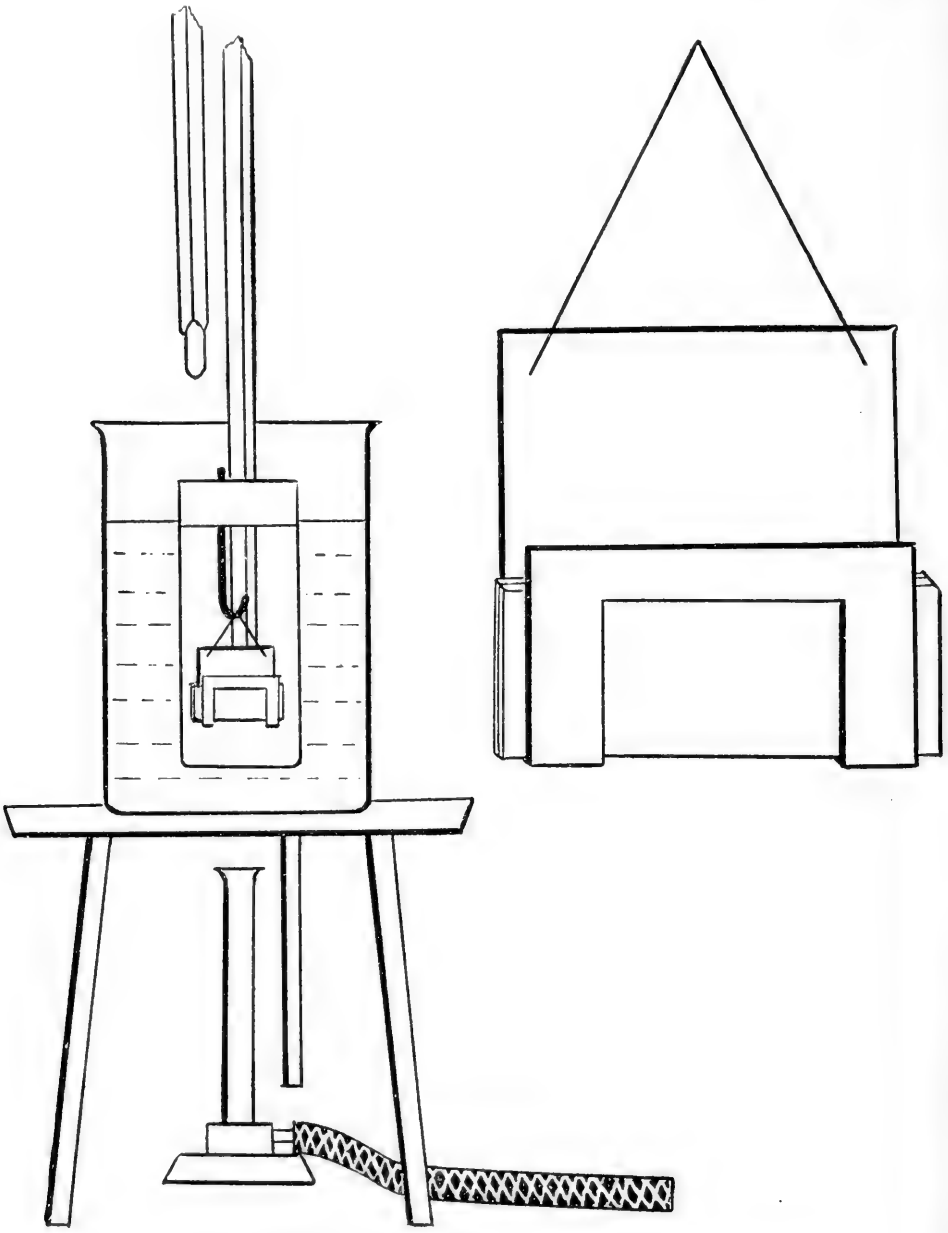

FIG. 16.—The method of Kuhara and Chikashigé.

ticable for general use. He, however, urges the "highest possible degree of uniformity in the method employed" for determining official melting points of pharmacopœial compounds.

It is probable that, except for some form of a capillary-tube method, no method for melting-point determinations is more generally advocated than that proposed in 1889 by Landolt([26]). The description

and illustration (fig. 17) of this method as published by Tyrer and Levy ([25]) are here reproduced:

The substance, being placed in a test tube of 30 millimeters bore and 174 millimeters length, this tube is surrounded by one of 40 millimeters bore, the whole being surrounded by a cylinder open at both ends and heated by a Bunsen burner so as to practically compose an air bath. The inner tube is closed with a cork, through which a thermometer and glass stirrer pass. For protecting the outer glass a small disk of asbestos, covered with wire at a small distance above the flame, is advantageous.

Landolt applied his apparatus to the determination of not only the melting point, but also the freezing point, of three substances, the approximate melting points of which were, respectively, 80°, 165°, and 200°. In successive experiments, involving both observations, he used widely varying amounts of material. The results obtained were remarkably concordant. It would appear that Landolt's method is the only logical one that may be considered to closely approximate theoretically accurate results. A practically prohibitive drawback to a general application of the method, however, is found in the comparatively large amount of material necessarily required, which would exclude its use for a large number of organic pharmaceutical products.

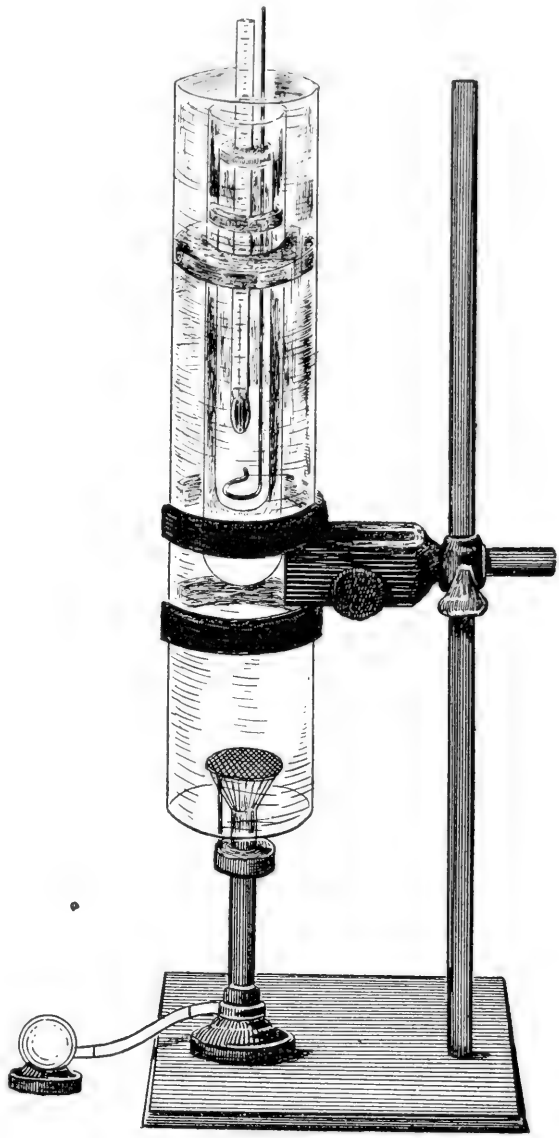

FIG. 17.—Landolt's method (after Tyrer and Levy).

As previously stated, the methods described above by no means include all that might be cited as of more or less common application; but it is believed that the number and variety considered serves fairly well the purpose of indicating the marked diversity of methods that prevail for melting-point determinations.

That the unrestricted use of different methods constitutes a real cause of divergence in the published melting-point values of the same compound may not appear from abstract consideration of the meth-

ods, but it is conclusively demonstrated by the excellent work of several reliable investigators. The investigation of Landolt([26]), previously referred to, was conducted for the purpose of determining the comparative efficiency of different methods. A preliminary part of the work involved the extremely careful and exact standardization of eight different thermometers. Besides his method, described above, he applied different forms of capillary tube methods (including Piccard's), using both liquid and air baths, and also the electrical methods of Löwe and of Wolff with various modifications. Many variations in procedure were applied. The same three substances (melting, respectively, at about 80°, 165°, and 200°) referred to above were used in all cases. Landolt found that the results obtained by different forms of capillary tube methods (including variation in size of capillary tube) were more divergent, and usually higher than those obtained when large amounts of the substance were employed for both the melting-point and freezing-point determinations (Landolt's method). The same statement applies to the various forms of electrical methods, as compared with both the Landolt method and the capillary tube methods. The widest variation in corrected results between the different methods for the three substances used approximated, respectively, 1°, 2°, and 5°. A slight modification of an electrical method for two determinations on Naphthalene (melting point about 80°) caused a divergence in results of over 2°.

Similar work by Reissert([2]), published in 1890, corroborates the evidence furnished by Landolt's results. Reissert did not apply as great a variety of methods as did Landolt, but his investigation included a comparative study of a much larger number of compounds (24 in all), which ranged in melting point gradually from 13° C. to 284.65° C. The results for the same compounds, as obtained by different methods, showed divergence ranging from a few tenths of a degree at low temperatures to several degrees at high temperatures. Reissert recommends the use of Landolt's method whenever possible.

Perhaps the most comprehensive comparative study of different methods that can be found in the literature is that of Tyrer and Levy([25]), published in the Year Book of Pharmacy, 1899 and 1900. In the course of their investigation nine different methods were used, including that described by the British Pharmacopœia, Græbe's, Landolt's, Piccard's, Christomanos's, Mill's, Kuhara and Chikashige's, and Levy's accoustical method. Twelve compounds were treated, ranging in melting point from about 40° C. to about 200° C.

Besides the divergence due to the use of different methods, they studied the effect upon the melting point due to varying physical conditions of the compound, to the extent that they determined the melting point of the commercial product, the same dried, and the same purified until there was no further rise in melting point. Fur-

ther reference to this feature of their work will be made in subsequent discussion.

The amount of divergence resulting from the use of different methods varied not only with rising temperature, but also between different compounds melting at about the same temperature, and ranged from about 0.5° at low to about 3° or 4° at high temperature. With only three compounds, however, was the comparison extended to all nine methods. These compounds were spermaceti, melting at about 43° C.; betanaphthol, at about 122° C.; and picrotoxin, at about 200° C. The range of divergence in this case extended from about 2° for the first two compounds to over 6° for picrotoxin. The increase in range of divergence with increase in the number of methods applied to the same compound under the same conditions is very striking and convincing.

As a result of their work the authors conclude that Græbe's method involves "the most convenient form of apparatus as applied to a majority of the pharmaceutical substances," while "electrical methods which eliminate to some extent the error of individual observation are to be recommended."

In each of the foregoing investigations it must be admitted that the factor of individuality (including nearly all individual variation in details of procedure) is practically eliminated from the results, and that therefore the divergence in the values obtained must be inherent to the different methods employed. Undoubtedly, then, the application of a great variety of methods to the determination of melting points, even though applied by the same investigator, may be a real and serious cause of divergence in the results obtained upon the same compound. Obviously, therefore, for the practical standardization of the melting points of any group or class of compounds it becomes primarily essential that a perfectly uniform or standard method shall be required in all cases for the determination of a standard value.

As applied to the United States Pharmacopœia, such a requirement seems entirely feasible. The present legal status of the pharmacopœia makes mandatory the application, for official tests, of any method the committee of revision may select and officially prescribe. It becomes necessary, therefore, only to decide upon the method or methods desired in order to eliminate the prevailing dissatisfaction with the United States Pharmacopœia melting-point requirements, in so far as that may be due to the variety of methods now used.

A careful consideration of all the methods for melting-point determinations described above leads to no definite conclusion as to which is best. Some seem to be better adapted for the purpose from one point of view, others from another. With reference to each of several very different methods the originator or advocates make the claim that its application insures the nearest approach to theoretical

accuracy in results. What constitutes theoretical accuracy in melting-point determination, however, seems to be a mooted question even among very reliable investigators. The claim of exactness is probably more frequently made of Landolt's method than of any other of those commonly applied. Nernst([32]), Reissert([2]), Crafts([33]), Dobbin([31]), and many other investigators seem to consider it in that light. But one of White's([1]) conclusions (see p. 18)—applied to pharmacopœial products—is to the effect that, in the melting of a mass of substance, there is no point at which the temperature remains constant—therefore, as an abstract deduction, the Landolt method would seem to offer the same wide range for the interpretation of the melting point that is possible to any capillary-tube method.

As a result of these considerations the writer has been led to the conclusion that theoretical accuracy in melting-point determinations, although highly desirable, is, in view of the uncertainty pertaining thereto, not as vitally important for the purpose of standardization of pharmacopœial products as is *uniformity of practice.* Therefore a method involving very simple apparatus, easily accessible to all concerned, *with carefully defined procedure*, would probably serve the purpose as effectively as a more complex device, a conclusion which is in accord with the suggestion (see p. 11) made by the committee of revision of the United States Pharmacopœia with reference to this work.

A careful consideration of the various methods found in the literature, together with the results of their application by different investigators, led to the further conclusion that some simple form of a capillary-tube method, with uniform procedure, would, doubtless, yield as consistent results for the same product as would any other practical method; and at the same time this type of method generally possesses the advantage of greater possible simplicity in the form of apparatus required, and the still greater advantage of being applicable to a larger class of organic compounds, than is true of most other methods. This line of reasoning eliminated, at the outset, all methods involving any very special or complicated form of apparatus (including some of the capillary-tube variety) and limited further consideration to the remaining forms of capillary-tube methods. Abundant objection on the part of many reliable investigators to any form of method involving the use of an air bath, and their aparent lack of general application, seemed to justify the further elimination of methods of that type. Experimental investigation was thus reduced to a few comparatively simple forms of capillary-tube methods.

It was further considered desirable, however, as tending toward an international standard, that the method adopted by the United States Pharmacopœia should conform to the method most commonly prescribed by foreign pharmacopœias, in so far as such a method discloses desirable features and does not conflict with the ideals of the

United States Pharmacopœial Convention. The following are brief statements of the melting-point requirements concerning methods of some of the foreign pharmacopœias:

British Pharmacopœia([34]): The substance is placed in a capillary tube of 1 mm. diameter, attached to a thermometer bulb, and immersed in a suitable bath, contained in a beaker. Constant stirring is applied. The substance is melted, allowed to cool and solidify, and is then remelted, the temperature of the second melting being considered the melting point. The thermometer used is required to be corrected by comparison with a standard thermometer, and the zero point should occasionally be determined. Correction for emergent stem of the thermometer should be applied unless the entire mercury thread is immersed in the bath.

German Pharmacopœia([35]): Substance dried for twenty-four hours in a desiccator over sulphuric acid. Sufficient substance introduced into a capillary tube of 1 mm. diameter to form a column 2–3 mm. high. Tube attached to a suitable thermometer and immersed in a bath of sulphuric acid contained in a test tube of 30 mm. diameter. Bath gradually heated with frequent stirring. That temperature at which the opaque substance becomes a transparent liquid and flows together in transparent drops is the melting point.

The methods prescribed by the Austrian([36]) and the Japanese([37]) Pharmacopœias are practically identical with that of the German, while the Helvetian([38]) Pharmacopœia differs essentially from the German only in prescribing the use of a double bath, that is, a form of Græbe's apparatus.

The Swedish Pharmacopœia([39]) requires that the substance be finely powdered and thoroughly dried. Sufficient is then introduced into a thin-walled capillary tube of 1 mm. diameter to form a firm layer several millimeters deep. The thermometer bulb and sample are immersed in a bath of sulphuric acid or liquid paraffin contained in a suitable vessel. The bath is briskly stirred while being heated. The melting point is defined as in the German Pharmacopœia.

In the Danish Pharmacopœia([40]) the substance is required to be finely powdered and well dried. Introduced into a capillary-tube of 1 mm. diameter, it is attached to a thermometer and heated in a Roth apparatus containing sulphuric acid in the outer tube.

The majority of pharmacopœias at hand, however, seemed to require the application of no definite method for the determination of official melting points.

The three methods finally selected for experimental investigation were all capillary-tube methods and involved the following simple forms of apparatus:

1. A simple round-bottomed glass bulb of 50 to 75 cc. capacity and having a short neck of about 20 mm. diameter. The method is prac-

tically that used by the investigators A and C in the preliminary experiment previously described (see p. 22).

2. One of the various forms of the Græbe apparatus, involving the use of an inner tube containing sulphuric acid and adjusted in a bulb which also contained sulphuric acid. The method is practically that used by the investigator B in the preliminary experiment referred to under 1.

3. A large test tube, about 100 mm. long, 30 to 35 mm. internal diameter, and provided with a glass stirrer. The apparatus corresponds practically to that prescribed by three of the foreign pharmacopœias.

It required little preliminary experimentation with these three methods to disclose the fact that constant stirring of the bath during a melting-point determination is a very desirable detail of the manipulation; and that the use of a double bath apparatus does not permit of as easy control of the rate of heating as a simple bath provided with a stirrer. The first and second methods were therefore rejected and further attention was confined to the development of the third. As the result of very extensive experimentation the following method was evolved and is now submitted to the committee of revision as:

The method recommended for incorporation in the official melting-point requirements of the United States Pharmacopœia.—It involves the use of a simple round-bottom straight-glass tube of 30 mm. internal diameter and about 100 mm. long, flaring slightly at the top like an ordinary test tube. The walls of the tube should be about 1 mm. thick (certainly not more than 1.5 mm. thick at any point) and must be made of glass which will stand heating over an open flame.[a] This tube or container is fitted with a stirring device which, with a little practice, can be easily made in a few minutes from a piece of small-sized thick-walled capillary glass tubing (about 2 mm. internal diameter preferred) of such length that a double bend above the top of the container brings the outer end of the stirrer within easy reaching distance of the hand for convenience in manipulation. When in use the container is filled with a suitable bath to a depth which will permit of such immersion of the bulb of the thermometer that the upper end of the bulb will be 2 to 3 cm. below the surface of the bath and the lower end of the bulb about equally distant from the bottom of the container. The capillary tube

[a] During the preliminary experimentation with this method perfectly satisfactory tubes were made in the laboratory by the investigators concerned from the water jacket of small broken Liebig condensers or other suitable tubing. Subsequently a small supply was made to order according to the specifications given above at very moderate cost. If the method is officially adopted by the United States Pharmacopœia the tube will doubtless be put on the market at still smaller cost.

is attached to the thermometer (by means of the capillarity resulting from sulphuric acid between two glass surfaces) in such position that the sample is centrally located by the side of the thermometer bulb.

The apparatus is set up by simply clamping the tube in an ordinary lamp stand, which also carries the thermometer suspended in any convenient manner from a second clamp attached to the stand. The container and stirrer and the adjustment described is shown in figure 18. The glass tube held by the clamped cork (shown at top of figure), and through which the thermometer passes, simply serves to prevent any swinging of the thermometer that might be induced by stirring, and at the same time does not interfere with raising the thermometer for convenience in adjusting the capillary tube.

For melting points up to 150° C. pure concentrated sulphuric acid was considered the most suitable and satisfactory bath. When fresh it can be used at much higher temperature, but then its very irritating fumes make it decidedly objectionable. After much experimentation no bath could be found suitable for work at temperatures much above 200° C. which was not more or less objectionable on account of fuming. This difficulty, however, was found

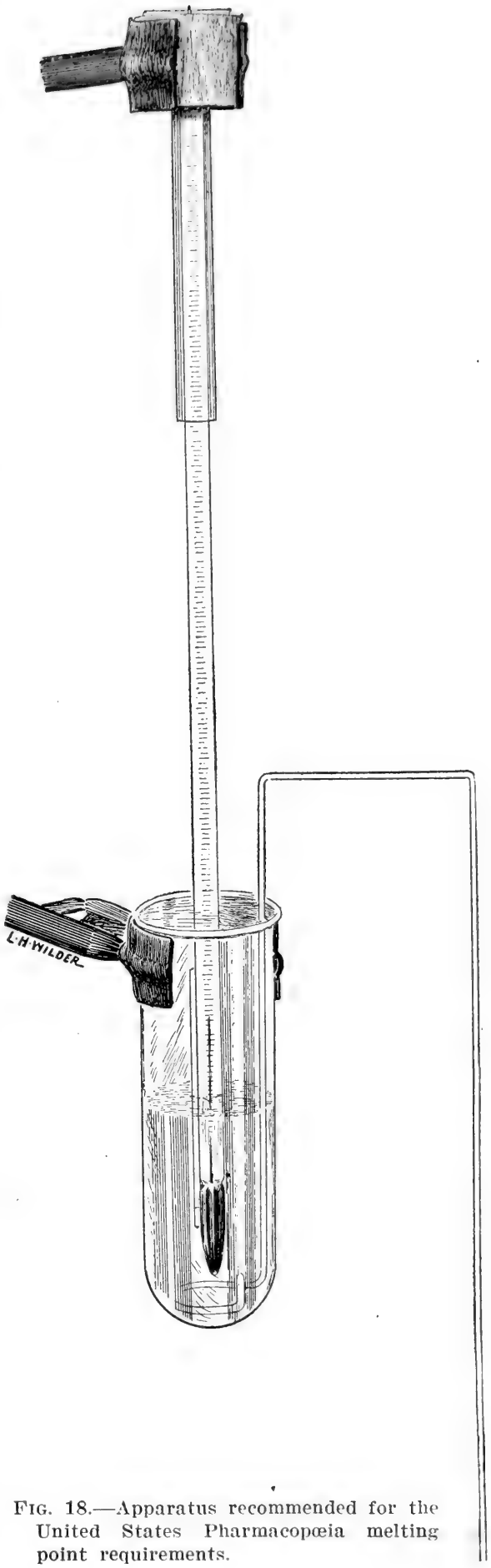

FIG. 18.—Apparatus recommended for the United States Pharmacopœia melting point requirements.

to be effectively overcome by a slight modification of the apparatus, which consisted in fitting the container with a cork, perforated for the thermometer and for the stirrer and with two or three small vents or grooves cut in the edge to prevent excessive pressure. With this modification, a very pure grade of cotton-seed oil,[a] freshly distilled paraffin, certain mineral oils, and a few other substances could be conveniently used up to 300° or over, but soon become muddy from decomposition and have to be frequently renewed.

The comparative efficiency of the different substances tested as high-temperature baths will, in some measure, be indicated by a brief consideration of some of the experimental work. A sample of the cotton-seed oil (referred to in footnote) was heated to about 260° C. and cooled to room temperature several times without any apparent change in color. It was sufficiently clear and colorless to make observation of the melting substance in a capillary tube almost as easy as in any other bath. The same sample of oil, heated as described above, was used as a bath in making from 15 to 20 melting-point determinations of a sample of saccharine, after which it was heated up to 360° C. with little evidence of boiling, and, though considerably darker than at first, was still sufficiently clear to permit of reasonably good observation of the melting point. So many melting-point determinations of saccharine offered a rather severe test of the bath, since, in most instances, the temperature rise from about 206° to about 221° C. invloved a time interval of from fifteen to thirty minutes.

Glycerine was also tested as a bath in determinations of the same compound, but discolored much more rapidly than did cotton-seed oil. Even when a very pure and high-priced grade was used there was a marked change after four or five determinations.

As a bath for melting-point determinations at moderately high temperature liquid petrolatum has the initial advantage of being perfectly clear and colorless. As a preliminary test it was slowly heated to about 275° C., at which temperature it did not fume excessively and remained perfectly colorless. Applied to melting-point determinations of cocaine hydrochloride (about 200°), however, it began to discolor after the second or third heating. After ten or twelve determinations at that temperature it is considerably darkened but still fairly clear (not muddy). Decomposition, however, seems to lower its flash point, and its use is therefore attended with more or less danger.

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[a] An exceptionally colorless and high-boiling sample of this oil was kindly furnished for this investigation by L. W. Bosart, jr., of Milliken, Staten Island, N. Y. I take pleasure in acknowledging with sincere appreciation the kind cooperation of Mr. Bosart in this connection.

A pure grade of paraffin freshly distilled makes a reasonably good bath. It begins to fume about 270° C. and boils about 350°. A sample used in melting-point determinations of hydrastine hydrochloride (about 210°) showed some discoloration after the fourth or fifth heating, but the change was not markedly increased by heating three or four times to 320° C. A fresh paraffin bath is perhaps clearer and more colorless than one of the pure cotton-seed oil, but discolors more rapidly with repeated heating, and is therefore inferior.

A sample of mineral oil—represented as probably better adapted than any other of that type for use as a melting-point bath—was received from a prominent refining company. The oil made an unfavorable impression at first glance, being quite dark, considerably fluorescent, and not very clear. It began to fume about 230° C., discolored rapidly on repeated heating to about 300° C., and was soon discarded as inferior to any of the baths previously tried.

Artificial (cotton seed) lard was also applied to the purpose and found to be reasonably efficient for a limited number of determinations up to 275° or 280° C. Olive oil and castor oil were also included in the investigation, but disclosed no advantages as baths over the substances already considered.

Of the various organic substances applied to the purpose of a bath the pure cotton-seed oil is undoubtedly the best, but the grade described above would doubtless be difficult to obtain in the open market and therefore would probably be comparatively expensive, while, according to the evidence obtained in this investigation, a poorer grade would be disproportionately inferior as a bath for melting-point work. Moreover, the cotton-seed oil at best requires more frequent renewal than would be considered desirable if many melting-point determinations at high temperature were involved. Attention was therefore directed to possible inorganic baths which might better serve the purpose.

Of the various fusible mixtures of inorganic salts[a] which frequently serve the purpose of a high-temperature bath in other lines of work, probably the one best adapted to melting-point work is that consisting of a mixture of equal parts of sodium nitrate, potassium nitrate, and sodium nitrite. This mixture melts at 180° C., though its melting point can be lowered to some extent by the addition of a little sodium chlorate. The mixture when first melted is slightly cloudy, but rapidly clears as the temperature rises, forming above 200° C. a perfectly transparent liquid with a slight yellow tinge. The bath is doubtless an excellent one for high-temperature work, but in its application to melting-point work it has certain serious

[a] For valuable suggestions in this connection my grateful acknowledgments are due Prof. H. W. Foote, of Yale University, whose kind interest and cooperation are sincerely appreciated.

drawbacks. The mixture must first be fused in a porcelain or other suitable vessel and then poured into the glass container, which, without careful manipulation, would often mean great liability to breakage and consequent danger of serious accidental burns. Without discussing other disadvantages disclosed by application of this bath, subsequent experimentation led to the conclusion that the high-temperature bath for the pharmacopœial method should be liquid below 180° C., and it was therefore concluded that this bath was not adapted to the purpose.

It was suggested that some of the so-called "specific-gravity baths," used for the separation of mineral mixtures, might supply the needs of a high-temperature bath for melting-point determinations. Access was had to the literature (references ([41]) to ([50]), inclusive) describing such baths, which also consist of fusible mixtures of inorganic salts, but, without experimental investigation, none was considered feasible.

The high-temperature bath finally selected and here recommended as admirably adapted to meet the needs of adequate pharmacopœial melting-point requirements up to from 320° to 370° C., depending on its preparation, is the one previously referred to as proposed by Mulliken ([8]). The author states that the bath—

> is prepared by cautiously boiling together for five to ten minutes under a hood a mixture of 70 parts by weight of concentrated sulphuric acid and 30 parts of neutral potassium sulphate and stirring constantly until the sulphate is completely dissolved; or by similar treatment of a mixture of 55 parts by weight of the acid with 45 parts of acid potassium sulphate. The mixture has the consistency of glycerin and does not fume so badly as to prevent the use of rubber bands for attaching melting-point capillaries to the thermometer. It is less corrosive and less easily discolored by traces of organic matter than sulphuric acid.

The bath is further described in a footnote, as follows:

> After long exposure to the air it may become semisolid through absorption of water, but is easily liquefied again by heat. When a melting point above 300° is to be taken with a bath that has not been heated much above 250° for some weeks it is advisable first to boil it for a few minutes. Otherwise the bubbles of steam given off in the neighborhood of 300° will cause bumping and interfere with the observation. Under certain obscure conditions the mixture may solidify to a hard mass, with a considerable rise of temperature. But this is a rare occurrence, and when it happens a short boiling will bring the bath back to its normal state.
>
> By increasing the proportion of neutral sulphate from 30 to 40 per cent this bath may be used for temperatures up to 370°. Such a bath remains pasty until the temperature has fallen to 90°–60°. Either of these sulphate baths, if slightly darkened by organic matter, may be cleared by a short heating above 300°.

Contrary to the claims made for it—though possibly due to some error repeatedly made in various preparations of it—this bath was found to fume in an open container at high temperature almost as

badly as the pure sulphuric acid. With the simple cork modification the fumes would char the cork and quickly spoil the bath, but by attaching a disk of thin asbestos, by means of copper wire, to the bottom of the cork and including a glass tube in the perforation for the stirrer both the fuming and charring were effectively overcome, and with reasonable care in heating the bath could be used as high as 350° C., or even to 370° C., with perfect convenience and safety. In all cases where the cork modification was applied the stirrer, in order to avoid inconvenience in attaching the capillary tube to the thermometer, was made in two parts—the first extending through and about one-half to 1 inch above the cork, the second part being the remainder of the stirrer, as first described. The two parts are easily joined, with ample security, by means of small pieces of small-bore rubber tubing. With practically no inconvenience this arrangement permits of attaching the capillary tube to the thermometer in the usual way, as shown in figure 19, which also shows, at the separate cut A, the special arrangement of the cork as above described, and, at B, the manner of assembling the two parts of the stirrer.

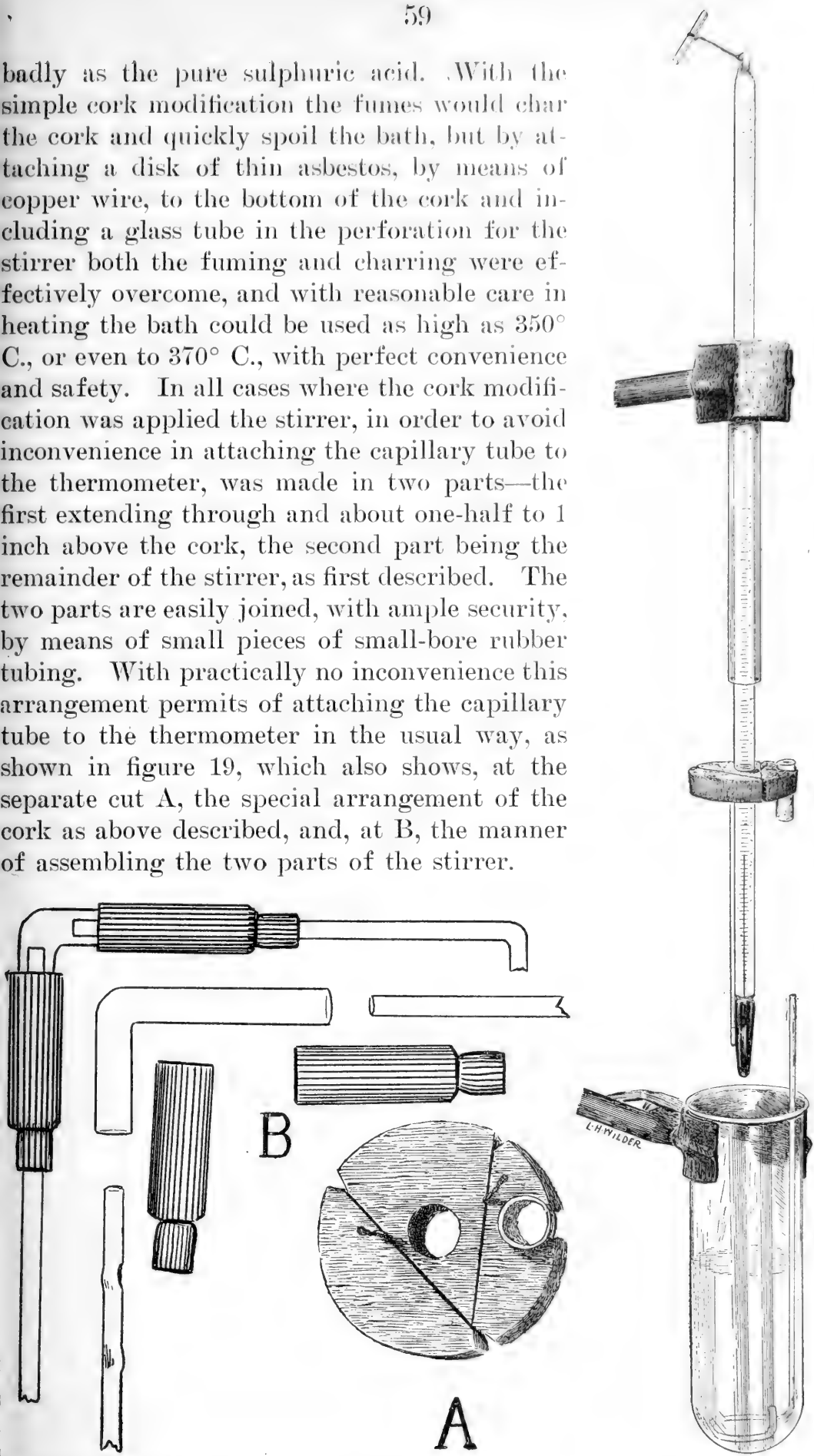

FIG. 19.—The cork modification and divided stirrer does not interfere with convenient manipulation.

The dark color gradually acquired by the bath from contamination with organic compounds can be readily cleared from time to time by adding a pinch of potassium nitrate—or less conveniently by heating, as described by Mulliken—and in this way continuous use for a large number of determinations is possible.

The perfectly clear liquid condition, at comparatively low temperature, of the more concentrated sulphate bath and the lack of inconvenience in manipulating the apparatus with the cork modification suggests the desirability of applying these conditions for temperatures above 150° C., thereby entirely eliminating practically all annoyance from the acid fumes which are evolved in heating sulphuric acid much above that temperature in an open container. The complete apparatus in operation, with the cork modification, is illustrated in figure 20.

Details of procedure to be recommended as a part of this method will be specified at the appropriate point in my discussion of the remaining causes of divergence (refer summary p. 17).

IV. *Variable practice in the use of thermometers.*—Considered in all its phases the thermometer is perhaps the most significant and serious, and in one respect, at least, the most obvious of all causes of divergence in melting-point values. Different thermometers, as applied by independent investigators, may induce divergence in various ways, including (1) variation in construction, involving materials, dimensions, range, etc.; (2) whether or not the thermometers have been standardized and the resulting calibration corrections are applied; and (3) whether or not correction for emergent stem is applied (when the mercury thread is not completely immersed in the bath).

The first consideration as a possible factor involving error in thermometric measurements does not seem to be generally appreciated. That it is effective, however, though perhaps in a comparatively minor degree, is one of the conclusions induced by this investigation. Variation in thickness of the wall in the bulb of different thermometers will undoubtedly lead to some degree of variation in the observation of a phenomenon (such as melting point), which is induced by a steadily rising temperature, the magnitude of variation being influenced, of course, by other factors. If a large part of the thermometer stem is outside the immediate source of heat (in this case the bath), variable thickness of wall in the stem will probably also exert a similar influence, not only on the direct observation, but also on the emergent stem correction, if one is made. In the latter case variations in the length of the stem and in the range of the thermometer would doubtless be much more effective than would a moderate difference in the thickness of the stem walls.

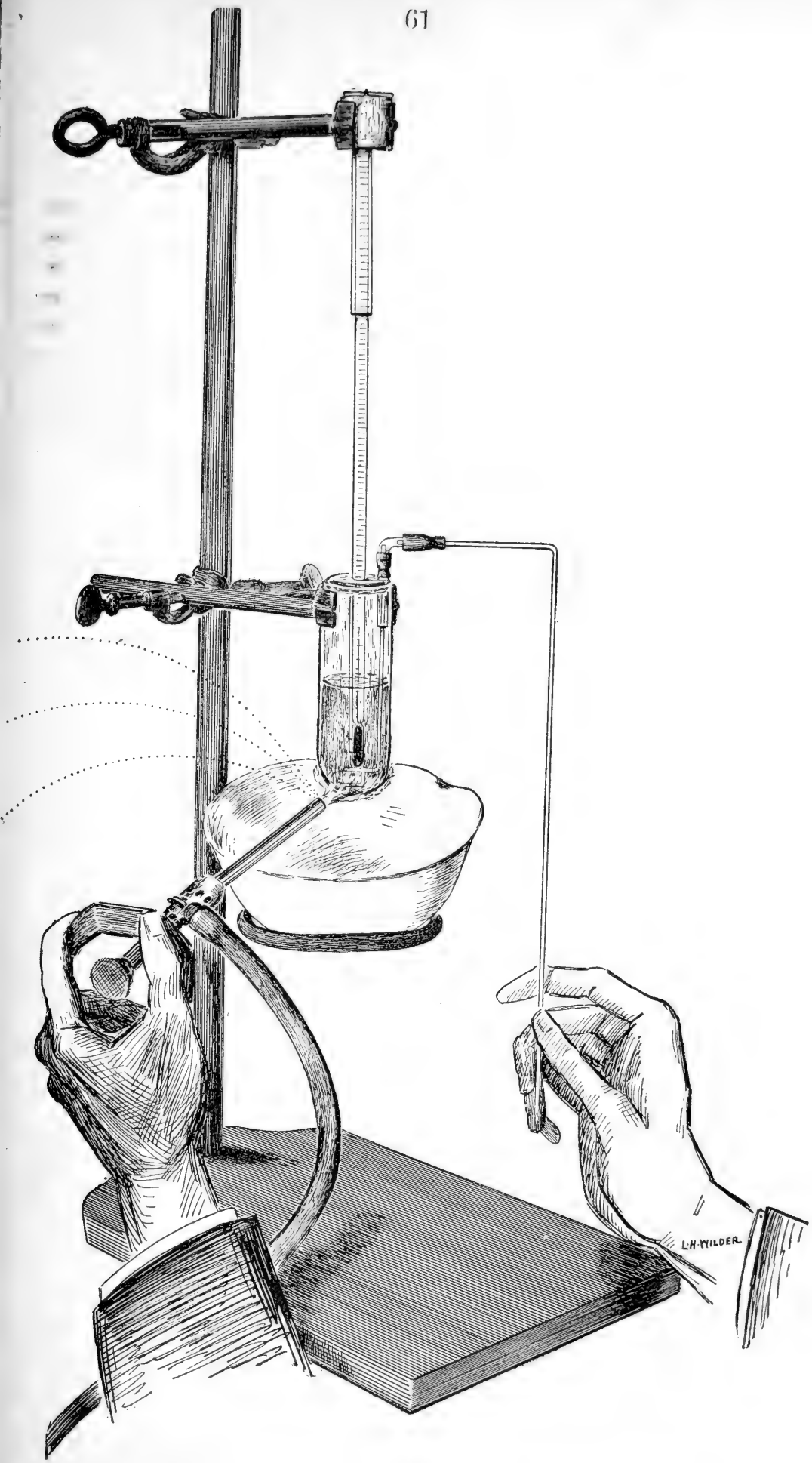

FIG. 20.—Complete apparatus in operation with cork modification.

Facts of the character outlined above were early indicated in the course of a large amount of preliminary experimental work involved in this investigation.

As stated in the introduction to this bulletin, two men were at first engaged in this work. The laboratory was supplied with standard thermometers[a] considered at that time to be especially well adapted to our purpose, but all differed more or less in construction. The two most used were, respectively, about 300 and 310 mm. long and were graduated in whole degrees, the first (No. 1) from —6° to 406° C., and the second (No. 2) from —15° to 263° C. The stem of the No. 1 thermometer was about 7 mm. and that of No. 2 about 6 mm. in diameter, while the bulb of the former was considerably smaller than that of the latter. These two thermometers, except perhaps in range, probably agree in dimensions about as closely as could ordinarily be expected of any two that were not the same. Nevertheless, their application by the two investigators to melting-point determinations of the same compound, by the same method, with uniform procedure, yielded results which were quite consistently, though moderately, divergent. The disagreement was considered to be due to the cumulative effect of errors of observation (fractions of a degree could only be approximated), emergent stem correction, the so-called "personal factor," etc. Nevertheless the two investigators could more closely approximate duplicate results by using the same thermometer.

The latter consideration subsequently led to an attempt by one of the investigators to eliminate the consistent divergence noted above by application to the two thermometers of more refined detailed procedure. The attempt was only partially successful. In the majority of a large number of melting-point determinations, covering a wide range of temperatures, the comparative results, obtained by alternate use of the two thermometers, still showed a divergence, in the same direction, of from 0.2° to 0.5°. Such a margin of disagreement for average melting-point determinations might reasonably be considered negligible; but if, in this case, the disagreement is due to variation in construction of the thermometers, a wider variation might cause the margin of error to exceed the limits of a reasonable standard. Whatever the explanation of this consistent disagreement, however, the facts led to the conclusion that for the purpose of determining official United States Pharmacopœia melting points official thermometers of uniform construction should be required. It is considered that for such a purpose the thermometer should not be too long (about 300 mm.), should not cover a range of more than about 150°, graduated in 0.5°, and the bulb- and stem walls should

[a] Special standard thermometers were kindly loaned the laboratory for this investigation by both the Bureau of Standards, Department of Commerce and Labor, and the Bureau of Chemistry, Department of Agriculture.

be as thin as is consistent with reasonable security from breakage. In anticipation of further melting-point work, sets of three such thermometers ranging respectively from −10.5° to 150.5° C., from 98° to 250.5° C., and from 198° to 350.5° C., have recently been acquired by this laboratory.

The importance of "standardization" as applied to thermometers, and the extent to which its indifferent application or omission may affect melting-point values, does not seem to be as generally recognized as it unquestionably should be. The extent to which even laboratories of high standing indiscriminately apply standardized or unstandardized thermometers to important work is surprising. Furthermore, it appears that the qualification "standardized" is sometimes interpreted to mean that a thermometer to which it applies gives correct readings directly, rather than that calibration corrections have been carefully made for it and should be applied to the observed readings.

The calibration correction below 100° C. for a thermometer of good make may be, and usually is, negligible for average melting-point work, but above that point even the best thermometers may show increasing error with rising temperature, frequently amounting in the neighborhood of 300° C. to 6° or 7°. Consideration of these facts makes it evident that the all-too-common practice of indiscriminately using standardized and ordinary thermometers for melting-point determinations constitutes a real factor in causing the prevailing divergence in published melting-point values. It is obvious therefore that for the determination of standard (official) United States Pharmacopœia melting points a standardized thermometer—or one that has been carefully compared with a standardized thermometer—with invariable application of the resulting calibration corrections, should be required.

Among the many possible causes of divergence in melting-point values, it is highly probable that no one factor is more effective or more conspicuously obvious than the variable practice in the application of a correction for the emergent stem of the thermometer, a correction which is sometimes made, but far more commonly omitted. This correction, as measured in this laboratory for the two thermometers (Nos. 1 and 2) previously referred to, in their application to this work will give some idea of the magnitude of error involved by its variable application or omission. The following tabulation shows the correction as determined for the two thermometers at different temperatures. The first figures, read horizontally, represent the different temperatures at which the corrections were measured and the figures immediately below them represent, respectively, the correction at that point for the two thermometers, as indicated:

Temperature of bath.	80 degrees.	100 degrees.	120 degrees.	140 degrees.	160 degrees.	180 degrees.	200 degrees.
Correction for No. 1	0.43	0.54	0.90	1.38	1.97	2.63	3.39
Correction for No. 2	.48	.85	1.33	1.91	2.57	3.36	4.22

The corrections for the two thermometers at the same temperature necessarily vary, the variation being greater the higher the temperature, because, on account of the difference in length and range between the thermometers, the length of mercury thread emerging from the bath at any given temperature, and therefore the amount of correction, was greater with one thermometer than with the other.

The auxiliary thermometer measurements that were preliminary to the calculation of these corrections were made independently by two investigators applying as nearly as possible the same procedure that was previously followed in melting-point determinations. Considering the crudity of method their results were remarkably concordant and both sets of readings calculated to practically the same values, as recorded above. But, without making any claim of extreme accuracy in the measurements involved, the corrections above tabulated are considered to clearly illustrate the effect upon divergence in melting-point values that may result from variable practice in this single detail of procedure.

When it is clearly recognized that the correction which should be applied to different thermometers at the same temperature may vary within wide limits, depending upon the varying construction and immersion of the thermometer, the possible extent of confusion in results becomes very striking and leads to the very definite conclusion that for the purpose of pharmacopœial standardization of melting points the correction should be officially required. It is true, however, that the single purpose of insuring uniformity would be just as well, possibly better, served by requiring only the regulated use of an official standard thermometer without application of the correction for emergent stem; but, as before stated, theoretical accuracy should be attained as nearly as is practicable, and therefore the correction should be required even in connection with an official thermometer.

The manner of determining the emergent-stem correction may also constitute a cause of divergence in melting-point values. In this investigation the corrections were made by application of the Kopp ([51]) formula:

$$\text{Cor.} = a\ N(T-t),$$

in which T represents the temperature registered by the main thermometer; t, the mean temperature of the emergent stem as measured by an auxiliary thermometer (i. e., the temperature at a point midway

between the surface of the bath and the top of the mercury thread of the main thermometer); N, the length of emergent stem measured in degrees (i. e., if the main thermometer is immersed in the bath to the 10° mark and the temperature is 200°, then N=200—10, or 190); and a represents the apparent expansion of mercury in glass for 1°, which is given for this formula as 0.000154.

The accuracy of the Kopp formula has been challenged by various investigators, and other—generally more complicated—formulas have been proposed to replace it (references 52 to 56, inclusive). In 1889 Rimbach([57]) published a review of several of these formulas, in which he discussed their development and comparative accuracy. Although doubtless inaccurate for extremely refined measurements, it is probable that the original Kopp formula still finds more general application than any modification of it. It is obvious, however, that in so far as different formulas may induce significant variation in the correction (Thorpe([53]) claims for the Kopp formula an "over-correction" of 0.5° at 200°) their indiscriminate application constitutes another slight cause of divergence. To insure complete uniformity, therefore, it becomes necessary to designate some one formula to be applied to emergent-stem corrections. As applied to pharmacopœial standardization, it seems more desirable to adopt simplicity in uniformity of practice than to attain extreme theoretical accuracy when the two are opposed, and therefore the Kopp formula is recommended for official adoption in connection with the United States Pharmacopœia melting-point requirements.

If concordant values are to be obtained, at the hands of different manipulators, in measuring the mean temperature of the emergent stem (t in the formula), several factors must be carefully considered. Slight variations in the manipulation of the flame by which the bath is heated are likely to cause comparatively wide fluctuations in the readings of the auxiliary thermometer; cold drafts or currents of air playing directly upon the bulb of the auxiliary thermometer will induce the same effect in even more marked degree. Fluctuations from both these causes can be largely eliminated by adjusting to the stem of the main thermometer a movable platform made of paper, sufficiently firm to maintain its shape, and having its edges folded so as to form a box-like shield surrounding the bulb of the auxiliary. Manipulation of the flame should also be uniform, however, and should follow, as nearly as possible, the procedure prescribed for melting-point determinations; in both cases the flame, when not applied to the bath, should not be held too close to the apparatus (easily avoided by a turn of the hand, see dotted line fig. 20).

The auxiliary thermometer should be small (Anschutz thermometers were used in this investigation) and should be so suspended that

the center of the bulb is as close as possible to the stem of the main thermometer at the point where the temperature is to be measured.

In this investigation when the bath was used without the cork modification it was at first considered necessary, for obvious reasons, to make separate corrections for the part of emergent stem which was below the top of the bath container and the part which extended above it, the two sets of values being added to obtain the complete correction. A subsequent correction, however (in which only one set of auxiliary readings was made for the entire emergent stem), indicated that, for practical purposes, a separate correction for that part of the stem included within the empty space of the container was unnecessary. When the cork modification was used the emergent stem was considered to begin at the bottom of the cork.

To make a correction for emergent stem in connection with every melting-point determination would, indeed, be an intolerable nuisance. For practical purposes, however, and providing the same thermometer or one of very similar construction is always used, such a procedure is entirely unnecessary. A set of corrections covering the entire range of the thermometer at intervals of 10° or 20° could be made once for all and the results plotted in a curve on coordinate paper, from which the correction for any temperature could be determined by inspection. The only material source of error in such a procedure is variation in room temperature. But the extreme fluctuation in the temperature of an operating laboratory will seldom exceed 20° F. (65° to 85°). If, therefore, the set of corrections is made at an average temperature, the limit of error for the correction will seldom exceed 0.1° C. and will usually be much less.

The procedure for making emergent-stem corrections in this investigation was that outlined above. Several sets of auxiliary readings for the two thermometers used were obtained at intervals of 20°, on successive days. From the average of these readings the corrections were calculated by application of the Kopp formula. To the results obtained the calibration corrections were algebraically added (instead, they could be so added either to the observed values or after correction for emergent stem had been made) and the final values plotted on coordinate paper, as suggested above. The resulting curve showing corrections for one of the thermometers from 80° to 200° is shown in figure 21, the observed readings on the main thermometer being plotted as abscissa, the heavy lines representing 10° intervals, and the correction being plotted as ordinate, the heavy lines representing 1° intervals. Horizontally the small divisions represent 1° C. readings (observed temperature), while perpendicularly they represent 0.1° (correction). According to the curve the total correction for this thermometer (under the conditions of the experiment) at, say, 172° C. is 2.4°. In like manner the correction at any point can be read directly from the curve.

Presumably for the purpose of facilitating and encouraging more general application of the emergent-stem correction in ordinary melting-point work, several investigators have published tables of corrections, and others have recommended their use, from which the correction at certain intervals of a more or less wide range of temperature can be read directly. Two tables by Rimbach (57) give the correction for two kinds of thermometers at intervals of 5° over a range of from 70° C. to 220° C. It would appear, however, that such tables can apply accurately only to the thermometers which conform closely in construction to the type used in determining the corrections tabulated. As applied to official United States Pharmacopœia melting points such a condition amounts to prescribing an official thermometer, which has already been recommended. Should such a requirement be adopted, the determination of official corrections for emergent stem and the publication in the Pharmacopœia of such corrections, in the form of a table or a curve, would be eminently desirable, as tending not only to the great convenience of all concerned with the pharmacopœial standard, but also to a higher degree of uniformity in this particu-

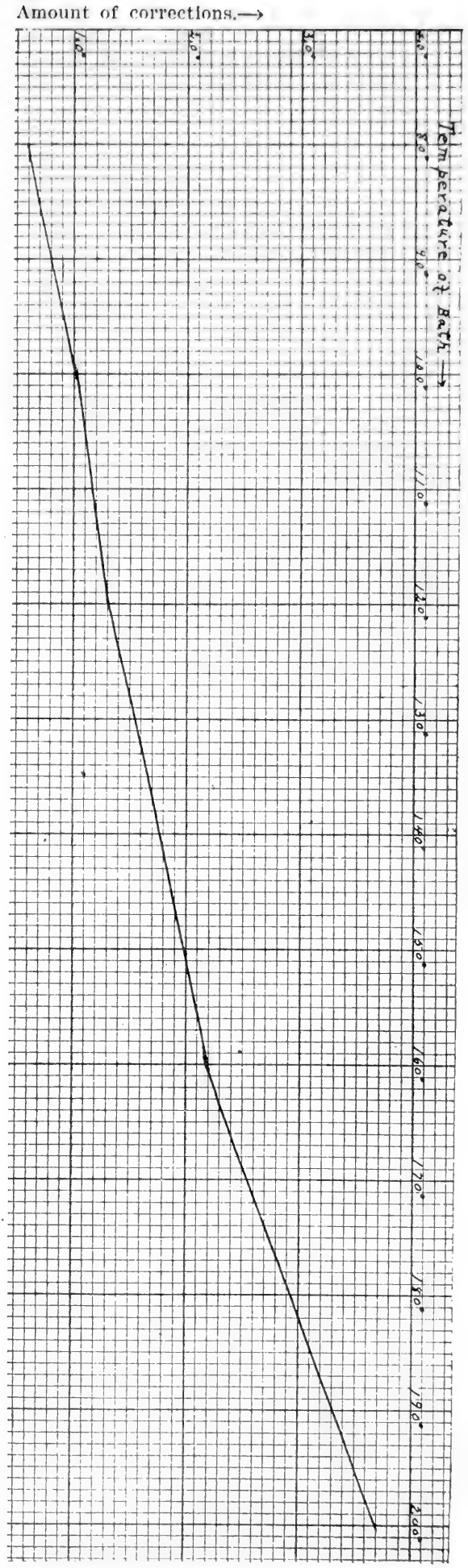

Fig. 21.—Correction curve, showing total correction (emergent stem plus calibration corrections) from 80° to 200° C.

lar than would otherwise be possible. Of the two, the publication of an official curve, rather than an official table, of corrections would seem to be the more desirable, for the following reason—the correction for temperatures lying between the intervals experimentally determined could be read directly from a curve, while from a table they would have to be calculated by interpolation.

In summary of the recommendations regarding the use of thermometers in official United States Pharmacopœia melting-point determinations, it may be stated that:

1. Some degree of uniformity in construction of the thermometers used in official melting-point tests should be required.

2. Thermometers should have been accurately standardized, or carefully compared with an accurately standardized thermometer before being used in official tests, and the calibration corrections should always be applied.

3. If an official thermometer is prescribed a table showing corrections for emergent stem at frequent temperature intervals, or a curve showing such corrections for all temperatures, should be published in the Pharmacopœia. If an official thermometer is not prescribed, the official melting-point requirements should include a clear description of the procedure to be followed in making emergent-stem corrections.

V. *Varied individual manipulation.*—Consideration of this subject, as a cause of divergence in melting-point values, includes only those details which usually depend entirely upon the individual and are largely independent of the type of apparatus or thermometer used and of the physical condition of the compound. Probably the most important of these details concern the variable rate of heating and the equally variable practice of stirring. The so-called "personal factor" or "personal equation" (or other synonymous phrase) is also a detail that may be considered under this head.

Of the two first-mentioned details of individual manipulation, undoubtedly the rate of heating is the more significant cause of divergence. Perhaps no more striking illustration of the effect of a variable rate of heating upon the melting point of the same compound could be offered than that found in Græbe's[14] discussion of the results obtained by different investigators in determining the melting point of phthalic acid. Græbe states that according to Lossen[60] the melting point of this compound is 184°, while according to Ador[61] the powdered acid melts at 203° and the large crystals at 213°. Græbe considers the high results by Ador to be due to rapid heating. He states further that the melting point of this acid, as commonly determined, varies from 190° to 195°, but that by a proper regulation of the temperature during the determination even the large crystals can be made to melt below 184°.

Replying to Müther and Tollens[58] in defense of the "Maquenne bloc"[a] for melting-point determinations, Maquenne[59] points out that for the compound phenylglucosazone melting-point values may be obtained ranging from 200° C. to 230° C. *according to the rate of heating*. Seven different values within these limits are given for this compound, together with the time required for melting in each case, using the "Maquenne bloc."

Conclusive evidence corroborating these contentions regarding a variable rate of heating—at least in the neighborhood of the melting point—was obtained in the course of the experimental work involved in this investigation, and such evidence was not limited to those compounds which show a tendency to decompose at the melting point. Other instances might be cited to prove that the present erratic practice in heating a melting-point bath is a real and serious cause of divergence in melting-point values; but the evidence already submitted is considered amply sufficient to induce the firm conviction that, if we are to approach standardization in pharmacopœial melting points, it is a prime essential that a definite rate of heating be included in the United States Pharmacopœia melting-point requirements. The disadvantage in such a requirement, involved by the necessity for continual reference to a timepiece during part of a melting-point determination, is more apparent than real. In connection with this work considerable experimentation was pursued with the purpose in view of establishing a method of heating which would eliminate the timepiece and at the same time insure a reasonably adequate and consistent regulation of the rate; but no feasible method was found.[b] A very little practice, however, quickly demonstrated the fact that the adequate regulation of the rate by reference to a timepiece was easily practicable, and upon this basis further experimentation proceeded for the purpose of establishing a rate which would accomplish the desired end. A procedure was selected and many melting points determined in accordance with it by two investigators. Subsequent comparison of the results, however, led one of the investigators to the conclusion that a slight modification in the regu-

[a] Described and illustrated by Dr. J. Konig in his "Chemie der Menschlichen Nahrungs- und Genussmittel," Vol. III, 1st pt., p. 55, (ed. 1910).

[b] It is doubtless feasible, in this connection, to devise additional apparatus which when properly regulated would insure a definite rate of heating without constant reference to a timepiece and with which the rate could be varied at will. The work in this laboratory has shown, however, that such additional apparatus, although it would probably simplify procedure, is not essential to the proper application of the method recommended. Since such a device would involve more or less special features, and therefore increased expense, its incorporation in these recommendations would seem to be inconsistent with the fundamental ideas of simplicity and economy which should characterize the official method.

lation of the heating would probably insure more perfect concordance. The modified procedure, which is here recommended for official adoption, is as follows:

The bath is heated by direct intermittent application of a small Bunsen flame to the bottom of the container, the temperature being at first raised at any desired rate consistent with reasonable caution and safety in heating glass with an open flame. As the temperature approaches a point 25° below where the substance begins to melt the rate is brought under control and from that point the rise in temperature is carefully regulated to a rate of 3° a minute. It requires very little practice—using a thermometer of the construction suggested—to insure easy control of such a rate within 0.5°. The rate of 3° a minute is maintained until the sample begins to melt—a point which is fixed in accordance with the definition of melting point previously suggested. Close observation of the sample to determine this point may cause loss of control of the temperature for not more than one-half minute. With reasonable care, however, no serious fluctuation will be involved and especial care should be observed to prevent sudden rise in temperature at this point. From the beginning of melting to the end the rate of rise in temperature is changed from the 3° a minute to 0.5° a minute. Most of the pharmacopœial compounds investigated, and which do not show decomposition at the melting point, melted, under this procedure, within a range of 1° or less.

The specific effect upon melting-point values of variable practice in the application of stirring during a melting-point determination has not been experimentally studied in the course of this investigation. It has been shown, however, that without stirring the process of heating the bath as described above will induce widely varying temperatures at different points in the bath, due, of course, to the fact that the lower portion of the bath is acquiring heat rapidly because of its close proximity to the source of heat, while heat is being distributed through the upper portions mainly by means of convection currents from the lower portions. The convection currents themselves, which really constitute channels of heated substance, induce different temperatures at different points in the upper portions of the bath. The degree to which variation in temperature at different points in the bath may be induced by heating without stirring can easily be demonstrated. If a bath be heated for a few minutes and the flame removed and then vigorous stirring be immediately applied the temperature will rise sharply to the extent of 10°, 15°, 20°, or more, according to the manner and amount of heating previously applied.

It seems a reasonable assumption that under such conditions a greater or less degree of divergence will result in melting-point determinations, depending on whether the convection currents happen to

strike the thermometer bulb and the capillary containing the sample simultaneously or the one before the other. Whatever effect variable practice in this respect may have upon melting-point values, it can readily be eliminated and at the same time a perfectly uniform temperature in all parts of the bath can be maintained if constant stirring is applied. Moreover the practice of constant stirring while heating is highly conducive to more perfect control in maintaining a definite rate of rise in temperature.

It is therefore recommended that for official melting-point determinations constant stirring of the bath throughout the experiment be made a detail of the United States Pharmacopœia melting-point requirements. To those not accustomed to apply this detail of procedure the requirement will doubtless be more or less annoying, because of the initial difficulty involved in coordinating the different movements with each hand. A little persistence, however, quickly overcomes this difficulty and the manipulation becomes practically automatic. This fact makes it easier to apply the stirring *constantly* than intermittently. In any case, however, *constant stirring* is necessary in order to properly control and distribute the temperature of the bath.[a]

The "personal factor," in so far as the phrase implies psychological variations inherent to different individuals, is doubtless a material and legitimate cause of divergence, to some slight and varying degree, in any equally conscientious comparative work. In application to the melting-point problem, however, it would appear to be equally doubtless that the ready adaptation of the phrase to the service of a shield or screen hiding careless, indifferent, and hurried manipulation, is likely to lead to its abuse. The material significance of the "personal factor," as a cause of divergence in melting-point work, is probably proportionate to the number of details of procedure left to the choice of the individual. Therefore, if all the important details of procedure are clearly defined and rigidly prescribed it would seem to be a reasonable assumption that the amount of divergence then honestly due to such a cause would not exceed the limits of a reasonably rigid standard, and so for practical purposes it may be disregarded.

VI. *Variation in physical condition and in preliminary treatment of the compound.*—Treatment of this subject, as a cause of divergence, involves three main considerations: 1. Variation in the size of the individual particles of the compound. 2. Variation in the amount of adherent moisture contained in the sample. 3. Variation

[a] The detail of stirring may be made independent of the operator by mechanical device. Such a device, however—as with any for regulating the rate of heating—is more a matter of convenience than of necessity, and is therefore a complication which should not be *required* as a part of the official method.

in the amount and kind of impurity present. Under this heading, also, may be included, 4, variation in amount of sample used for the test, which, being in some measure dependent upon the size of the capillary tube, logically involves consideration of that detail of apparatus.

1. Evidence that difference in fineness of division of a compound may cause wide variation in melting-point values as ordinarily determined is abundant and conclusive. A striking instance has already been noted where, in the case of phthalic acid, the melting point of the finely powdered substance (203°) and that of large crystals (213°), as determined by Ador ([61]), varied by the wide margin of 10°. The results obtained by Pawloff ([62]), however, in a specific investigation covering this phase of the melting-point problem, have a broader application and therefore offer more convincing evidence of the fact. This author demonstrated experimentally with different compounds that the melting point varies with the size of the individual particles of the compound, being higher for the coarser grains than for the finer. In some cases he found that a powder, in which the individual particles did not exceed a diameter of 2μ, melted 4° to 7° lower than a sample of the same compound composed of particles 0.5 to 2 mm. in diameter. For the common pharmaceutical compounds, salol, antipyrine, and phenacetine, such variation in the size of particles caused a variation in melting point of 7°, 5°–7°, and 4°, respectively.

Evidence in corroboration of that cited above was obtained early in this investigation. Before giving special consideration to fineness of division as a cause of divergence it was observed in a large number of melting-point determinations that the portion of material adhering to the inner walls of the capillary tube above the main part of the sample appeared to melt at a lower temperature than the mass of material in the bottom of the tube. This observation was at first explained upon the assumption that the difference in quantity of material would, under the conditions of the experiment, cause a difference in the melting point. The results in a special series of experiments, however, involving many determinations, indicated that, within comparatively narrow limits, a difference in the amount of sample used made no material difference in the observation of the melting point and, in view of the results obtained by Ador and by Pawloff and others, led to the final conclusion that the difference in melting point noted above was due to a difference in fineness of the particles, the finer particles clinging to the walls and the larger particles falling to the bottom or out of the tube (the latter in those cases where for experimental purposes the tube was charged, then emptied, and the melting-point determination made upon the portion clinging to the walls.)

Considering the wide difference in size of the particles of the same pharmaceutical product, as found in the open market under different labels, the above discussion makes it clear that, for adequate standardization of pharmacopœial melting points some degree of uniformity of fineness in the samples to be tested should be officially required.

It is therefore recommended that the official United States Pharmacopœia melting-point requirements provide that all substances be so finely powdered that the particles can be passed through a 100-mesh sieve before being subjected to the melting-point test.

2. In their investigation of different methods for melting-point determinations Tyrer and Levy ([25]), as previously noted, also studied the effect upon the melting point due to the presence of varying amounts of moisture, to the extent that they made comparative determinations upon the commercial product and the same dried. In this connection their results upon acetanilide, antipyrine, and phenacetine show a variation in melting point between the two forms of about 1° C.; that is, the difference in moisture content between a compound that has been dried and the commercial form of that compound may be sufficient to cause a material difference in the melting point of the two, and therefore variable practice in the matter of drying constitutes a real cause of divergence in melting-point values. Moreover, considering possible varying conditions in their manufacture and also varying atmospheric conditions, it is not reasonable to presume that commercial products will always contain the same degree of moisture. For the purpose of standardization, therefore, it becomes necessary to prescribe a manner and period of desiccation which will insure a reasonable degree of uniformity in the condition of dryness of the sample for all official tests. As previously indicated, the more progressive of the foreign pharmacopœias, at least in the matter of melting-point standardization, require that the substance be finely powdered and dried in a desiccator over pure concentrated sulphuric acid for twenty-four hours as a preliminary to the melting-point test.

It may be contended that if the moisture content of the commercial product is admittedly variable it would logically require different periods of desiccation to insure a uniform degree of dryness which would make standardization in this respect impracticable. True; but during this investigation the results obtained indicate that a 24-hour period of proper desiccation is amply sufficient, for practical purposes, to cover any reasonable variation in the moisture content of a commercial product of pharmacopœial standard. The period suggested would certainly be sufficient if the committee of revision would adopt as official some reasonable limit of moisture content for the different classes of pharmacopœial products. During investiga-

tion of this detail of procedure the drying period for many different commercial samples was allowed to exceed the 24-hour limit in varying degree, but without significant effect upon the melting point. In some cases the samples were dried for two months, but even in those cases the effect, on the melting point, of the period in excess of the twenty-four hours was less than 0.5°.

It is therefore recommended that the United States Pharmacopœia melting-point requirements provide further that all substances be dried in a desiccator over concentrated sulphuric acid for a period of twenty-four hours as a preliminary to the official test.

3. It is, of course, well known that the melting point of a pure substance is lowered by the presence of impurity, and that, generally speaking, the amount of lowering is proportional to the amount of impurity present. The value and efficiency of the melting-point determination, by a practical method, as a means of measuring quantitatively, or of detecting moderate variations in the amount of impurity present, however, seems not to have been definitely established. But the *percentage* of impurity is not the only factor that enters into this phase of the melting-point problem: the *kind* of impurity may also be significant. Considering a molten mixture as a solution, the principal compound representing the solvent and the impurity the solute, it is apparent that the lowering of the melting point (freezing point) of the solvent (principal compound) will be subject to the same laws that apply to ordinary solutions; that is, the amount of lowering of the melting point will depend not only upon the percentage but also upon the molecular weight of the dissolved substance (impurity).

Ostwald([63]), however, by many series of melting-point determinations on appropriate binary mixtures of different organic acids has shown that within certain percentage limits a difference in the molecular weight of the impurity will cause practically no difference in the lowering of the melting-point, even in mixtures containing 15 to 20 per cent or more of the different impurities. For example, consider the pure substances I, II, and III, each having a different molecular weight. If two mixtures of these substances be made consisting, respectively, of 80 per cent I with 20 per cent II and 80 per cent I with 20 per cent III, the lowering of the melting point of I in each case will be practically the same. So also with similar mixtures of II with I, II with III, etc. In Ostwald's experiments the effect of variation in molecular weight was negligible in some cases, even when the mixtures contained 40 per cent of impurity. Other investigators have been active in this large field of research, but their results are not so easily applied to this discussion as those by Ostwald. No specific investigation along this line has been pursued in this laboratory, but there seems to be no good reason to question the gen-

eral application of Ostwald's results. Therefore, considering that the purity of synthetic organic drugs of pharmacopœial standard is or should be well within the limits above quoted, it would appear obvious that variation in kind of impurity as a factor in the attempted standardization of pharmacopœial melting points may for the present be entirely ignored.

The efficiency of the melting-point determination as a quantitative method of analysis for binary mixtures of organic compounds has been tested by various investigators. As previously indicated (see p. 18), Tingle and Roelker ([3]) attempted to so utilize the test in determining the percentage composition of binary mixtures of the three isomeric nitranilines. In this work variations of 2 per cent in the composition of the mixtures within the limits of 90 to 98 per cent of either constituent affected the melting point, as determined by these authors, to the extent of from about 0.5° to 2°. From the results of their work the authors conclude that the composition of such mixtures may be determined within 2 per cent by the melting-point test. Such a conclusion, applied to pharmacopœial compounds, would indicate that the melting-point test alone may be relied upon to standardize the compound within 2 per cent of the purity rubric—a striking commentary upon the value and efficiency of the test. The contention of White ([1]) that the addition of impurity has a much greater effect in extending the melting *interval* than in lowering the melting *point* suggests that if in the above work the melting interval had been measured instead of a rather indefinite point the efficiency of the method for determining the percentage composition of the mixtures might have been proportionately increased. Similar but less complete work by Holleman and Sluiter([64]) yielded results which indicate practically the same degree of efficiency for the melting-point test that is shown in the Tingle-Roelker results.

When acetanilid is mixed with phenacetin much difficulty has been found in detecting and quantitatively determining the former by analytical methods. For this reason the melting points of mixtures, in all proportions, of these two substances have been made an object of study by various investigators to determine the efficiency of this test for the purpose of detecting the acetanilid. Special investigations of such mixtures have been made by Schweitzer ([65]), by Kebler ([66]), and by Beringer ([67]). The results obtained are tabulated by the different investigators as here reproduced.

TABLE III.—*Melting points of mixtures of phenacetin and acetanilid (Schweitzer).*

No.	Mixture consisting of—		Begins to melt at—	Completely transparent at—
	Phenacetin.	Acetanilid.		
	Per cent.	*Per cent.*	°C.	°C.
1	99	1	92	134
2	95	5	92	133
3	85	15	92	128
4	66⅔	33⅓	92	126
5	60	40	92	125
6	50	50	92	122
7	40	60	92	118
8	33⅓	66⅔	92	114
9	15	85	92	110
10	5	95	92	106
11	100	0	133	134.5
12	0	100	112	114

TABLE IV.—*Melting points of mixtures of phenacetin and acetanilid (Kebler, drug laboratory).*

No.	Percentages in mixtures of—		Melting point.	
	Phenacetin.	Acetanilid.	Begins to melt at—	Completely transparent at—
	Per cent.	*Per cent.*	°C.	°C.
1	90	10	96	127
2	75	25	92	121
3	50	50	90	92
4	25	75	90	99
5	10	90	95	110
6	100	0	133.5	134.8
7	0	100	112	113.8

TABLE V.—*Melting points of mixtures of acetanilid and phenacetin (Beringer).*

Acetanilid.	Shrinks.	Softens.	Melts.	Complete fusion.
Per cent.	°C.	°C.	°C.	°C.
1	126	128	131–132	134
2	115	125	130–131	132
3	110	121	128–129	131
4	105	120	125–126	130
5	100	108	112–114	124
10	100	105	110–112	120
15	95	105	110–112	120
20	92	100	105–106	118
25	90	94	95–96	115
30	90	92	94–95	115
35	85	90	92–94	108
40	84	90	91–93	105
45	82	90	92–93	100
50	80	86	90–92	96
55	77	86	90–92	95
60	82	88	90–92	94
65	82	87	90–92	94
70	87	90	91–92	95
75	88	90	92–93	96
80	88	90	91–92	99
85	90	92	95–96	105
90	92	95	100–101	108
95	96	102	107–108	111

As is indicated by the values in the column under "Begins to melt at—" in the Schweitzer table, he made the remarkable observation that all mixtures of phenacetin and acetanilid begin to melt at the same point. He therefore concludes that the detection of acetanilid in phenacetin needs no further examination than a determination of the melting point. The other investigators seem not to have consistently found such an ideal condition, except perhaps for a limited range of mixtures approximating the eutectic. It is difficult to conceive that perfectly uniform mixtures of equally pure compounds in the same proportions would show as widely divergent behavior on melting as is indicated by comparison of some of the results given in the different tables. Without an exact knowledge, however, of the method and detailed procedure applied by each of the different investigators comparative study of such data is of little value, except perhaps to still further illustrate the confusion that now prevails in melting-point work and to indicate the urgent need of investigation and standardization.

To the extent that Beringer's table gives the more complete data, including records of observation of all possible changes and a more consistent variation in the results for different mixtures, his investigation might be considered to represent the more exhaustive and the more careful work, and therefore to yield the more reliable results. Upon such an assumption his results indicate a high degree of efficiency for the melting-point test as a means of detecting variations in the impurity content.

It will be noted in Beringer's table that for each variation of *1 per cent* in the composition of the first four mixtures the melting point of phenacetin (final column) is lowered by the comparatively wide margin of from *1° to 2°*. These results also offer some measure of corroboration of the claim that the low end of the melting interval is extended by impurity more than the upper limit is lowered—one or more of the results recorded for the first three points of observation being more affected by the 1 per cent variation in composition than the final value. In further exhaustive investigation of many market samples of phenacetin Beringer (loc. cit.) found, in some cases, that the presence of about 3 or 4 per cent of acetanilid lowered the melting point of phenacetin about 3° or 4°, thereby corroborating some of the results obtained in his special investigation of the melting points of known mixtures.

The evidence obtained from Beringer's results, and also from those by Tingle and Roelker[3], Holleman and Sluiter[64], White[1], Ostwald[63], Landolt[20], Tyrer and Levy[25], and many others, even if not entirely convincing, certainly encourages the conclusion that the melting-point test properly applied is quite adequate to the detection, within moderately exact limits, of varying amounts of impurity,

and if methods and procedure are sufficiently refined is therefore well adapted to the exact standardization of such pharmacopœial compounds as admit of its application.

4. The special series of experiments referred to on page 72, in connection with fineness of division, also included consideration of (*a*) the practice of remelting in determinations of the melting point; (*b*) the effect upon the melting point of variation, within narrow limits, in the amount of sample used; and (*c*) the effect of variation in the size of the capillary tubes. The results of many determinations upon salicylic acid led to the conclusion that the practice of remelting would, for pharmacopœial purposes, involve complication without compensating advantage, and is therefore opposed. Other investigators have opposed the practice on the ground that it causes high results. In these experiments, however, the results, as compared with direct determinations, were low, due possibly to our failure to allow sufficient time for complete cooling and solidification after the first melting.

As previously indicated, the degree of variation in the amount of sample used in different experiments was considered to induce no material variation in the observation of the melting. It was subsequently learned, however, that the limits of charge applied in these experiments were not nearly as widely divergent as may commonly prevail in the application of capillary-tube methods. The conclusion derived is therefore not entirely convincing. In this connection, however, as in all others, the keynote of standardization is uniformity of practice, and therefore the amount of sample to be used in determinations of official melting points should be at least approximately prescribed.

What constitutes an adequate and convenient charge of sample for a melting-point determination by a capillary-tube method depends in some measure upon the size of the capillary tube. In the experiments under discussion the tubes used varied in diameter from about 0.5 mm. to about 1.5 mm., which were then considered to include the limits of a reasonable interpretation of the term "capillary tube" as applied to melting-point work. It is now recognized that, in ordinary practice, at least the higher of these limits may be considerably extended. In our experiments there was no variation either in temperature interval or time interval over which the sample melted in tubes of different size, other details of procedure being the same. These observations agree practically with those of Landolt,[26] who used tubes of the following diameters: 0.6 mm., 0.8 mm., 1 mm., 1.5 mm., and 3 mm. The results of some of the experiments referred to above are given in the following table. The first evidence of change in the sample is recorded in the table as "first sign." The "beginning of melting" at the time these experiments were made was not

so carefully defined as is recommended in this bulletin (see p. 21), and other details of procedure varied somewhat from those finally recommended. For the purpose intended, however, these details were not significant. The practice of determining the melting point by observation of the particles clinging to the walls of the capillary was discarded in favor of the ordinary method, because the former was considered more difficult to define. In all cases the rate of heating was the same.

TABLE VI.—*Uncorrected melting points of salicylic acid as determined by different procedures with a capillary-tube method.*

Procedure.	No.	First sign.	Beginning of melting.	Complete fusion.
Powder adhering to capillary walls	1	148	154	156
	2	149	154	156
	3	148	154	156
	4	148	154	156
The same when remelted after crystallization	5	154	154	155.5
	6	154	154	155.5
	7	154	154	155.5
	8	154	154	[a] 155.5
Usual method	9	148	154	157
	10	148	154	157
	11	148	154	157
Large charge of sample	12	148	154	157
Small charge of sample	13	148	154	157
	14	148	155	157

[a] In this instance, as in others, one person observed changes in the sample, while another regulated the heating and recorded the temperature corresponding to the different points of observation.

According to Tyrer and Levy([25]) a very important detail in the construction of the capillary tube is that the walls of the tube should be as nearly as possible the same thickness as the walls of the bulb of the thermometer used. It is probable that variation in the thickness of the walls of the tube constitutes the greatest danger of divergence in melting-point values in so far as the capillary tube may be considered the cause. Whatever may be the effect, however, of variation in the amount of sample for a capillary-tube charge, or of variation in the dimensions of the capillary tube, in producing the prevailing divergence in melting-point values, it can doubtless be practically eliminated by officially prescribing reasonable limits for the dimensions of the capillary tube and for the amount of sample to be used in official melting-point determinations.

As before stated, it seems desirable—as tending toward an international standard—to conform to the requirements of foreign pharmacopœias when such a policy does not retard progressive development. Certain of the foreign pharmacopœias (see p. 53) require the use for official melting-point determinations of a capillary tube having a diameter of 1 mm. and a charge of sample sufficient to form a column 3 mm. high in the bottom of the tube. In view of the practically negligible effect produced upon the melting point by slight

variations in the size of the capillary-tube, and the consequent variation in the charge of sample, such exact requirements seem unnecessary, if not unreasonable and impractical. It is much more reasonable and practical and accomplishes the purpose of pharmacopœial standardization probably quite as well to require the use of a capillary tube closely approximating 1 mm. in diameter at the sealed end (not less than 0.8 mm. nor more than 1.25 mm. internal diameter for a distance of at least 5mm.). Likewise the charge of sample required should be sufficient to form a column approximately 3 mm. high (not less than 2.5 mm. nor more than 3.5 mm.) in the bottom of the tube. The difference between the two requirements considered on an absolute basis is very slight, but when applied in practice would be found to be very considerable.

Of two capillary tubes having the same internal diameter the relative thickness of their walls will doubtless depend jointly upon the kind of glass from which they were made and upon the manipulation applied in making them. The preparation and charging of a melting-point tube is described by Mulliken([15]) as follows:

> They are most readily made from rather soft glass tubes having an internal diameter not less than 1 cm., not, as is often done, from small-bore tubing. A piece of such tubing, of convenient length, is supported at both ends and rotated in a blast-lamp flame until a ring 2 cm. long has been heated to dull redness. Then, upon separating the hands rather slowly, until both arms are outstretched at full length horizontally from the shoulders, a capillary of the desired diameter and nearly 2 meters in length may be drawn out in a single operation.
>
> To charge the melting-point capillary force its open end downward into a small mound of the finely powdered substance. Then, holding upward the open end, which will now be closed by a short plug of the compacted powder, draw the flat side of a file horizontally across the tube a little below the substance. The powder, loosened by the vibrations set up in the glass, will quickly slide down into the desired position.

For this investigation, involving the use of a large number of capillary tubes, a similar but somewhat larger tube (about 20 mm. internal diameter, with walls about 2 mm. thick) was softened in a blast-lamp flame over an area a little larger than that suggested by Mulliken and quickly drawn out by two men to the extent of 12 to 15 feet. When the operation is properly performed very little of the resulting capillary will vary from the desired dimensions, either in internal or external diameter. The capillary tube thus prepared was cut or broken into appropriate lengths, approximately double the length required for a single test, and the two ends carefully sealed to prevent accumulation of dust and consequent contamination of the sample when introduced. In sealing a capillary tube the mistake is often made of fusing too much of the glass, thereby thickening the walls, reducing the diameter of the tube, and causing the formation of a glass bead at the sealed end. In sealing

the tube only the edges should touch the flame, and it should be heated only enough to close the end. When required for use the double-length tube is broken at the center and serves for two determinations. Of a great many tubes prepared as described comparatively very few of them exceeded the dimensions recommended above. Figure 22 represents one of the tubes in exact dimensions as closely as it could be drawn. It is very nearly 1 mm. in diameter. The shaded portion represents a charge of sample forming a column very close to 3 mm. high when packed down as closely as light tapping of the tube on a solid surface will induce.

A summary of the recommendations for the revision of the melting-point requirements of the United States Pharmacopœia, which have been submitted in this bulletin, consists of the following:

1. The so-called "melting point" should be carefully defined. Instead of a single value the definition should specify a melting interval representing a range of temperature within which a substance, in order to be considered of pharmacopœial standard, must melt completely, the beginning and end of melting being also clearly defined (see pp. 17–21).

FIG. 22.—Size of capillary tube and amount of charge of sample recommended.

2. The decomposition point should be clearly differentiated from the melting point and its determination should not be required as an official test, or at least not as a test of purity, until its value for such a purpose has been more definitely determined. As a means of distinguishing between compounds of similar appearance but which decompose at different temperatures the decomposition-point determination is of value (see pp. 21–29).

3. For all official melting-point determinations the requirements should prescribe the application of a perfectly uniform method (or methods). The official method should involve apparatus which is as simple in units and in construction, as readily available, and as economical as is consistent with reasonable efficiency. In this connection recommendations with regard to the melting-point bath for the class of compounds investigated are also included (see pp. 29–61).

4. If feasible the use of official centigrade thermometers should be required for official melting-point tests. Such a requirement should provide for uniformity of construction, including quality and thickness of glass, length of thermometer, range of registration, size of bulb, etc., and standardization. Correction of observed readings, because of emergent stem of the thermometer, should be required in accordance with a prescribed formula. If the requirement of an official thermometer is adopted an official table or curve of such corrections should be prepared and published in the pharmacopœia—if

not, then the correction should be required of the individual manipulator (see pp. 61–68).

5. Details of manipulation as applied to the official apparatus should be clearly described and uniformity of practice therein rigidly required. Constant stirring of the melting-point bath during an official test, and a uniform rate of heating of 3° per minute, from 25° below the melting point to the beginning of melting, and of 0.5° per minute, during the melting interval, is recommended (see pp. 68–71).

6. For the class of compounds considered in this bulletin the requirements should provide that, as a preliminary to the official test, the compound must be so finely powdered as to pass through a 100-mesh sieve; and that the powdered substance must be dried for twenty-four hours in a desiccator over concentrated sulphuric acid. A sample of the powdered and dried substance for an official test should be sufficient to form a solid column (tapped down) 3 mm. high in the bottom of a thin-walled capillary tube, the internal diameter of which is not less than 0.8 mm. nor more than 1.25 mm. (see pp. 71–79).

Adaptability to different classes of compounds of the method recommended: It is probably true that no single method for melting-point determinations can be readily adapted with equal efficiency to all classes of compounds. In approaching this investigation it was assumed that the solid crystalline organic compounds comprise the largest class in application to which the melting-point determination is of the greatest practical importance. Therefore, in the development of the method and procedure recommended, attention was concentrated upon this class of compounds, and as yet experimental investigation has extended to no other. It is considered, however, that the efficient application to other classes of compounds (such as fats, waxes, etc.) of the method suggested involves only slight modification in details of procedure and not any material change of apparatus. Furthermore, there would appear to be no serious difficulty in its adaptation as a modification of Landolt's method in those cases where it would seem feasible and desirable to determine the melting point or freezing point of a compound by using a comparatively large charge, with the thermometer dipping directly into the substance under investigation. These are details, however, which can be satisfactorily determined only by further experimentation.

STANDARDIZATION OF MELTING POINTS OF PHARMACOPŒIAL COMPOUNDS.

For this important part of the work involved in this investigation it was considered extremely desirable that a market sample from every manufacturer of each product should be obtained. To that end we entered into correspondence with several manufacturing firms, but their extreme conservatism in giving information on this point

soon disclosed the impracticability of the plan, and we were forced to adopt the unsatisfactory alternative of considering that every sample in the market bears the label of the firm that makes it. Acting upon this alternative, we were able to conveniently obtain, in most cases, from 5 to 8 different samples of each of the compounds selected for immediate standardization, though in a few instances two or more samples bore the same label. As before stated, such selection at first comprised a list of 37 of the more important organic pharmacopœial compounds, of which list, however, 13 compounds could not be immediately standardized for reasons already discussed (see pp. 13–14). From 4 to 8 or more determinations of the melting point were made upon each of the samples of the compounds finally selected for immediate standardization, the method and procedure being in accordance with that recommended in this bulletin. The melting points having been so determined, each of the samples were standardized in duplicate, according to pharmacopœial tests.[a] In all melting-point determinations the first observable evidence of change was recorded as the "first sign," an approximate point only.

These melting-point determinations, including some upon compounds which decompose, are briefly summarized as follows, only the average uncorrected results of all determinations on an individual sample being given in each case, the average of closely agreeing results for different samples being corrected for the standard value:

Acetanilide (6 samples):

Sample.	First sign.	Beginning of melting.	Complete fusion.
	Degrees.	*Degrees.*	*Degrees.*
	[a] 100±	110.9	112.5
(recrystallized)	112±	113.0	113.5
	110±	112.3	113.0
	109±	111.8	112.8
	106±	110.6	112.5
	110±	112.1	113.0

[a] The sign ± indicates approximate values.

The average of a and e corrected=104°±, 111.9°–113.7°.
The average of c, d, and f corrected=111°±, 113.3°–114.1°.
In all cases the corrected values represent, as in the tables, the first sign, beginning of melting, and complete fusion, respectively.

Sample b was a purified sample. The final result in all cases was a clear, colorless liquid. All samples conformed to other United States Pharmacopœial tests. In spite of that fact, however, it will be observed that for one group of standard samples the average melting point differs quite considerably from the average melting point

[a] A procedure which incidentally disclosed several other chemical defects in pharmacopœial tests.

of another group, also standard, the melting point of the individual members of each group being practically the same. Such a condition was found to prevail, as will be noted, for many of the compounds standardized, and is suggestive of several theories by way of explanation, the most tenable of which probably is that the melting-point determination is a more delicate test than others prescribed by the pharmacopœia. Whether or not the official values for the melting point in such cases should include the limits of all values found for different samples, otherwise standard, is a question for the committee of revision to decide.

Phenacetine and acetphenetidine (7 samples):

Sample.	First sign.	Beginning of melting.	Complete fusion.
	Degrees.	*Degrees.*	*Degrees.*
a (not labeled)	128±	132.0	133.0
b (impure)	90±	114.0±	125.0
c	130±	132.5	133.1
d	131±	132.7	133.3
e	131±	132.5	133.2
f	131±	132.4	133.1
g	132±	132.7	133.2

The average c, d, e, f, and g corrected=133°±, 134.2°–134.8°.
The readings for sample a corrected=129°±, 133.6°–34.6°.

Sample a is considered separately, because, while the margin of difference it shows as compared with the others is not very great, that difference, as disclosed by many determinations, was perfectly consistent. In all cases except b there was little sign of change below the melting point and the final result was a clear, colorless liquid. Sample b contained acetanilide. The effect upon the melting point was very striking, but the contamination was not so easily detected by other pharmacopœial tests. All other samples conformed to other pharmacopœial tests.

Acid, benzoic (5 samples):

Sample.	First sign.	Beginning of melting.	Complete fusion.
	Degrees.	*Degrees.*	*Degrees.*
a	105±	120.2	121.
b	105±	120.5	121.
c	105±	120.6	121.
d	105±	120.4	120.
e	105±	120.0	120.

The average for all samples corrected=106°±, 121.3°–122.2°.

All samples melted sharply to a clear colorless liquid, but show some measure of sublimation below the melting point.

Duplicate analyses of all samples showed them to contain 98 per cent or more acid.

Acid, camphoric (6 samples):

Sample.	First sign.	Beginning of melting.	Complete fusion.
	Degrees.	*Degrees.*	*Degrees.*
	170±	183.8	185.0
	155±	181.9	183.4
	174±	183.5	184.5
	170±	180.5	182.5
	165±	181.0	182.0
	165±	181.3	182.8

Reading for sample a corrected=172°±, 186.4°–187.7°.
Average of d, e, and f corrected=167°±, 183.5°–185.4°.

The melting-point determinations on this compound were unsatisactory. In all cases the change from "first sign" to "beginning of nelting" was a gradually increasing one, which made it more difficult to exactly determine the latter point. The final result was a lear colorless liquid. Samples b and c were recovered from other ork and therefore can not justly be considered of pharmacopœial tandard. The other samples were all tested from original packges. Corrected readings for sample a are given separately, because n many consistent determinations it showed a constant variation rom the other samples of considerable magnitude. The readings or samples d, e, and f, although not as concordant as desirable, are veraged in making the correction. Corrected values, including the xtreme limits of melting interval shown by all three samples (180.5°–83°), are 183.4°–186°.

Duplicate analyses of all market samples showed them to contain 8 per cent or more acid.

Acid, salicylic (5 samples):

Sample.	First sign.	Beginning of melting.	Complete fusion.
	Degrees.	*Degrees.*	*Degrees.*
	140±	156.0	156.9
	140±	156.5	157.1
	140±	156.4	157.0
	140±	155.5	156.4
	140±	156.0	156.8

Average of a, b, c, and e corrected=142°±, 158.2°–158.9°.
Value of sample d corrected=142°±, 157.5°–158.4°.

With these samples of salicylic acid there was a gradual contraction of the capillary charge above the first sign, becoming pronounced ust below the beginning of melting. The final result in all cases vas a practically clear and colorless liquid.

Duplicate analyses of all samples showed them to be 98 per cent ure or better.

Acid-tartaric (6 samples).—After much experimentation with different samples of this compound the tentative conclusion was reached that it should be placed with the class of compounds which decompose on melting. In the case of tartaric acid the effervescence at the melting point was not very vigorous with any sample; but bubbles were held in the mass, giving the appearance of a mushy or partly molten condition, which gradually cleared upon standing, even though the bath was cooling, leaving a clear, colorless substance. This behavior made the exact determination of the melting point very difficult, although concordant results for the same sample by the same individual were easily obtainable. It is in cases of this sort, however, that the "personal factor" would constitute a very significant cause of divergence. The corrected values obtained for the melting interval with different market samples ranged from 165.7°–167° for the lowest to 167.9°–168.4°, the highest.

The samples were not subjected to the other pharmacopœial tests.

Aconitine (6 samples: 3 white, crystalline; 3 amorphous, with slight tinge of yellow).—Of these samples it is, of course, only the crystalline form that is required by the United States Pharmacopœia to conform to a melting-point standard.

The behavior of this compound on melting has already, perhaps, been sufficiently discussed (see pp. 22–24). It decomposes vigorously at the melting point, and a wide range of values may be obtained according to the manipulation applied. Heated at the rate of 3° per minute from about 165° C., the corrected values obtained for the three crystalline samples approximated 188.6°–189.6°. The point required by the pharmacopœia is 195° C.

The samples were not subjected to other pharmacopœial tests.

Ammonium benzoate (4 samples).—As in the case of aconitine, the melting behavior of ammonium benzoate has already been made the subject of considerable discussion (see pp. 25–29). Before the factor of decomposition, as a complication in melting phenomena, was fully appreciated, however, many determinations upon the four samples of this compound had been made in the attempt to establish adequate procedure for obtaining a standard value. Corrected results obtained by the same manipulation applied to aconitine approximated 192.3°–193.3° C. The value required by the pharmacopœia is 193°–194° C.

The samples were not subjected to other pharmacopœial tests.

Antipyrine (6 samples):

Sample.	First sign.	Beginning of melting.	Complete fusion.
	Degrees.	*Degrees.*	*Degrees.*
	108.5±	109.1	109.7
	108.5±	109.0	109.7
	108.5±	109.1	109.7
	108.5±	109.2	109.8
	108.5±	109.2	109.8
	108.5±	109.3	109.8

The average of all samples corrected=109.5°±, 110.2°–110.8° C.

All samples melted to a clear, colorless liquid.

All samples conformed to other pharmacopœial tests.

Apomorphine hydrochloride (6 samples).—This compound was investigated only sufficiently to show that it decomposes at the melting point.

Atropine (3 samples).—Only three samples of this compound bearing different labels were conveniently obtainable.

Sample.	First sign.	Beginning of melting.	Complete fusion.
	Degrees.	*Degrees.*	*Degrees.*
	112±	113.8	114.8
	111±	112.9	113.9
	112±	113.9	114.8

The average of a and c corrected=113°±, and 115°–116° C.
Observed readings of b corrected=112°±, and 114.1°–115.1° C.

All samples melted to a clear, colorless liquid and were considered standard, though several of the other pharmacopœial tests were considered unsatisfactory.

Betanaphthol (8 samples):

Sample.	First sign.	Beginning of melting.	Complete fusion.
	Degrees.	*Degrees.*	*Degrees.*
	118±	119.9	120.4
	119±	119.9	120.4
	119±	119.9	120.4
	119±	119.8	120.4
	119±	120.0	120.4
	119±	119.9	120.4
	115±(119)	120.2	120.9
	115±(119)	120.0	120.7

The average for all samples corrected=119°±, 121.2°–121.8° C.

All samples melted sharply to a clear liquid having a slight brownish tinge. A very slight red-brown sublimate also appears on the sides of the capillary below the surface of the bath.

All samples conform to United States Pharmacopœia standard, according to other tests.

Benzosulphinide (saccharin) (7 samples).—The melting behavior of this substance was found to be extremely unsatisfactory. Different samples showed the first sign of change about 180° to 190° C., the change gradually increasing to the "beginning of melting" point, which was therefore difficult to define, and for different samples was observed at about 208°, 212°, 213°, 215°, 217°, and 220° C., respectively. The point of "complete fusion" as observed was much more concordant, varying only from about 220° to 222° C. The melting interval for the different samples varied from 2° to 12°, though reasonably constant for each sample. Although remelting disclosed no definite evidence of prior decomposition, it was concluded that this compound should be made the object of special investigation to determine the cause of such peculiar behavior before attempting final standardization of its melting point. The Pharmacopœia gives the melting point as 219° to 220° C., while the melting interval for the above samples, determined in accordance with the recommendations made in this bulletin, varied from 220°–222° to 208°–220.5° C. (uncorrected readings; corrections were not determined at this temperature, but by extrapolation would doubtless be in the neighborhood of 4° and 5° for the thermometer used, which would make the corrected readings about 225°–227° to 212°–225°).

Camphor (4 samples).—The resinous character of this substance was considered to exclude it from convenient investigation by a method involving the use of a closed capillary tube. It was further assumed that it could be conveniently investigated by a method which could be applied to fats and waxes, and it was therefore tentatively placed in that class of compounds.

Camphor-monobromide (5 samples):

Sample.	First sign.	Beginning of melting.	Complete fusion.
	Degrees.	*Degrees.*	*Degrees.*
a	74±	75.1	75.7
b	73±	74.4	75.1
c	73±	74.4	75.1
d	73±	74.4	75.1
e	74±	75.0	75.7

The readings for b, c, and d, corrected=73.5°±, and 74.8°–75.6°.
The readings for a and e, corrected=74.5°±, and 75.5°–76.2°.

Sample a was a recrystallized sample, but sample e was a market sample. All samples melted sharply to a colorless liquid and conformed to other pharmacopœial tests.

Chloral-formamide (chloralamide) (1 sample).—Apparently this compound can be obtained in the open market only under the same

label; therefore only one sample was investigated. It shows the first sign of change about 100° C.—more sharply at 108°—and melts at 115.2° to 116°.

Corrected readings = 101°±, and 116.4°–117.2°.

The first evidence of change gradually increased to beginning of melting, becoming very definite at 108°, and at the point of complete fusion the charge is a clear, colorless liquid. There is slight evidence of decomposition, which becomes very definite on heating the molten mass to about 175°.

The sample conformed to other pharmacopœial tests.

Cocaine (6 samples):

Sample.	First sign.	Beginning of melting.	Complete fusion.
	Degrees.	*Degrees.*	*Degrees.*
a	93±	95.0	95.7
b	94±	95.9	96.6
c	94±	95.5	96.1
d	93±	95.1	95.7
e	95±	96.0	96.8
f	93±	96.5	99.0

Average readings for a, c, and d, corrected=94°±, and 96.2°–96.8° C.
Average readings for b and e, corrected=95°±, and 97°–97.7° C.
Readings for sample f, corrected=94°±, and 97.5°–100° C.

The final result in all cases was a clear, colorless liquid.

Three sets of consistent results were obtained. The average of the determinations for each of the samples a, c, and d do not agree quite as closely as those for each of b and e, but are nevertheless considered to be reasonably concordant, and are therefore averaged for determining corrected values which may apply to all three samples. The extremes of the two corrected averages (a, c, d, and b, e) might possibly be included in a single range without doing violence to a reasonable standard for pharmacopœial compounds. The divergence between the values obtained for sample f and those obtained for all other samples, however, would seem to be too great to pass sample f unchallenged as a standard product. The fact that all samples conform to other pharmacopœial tests, as nearly as could be determined, is by no means conclusive. The pharmacopœial tests for cocaine are considered to be extremely unsatisfactory, because of very inadequate directions for converting the cocaine into cocaine-hydrochloride. The pharmacopœial requirements in this connection should certainly be given careful consideration in the next revision.

Cocaine-hydrochloride (8 samples).—This compound was found to decompose at the melting point, and therefore was not standardized. Some of the results obtained with these samples, however, are of interest in further elucidation of the subject of decomposition point. Different samples were casually investigated while testing high-tem-

perature baths. In each experiment the rate of heating was not definitely regulated, but was applied as is ordinarily done in the average melting-point determination. In three experiments with sample b the "first sign" was observed, respectively, at 195°, 186°, and 190° C., the corresponding decomposition points being 197°–198°, 187°–188°, and 195°–196° C. A fourth experiment was accidentally interrupted for a few minutes when the temperature was at 180°, the bath being allowed to cool during the interruption. On renewing the heating the first sign was observed at about 170°, and decomposition was complete at 179° C. In another experiment a sample which decomposed at about 198°–199° was mixed with a considerable but indefinite proportion (probably 25 per cent) of cocaine (melting point about 96°), and a capillary charge of the mixture was introduced into a bath which was at a temperature of about 90° C. Sharp contraction of the sample occurred immediately, but on fairly rapid heating no further change was observed until decomposition occurred at about 208° C., a result which in some measure corroborates the work with ammonium-benzoate-benzoic-acid mixtures.

The United States Pharmacopœia states that cocaine-hydrochloride "melts at about 189.9° C.," and adds that "minute quantities of impurities may reduce the melting point to 180° C."

Hexamethylenamine (6 samples).—This compound decomposes below the melting point and was therefore not standardized. The first sign of change occurred at about 165° C. Ammonia is gradually and continually given off up to over 260° C. (limit of range of the thermometer used) without complete fusion of the sample.

The United States Pharmacopœia states that it sublimes, with partial decomposition and without melting, at 263° C.

Homatropine-hydrobromide (5 samples):

Sample.	First sign.	Beginning of melting.	Complete fusion.
	Degrees.	*Degrees.*	*Degrees.*
a	200±	(205) 206.5	209.0
b	203±	(206) 208.0	210.5
c	203 ±	(205) 206.5	209.5
d	203 ±	(205) 207.0	210.0
e	202 ±	(205) 206.5	208.7

Average readings for a, c, and e, corrected, =206° ±, and (209) 210.4°–213.1° C.
Average readings for b and d, corrected, =207° ±, (210) 211.4°–214.5° C.

The behavior on melting of these five samples of this compound was very unsatisfactory. The change above the first sign is gradual, and renders the beginning of melting difficult to define, hence the additional values at that point. The samples all melted to a red-brown liquid with slight decomposition, the latter being confirmed by remelting, though vigorous and sharply defined decomposition

does not occur util above 250° C. Because of the decomposition at the melting point this compound should probably be further investigated before a standard melting point is officially adopted. Many uniform determinations on different samples, however, seemed to justify their consideration at this point.

All samples conformed to other pharmacopœial tests, except that repeated attempts to obtain the free base, according to the directions by the Pharmacopœia, resulted in absolute failure in all cases, another detail of pharmacopœial tests that should receive attention.

Hydrastine (5 samples).—Hydrastine requires further investigation because of striking contrast in the behavior of different samples of pharmacopœial standard. By other than the melting-point test all five samples investigated were found to be of pharmacopœial standard. The Pharmacopœia states that "hydrastine melts at 131° C." Only one of the five samples melted completely (i. e., to a perfectly clear liquid) at about that temperature. There was slight change in color at the "first sign" and slight effervescence at the melting point. On allowing the molten mass to cool and completely solidify and then remelting, the melting point observed was about 142° C. This result suggested simply that the compound decomposes at the melting point, forming a product which melts at the higher temperature. But two other commercial samples showed only sharp contraction at about 130°, and practically no further change until melting occurred at about 142°. The remaining two samples melted at about 131° to a turbid liquid, which did not become clear until the higher temperature was reached. If such divergent and peculiar behavior of different samples of the same drug is consistent with a "pharmacopœial standard" and is in the interest of the public health, it would appear that the application to such compounds of the melting-point test, as a means of standardization, is quite useless.

Hydrastine-hydrochloride (6 samples).—Different market samples of this compound were found to decompose at temperatures varying, presumably with varying rate of heating, from 175° to 220° C. Obviously therefore further investigation is needed. The United States Pharmacopœia requirements state that "hydrastine-hydrochloride melts at 212° C."

Hyoscyamine-hydrobromide (3 samples):

Sample.	First sign.	Beginning of melting.	Complete fusion.
	Degrees.	*Degrees.*	*Degrees.*
a	147±	148.8	149.8
b[a]			
c[a]			

[a] Could not be determined because of initial resinous condition.

Observed readings for sample a corrected=149°±, and 150.8°–151.8° C.

Sample a was a perfectly white crystalline product. It collapsed sharply at the melting point to an opaque mass which did not become a clear liquid on further heating to 190° C. On cooling under a stream of water and remelting the solidified mass no difference in behavior could be noted. The sample conforms to other pharmacopœial tests.

Samples b and c, although labeled "hyoscyamine-hydrobromide" and purchased at the same price as that paid for sample a, were brown, semiplastic, smeary products—impossible of investigation by a capillary-tube method and obviously below United States Pharmacopœia standard. None of the samples, however, were labeled "U. S. P."

Iodoform (6 samples), *strychnine* (6 samples), *saccharum lactis* (9 samples). Of the list of 37 compounds originally selected for immediate investigation and standardization these are the only three remaining compounds which decompose at the melting point, and therefore require further investigation.

Naphthalene (5 samples):

Sample.	First sign.	Beginning of melting.	Complete fusion.
	Degrees.	*Degrees.*	*Degrees.*
a	77.0±	79.2	79.8
b	77.0±	79.2	79.8
c	78.0±	79.5	80.0
d	79.0±	79.5	80.0
e	78.5±	79.3	80.0

Average readings for all samples, corrected, =78.5° ±, and 79.9°–80.5° C.

All samples melted sharply to a clear colorless liquid, and conform to other pharmacopœial tests.

Phenylsalicylate (salol) (6 samples):

Sample.	First sign.	Beginning of melting.	Complete fusion.
	Degrees.	*Degrees.*	*Degrees.*
a	41.5±	42.0	42.5
b	41.5±	42.0	42.5
c	41.8±	42.0	42.6
d	41.5±	41.8	42.5
e	41.8±	42.0	42.7
f	41.8±	42.0	42.6

Average readings for all samples, corrected, =41.5° ±, and 42.2°–42.8° C.

All samples melted sharply to a clear colorless liquid, and all conform to other pharmacopœial tests.

Piperine (4 samples):

Sample.	First sign.	Beginning of melting.	Complete fusion.
	Degrees.	*Degrees.*	*Degrees.*
a	124.0(±)	126.0	128.0
b	127.5(±)	128.6	129.1
c	128.0(±)	128.5	129.1
d	127.0(±)	128.4	129.0

The average readings for samples b, c, and d, correcetd=129° ±, and 130°–130.6° C.

The readings for sample a, corrected, =125.5° ±, and 127.4°–129.5° C.

Sample a was not in the original package and therefore could not positively be considered a market sample. All samples, except a, melted sharply to a clear liquid having a pale greenish tinge. All (including a) conform to other pharmacopœial tests, which, however, are open to criticism in many details.

Pyrogallol (6 samples):

Sample.	First sign.	Beginning of melting.	Complete fusion.
	Degrees.	*Degrees.*	*Degrees.*
a	110±(125)	129.4	130.4
b	110±(127)	130.0	131.0
c	112±(127)	130.5	131.2
d	112±(128)	130.5	131.3
e	112±(128)	130.5	131.5
f	112±(128)	130.5	131.5

The readings for sample a, corrected, =111° ± (126.5°), and 130.9°–131.9° C.

Average readings for all other samples, corrected, =113° ± (129°) and 131.9°–132.8°.

Sample a differed consistently by a considerable margin from all other samples. In all cases the "first sign"—very slight when first observed—became more pronounced at the higher temperature given in parentheses.

All samples melted to a clear liquid having a slight brownish tinge, and all were of United States Pharmacopœial standard as determined by other pharmacopœial tests.

Resorcinol (6 samples):

Sample.	First sign.	Beginning of melting.	Complete fusion.
	Degrees.	*Degrees.*	*Degrees.*
a	105±	109.0	109.4
b	104±	109.0	109.4
c	105±(108)	108.8	109.5
d	103±	108.9	109.3
e	104±(108)	108.9	109.4
f	105±(108)	108.9	109.4

The average readings for all samples, corrected, =104° ±, and 110°–110.5° C.

Samples c, e, and f showed more definite change at the temperature indicated in parentheses than at the "first sign." All samples melted sharply to a clear, colorless, or very slightly amber-colored liquid, and all conform in other tests to United States Pharmacopœial standard.

Salicine (4 samples):

Sample.	First sign.	Beginning of melting.	Complete fusion.
	Degrees.	*Degrees.*	*Degrees.*
a	190±	196.5	197.0
b	190±	196.5	197.1
c	191±(195)	196.6	197.3
d	192±	196.0	196.8

The average readings for all samples, corrected, =194° ±, and 199.9°–200.6° C.

In all cases evidence of change was gradually progressive above the "first sign," but the "beginning of melting" point was sharply defined and consistent. During the melting interval very fine bubbles appeared in the mass, slowly rising to the surface, suggestive of slight decomposition, but no such indication was disclosed by remelting.

All samples melted sharply to a colorless liquid, and all conformed, in other tests, to pharmacopœial standard.

Santonine (5 samples):

Sample.	First sign.	Beginning of melting.	Complete fusion.
	Degrees.	*Degrees.*	*Degrees.*
a	165±	168.5	169.5
b	165±	168.7	169.6
c	165±	168.7	169.5
d	166±	168.9	169.7
e	166±	168.9	169.7

The average readings for all samples, corrected, =167.5° ±, and 171.2°–172.1° C.

In all cases there was more or less slight change between the first two points recorded, but the second point was always sharply defined. The final result was a clear, colorless liquid. All samples conformed to standard according to other pharmacopœial tests.

Sulphonethylmethane (5 samples):

Sample.	First sign.	Beginning of melting.	Complete fusion.
	Degrees.	*Degrees.*	*Degrees.*
a	75.5±	76.0	76.8
b	75.5±	76.0	76.6
c	75.5±	76.0	76.8
d	75.0±	75.7	76.2
e	75.0±	75.5	[a]76.2

[a] Not clear.

The average of values for samples a, b, c, and d, corrected=76° ± and 76.4°–77.1° C.

These samples melted sharply to a clear, colorless liquid. The behavior of sample e was unsatisfactory, in that the point of complete fusion was obscured by the formation of a foam or by a residue of substance infusible at that temperature. This mushy condition gradually cleared on continuing the heating to 190° (observed reading).

All samples conformed to other pharmacopœial tests, two of which, however, are open to minor criticism.

Sulphonemethane (6 samples):

Sample.	First sign.	Beginning of melting.	Complete fusion.
	Degrees.	*Degrees.*	*Degrees.*
a	124.5±	124.8	125.3
b	124.5±	124.8	125.3
c	124.5±	124.8	125.3
d	124.5±	125.0	125.5
e	124.5±	125.0	125.5
f	124.5±	125.0	125.5

The average of values for all samples, corrected=126° ±, and 126.3°–126.8° C.

All samples melted with remarkable precision and uniformity to a clear, colorless liquid; and all conformed to other pharmacopœial tests, regarding which the same criticisms may be made that apply to those for the preceding compound.

Thymol (7 samples):

Sample.	First sign.	Beginning of melting.	Complete fusion.
	Degrees.	*Degrees.*	*Degrees.*
a	49±	49.9	50.7
b	49±	50.0	50.7
c	49±	50.1	50.8
d	48±	49.9	50.4
e	49±	49.9	50.4
f	49±	49.9	50.5
g	49±	49.8	50.3

The average of values for all samples, corrected=49° ±, and 50.1° and 50.8° C.

All samples melted sharply to a clear, colorless liquid and all conformed to standard to the extent that all behaved exactly alike under other pharmacopœial tests. In several tests, however, the reactions obtained differed considerably from those described by the pharmacopœia, and several attempts to liquefy each of the above samples by triturating with chloral hydrate failed completely.

Vanillin (5 samples):

Sample.	First sign.	Beginning of melting.	Complete fusion.
	Degrees.	*Degrees.*	*Degrees.*
a	80±	81.2	81.9
b	80±	81.2	82.0
c	80±	81.5	82.1
d	80±	81.3	81.8
e	80±	81.3	81.9

The average of values for all samples, corrected=80.5° ±, and 81.9–82.5° C.

All samples melted sharply to a colorless liquid. The uniform relation of all samples of vanillin to other pharmacopœial tests is much the same as that described for thymol, except that in this case the discrepancy between the results obtained and those described by the pharmacopœia, for three important tests, is so great as to compel the conclusion either that the tests referred to are worthless as described or that all five samples under consideration are decidedly below United States Pharmacopœia standard.

The final corrected results obtained for the melting interval of the different compounds, exclusive of those which decompose at the melting point, are summarized in the following table (Table VII), which also includes the corresponding melting-point requirements of six different pharmacopœias and the values given in the more common and important sources of the chemical and pharmaceutical literature for this class of compounds.

The values obtained in this investigation are given in the column headed "By method recommended," the number of market samples conforming to each melting interval being indicated in the column immediately preceding the values given. The policy of giving two sets of values in those cases where consistent variation between different samples was observed is continued in the table. As before stated, probably several factors enter into the explanation of such variation, including greater delicacy in the melting-point test than applies to other pharmacopœial tests, the effect of different kinds of impurity (contradicted by Ostwald's work; refer p. 74), and possibly the presence of an impurity that is not detected by other pharmacopœial tests. The official melting interval in such cases must depend upon the degree of exactness or elasticity desired for the pharmacopœial standard. Of five samples of the same product, however, if four conform very closely to the same melting interval and only one consistently and considerably diverges from that value, whatever the cause of the divergence, it would seem reasonable to require the one to conform to the standard established by the four.

Consideration of this table should include recognition of the fact hat these compounds represent only the simplest of their class to vhich the melting-point test can be applied, and the further recog-iition of the fact that where the requirements of different pharma-opœias are identical it does not necessarily indicate independent ex-erimental determinations and, therefore, the correct value.

With the method recommended it is considered that a reasonable llowance for the "personal factor" need not extend the melting nterval more than 0.5° at any temperature. Any variation due en-irely to the "personal factor" (as interpreted on p. 71) is pre-umably constant, and therefore there would seem to be no good eason for making a variable allowance for it at different tem-eratures.

TABLE VII.—*Showing melting points of some pharmacopœial compounds, as determined by the method recommended and as given in different sources of the chemical and pharmaceutical literature.*

Compound.	Number of market samples tested.	By method recommended.	Official requirements of different pharmacopœias.						Value given in literature.		
			United States.	German.	French.	British.	Austrian.	Swiss.	Richter.	Beilstein.	Schmidt.
		Degrees.	*Degrees.*	*Degrees.*	*Degrees.*	*Degrees.*	*Degrees.*	*Degrees.*	*Degrees.*	*Degrees.*	*Degrees.*
Acetanilide	2	111.9–113.7	113	113	113	113.5	113–114	113–114	115 –116	112	113–114
	3	113.3–114.1									
Acetphenetidine (phenacetine)	5	134.2–134.8	134–135	134–135	135	135	135	134–135	135	.....	134–135
	1	133.6–134.6									
Benzoic acid	5	121.3–122.2	120–122	.....	121	121.4	.....	.....	121.4	121.4	.....
Camphoric acid	1	186.4–187.7	187	186	.....	186–187	.....	186.5	187	187	188
	3	183.5–185.4									
Salicylic acid	4	158.2–158.9	156–157	157	157	156–157	157	156–157	155 –156	155 –156	.....
	1	157.5–158.4									
Antipyrine	6	110.2–110.8	113	113	114	113	110	111–113	113	113	113
Atropine	2	115.0–116.0	113.8	[a] 115.5	115.5	115.5	[a] 115.6	[a]	115 –115.5	.....	115–115.5
	1	114.1–115.1									
Betanaphthol	8	121.2–121.8	122	122	122	122	122	122	122	122	123
Camphor-monobromide	2	75.5– 76.2	76	[a]	76	76	[a]	76	76	76	76
	3	74.8– 75.6									
Chloral-formamide (chloralamid) [b]	1	116.4–117.2	114–115	114–115	[a]	[a]	[a]	[a]	115 –116	.....	115
Cocaine	3	96.2– 96.8	98	[a]	98	98	[a]	[a]	98	98	98
	2	97.0– 97.7									
	1	97.5–100.0									
Homatropine-hydrobromide [c]	3	(209±) 210.4–213.1	213.8	.....	[a]	210–212	[a]	209–212	.....	.....	.....
	2	(210±) 211.4–214.5									
Hyoscyamine-hydrobromine	1	150.8–151.8	151.8	.....	[a]	151.8	[a]	.....	.....	.....	.....
Naphthaline	5	79.9– 80.5	80	80	[a]	80	80	79– 80	80	80.6	79.2
Phenylis-salicylate (salol)	6	42.2– 42.8	42	42	42.5	42	42.43	42.43	42.0– 42.5	42 – 42.5	42.5
Piperine	1	127.4–129.5	130	[a]	[a]	130	[a]	[a]	.....	128 –129.5	128–129
	3	130.0–130.6									
Pyrogallol	1	130.9–131.9	132	131–132	133	131	130	131–133	132.5–133.5	132.5–133.5	131–132
	5	131.9–132.8									
Resorcinol	6	110.0–110.5	109–111	110–111	119	119	110–111	110–111	110 (119)	110 (119)	111–112 (118)
Salicine	4	199.9–200.6	201.4	[a]	[a]	201	[a]	[a]	201	198 (201)	201
Santonine	5	171.2–172.1	170.3	170	170	170	170	170–174	169 –170	169 –170	170
Sulphonethylmethane	4	76.4– 77.1	76	76	76	76	76	76– 78	76	76	76
Sulphonemethane	6	126.3–126.8	125.5	125–126	125.5	125–126	125–126	125–126	125 –126	125 –126	125.5
Thymol	7	50.1– 50.8	50– 51	50– 51	51.5	44– 51	50– 51	50– 51	51.5 (50)	50 (51.5)	50– 51
Vanillin	5	81.9– 82.5	80– 81	[a]	80–81	80– 81	[a]	[a]	80 – 81	80 – 81	80– 81

[a] Not official. [b] Only one product obtainable. [c] Should probably be further investigated because of decomposition.

GENERAL SUMMARY.

In the introduction the present relation of the United States Pharmacopœia to the federal food and drugs act of June 30, 1906, is discussed and its logical effect upon pharmacopœial standards for drugs, with specific reference to the melting-point requirements, is indicated. The essential features of an effective legal standard, the deficiencies of the United States Pharmacopœia (eighth) melting-point requirements, and the relation of this melting-point problem to the efficiency of the United States Pharmacopœia standard is discussed.

The body of the bulletin first deals with the plan of procedure in attacking the problem at hand and giving direction to work required for its solution, leading to the disclosure and summary of serious superficial causes of divergence in the published melting-point values of the same compound. The individual causes are taken up separately for more or less exhaustive treatment, terminating in each instance with recommendations, the adoption of which it is believed will largely eliminate or control that cause of divergence in pharmaceutical practice. This discussion of individual causes of divergence includes:

1. Variation in melting-point interpretation.
2. Coordination of melting point and decomposition point.
3. A review and illustration of many of the various methods now in more or less common use for melting-point determinations, together with the description and illustration in detail of a method which is believed to be best adapted to present pharmacopœial needs.
4. Variable practice in the use of thermometers.
5. Variation in certain details of individual manipulation.
6. Variation in physical condition and in preliminary treatment of the compounds.

The method referred to as "best adapted to present pharmacopœial needs," together with carefully defined details of procedure—that is, uniformity of practice—is recommended for official adoption as a part of the melting-point requirements of the United States Pharmacopœia (ninth). This method and procedure were applied in the melting-point determinations of market samples of a group of the more important pharmacopœial compounds. The average observed readings for each sample and the average corrected values for concordant samples are given for each compound in connection with discussion of other pharmacopœial tests. The corrected averages are also tabulated, together with corresponding values, from different pharmacopœias and from various sources of the literature.

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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.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

No. 40.—1. The occurrence of a proliferating cestode larva (*Sparganum proliferum*) in man in Florida, by Ch. Wardell Stiles. 2. A reexamination of the type specimen

of *Filaria restiformis* Leidy, 1880=*Agamomermis restiformis*, by Ch. Wardell Stiles. 3. Observations 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.

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

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

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

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

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

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

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

No. 54.—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.

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

No. 56.—Milk and its relation to the public health. By various authors.

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

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

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

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

No. 61.—Quantitative pharmacological studies: Relative physiological activity of some commercial solutions of epinephrin. By W. H. Schultz.

No. 62.—The taxonomic value of the microscopic structures of the stigmal plates in the tick genus Dermacentor. By Ch. Wardell Stiles.

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

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

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No. 66.—I. The influence of age and temperature on the potency of diphtheria antitoxin. By John F. Anderson. II. An organism (*Pseudomonas protea*) isolated from water, agglutinated by the serum of typhoid fever patients. By W. H. Frost. III. Some considerations on colorimetry, and a new colorimeter. By Norman Roberts. IV. A gas generator, in four forms, for laboratory and technical use. By Norman Roberts.

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

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. ——.

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

HYGIENIC LABORATORY.—BULLETIN No. 71

JANUARY, 1911.

SOME KNOWN AND THREE NEW ENDOPARASITIC TREMATODES FROM AMERICAN FRESH-WATER FISH.—By Joseph Goldberger

ON SOME NEW PARASITIC TREMATODE WORMS OF THE GENUS *TELORCHIS*.—By Joseph Goldberger

A NEW SPECIES OF *ATHESMIA* (*A. FOXI*) FROM A MONKEY.—By Joseph Goldberger and Charles G. Crane

WASHINGTON
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United States Public Health and Marine-Hospital Service.

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SOME KNOWN AND THREE NEW ENDOPARASITIC TREMATODES FROM AMERICAN FRESH-WATER FISH.[a]

(Figs. 1–18.)

By Joseph Goldberger,

Passed Assistant Surgeon, Public Health and Marine-Hospital Service.

In 1905 Marshall and Gilbert described a fluke for which they established the genus *Leuceruthrus*.

Genus LEUCERUTHRUS Marshall and Gilbert, 1905.

Generic diagnosis.—Body thick, muscular, with bluntly rounded cephalic and pointed caudal end. Acetabulum preequatorial. Genital pore slightly preacetabular in mid ventral line. Oral sucker larger than acetabulum; pharynx present; esophagus short, in form of inverted Y. Ceca extend to near caudal margin.

Male organs: Testes, smooth, acetabular or postacetabular; testicular zones[b] overlap, fields abut or separate. Cirrus sac present, incloses vesicula, musculosa, and prostatica; no protrusile cirrus. Roomy, distensible genital atrium present.

Female organs: Ovary near caudal end, postacetabular, posttesticular, postuterine, intercecal, median to submedian; surface smooth. Shell gland compact. Laurer's canal present. Uterine coils transverse, intercecal, preovarian. Vitellaria elongate, postacetabular, in extracecal area.

Type species.—*Leuceruthrus micropteri* Marshall and Gilbert, 1905.

Systematic relations.—The position and relations of the testes and ovary would place this genus with the *Dicrocœliinæ*, but the thick muscular body, the totally preovarian uterus with only one (an ascending) limb very clearly separate it from this group. It seems probable that *Leuceruthrus* will be found to represent the type of at least a new subfamily, *Leuceruthrinæ*, and probably of a new family *Leuceruthridæ* of the superfamily *Fascioloidea*.

*

Among some worms sent to the Division of Zoology for determination there were examples of a distome resembling that described by Marshall and Gilbert (1905) under the name *Leuceruthrus micropteri*.

[a] Submitted for publication August 11, 1910.

[b] The topographic terminology is that suggested by Stiles. See Stiles and Goldberger, 1910.

In response to a request Professor Marshall very kindly sent some of his type material, comparison with which leaves no room for doubt as to the correctness of the identification. As a further result of this comparison I am able to add some new points to the original description and to extend this in some details.

LEUCERUTHRUS MICROPTERI Marshall and Gilbert, 1905.

[Figs. 1–6.]

SPECIFIC DIAGNOSIS.—The characters of the genus. Body 4 to 10 mm. long by 2 to 4 mm. broad. Surface cuticle 30 μ thick, unarmed. Acetabular aperture in mid ventral line about two-fifths of body length from cephalic margin. Genital pore slightly cephalad of acetabular aperture. Oral aperture ventro-subterminal, oral sucker relatively large, prepharynx absent, pharynx globular, esophagus very short, in form of an inverted Y; intestinal ceca run in slightly zigzag course to near caudal margin.

Male organs: Testes irregularly globular, occupy a zone slightly preequatorial equal to from one-tenth to one-eighth of worm's length; zone occupied by testes overlaps, abuts or separate from margin of acetabulum. Cirrus sac present, thin walled, contains vesicula, musculosa and prostatica. Genital papilla present, pierced by ductus ejaculatorius and metraterm that discharge by porus hermaphroditicus into distensible genital atrium. Protrusile cirrus absent.

Female organs: Ovary irregularly globular, in axial region, near caudal extremity, cephalad of excretory vesicle, between converging excretory canals. Shell gland close to cephalo-dorsal aspect of ovary. Receptaculum seminis absent. Uterus forms transverse coils, passes cephalad, fills intercecal area between the ovary and shell gland caudally, and the testes cephalically; metraterm discharges by porus hermaphroditicus. Laurer's canal present, pore opens in about median line of dorsum in or cephalad of zone of shell gland. Vitellaria made up of globular follicles in irregular longitudinal rows ventrad and externally of intestinal ceca, extend from level of caudal margin of acetabulum to cecal ends of intestines.

Egg: Uterine egg 63 by 37 μ, operculated with thickening and knob at opposite pole.

Excretory system: Excretory vesicle tubular, post ovarian; excretory pore caudoterminal. Longitudinal canals, vesicle and excretory duct together in form of letter Y; canals begin at level of cephalic margin of oral aperture.

HABITAT.—Stomach, mouth, gills of *Micropterus salmoides* (type host) and *M. dolomieu* from lakes around Madison, Wis. (type locality), and from stomach of *M. salmoides*, *M. dolomieu* and *Amia calva* from Lake Maxinkuckee, Indiana.

COTYPE.—U. S. P. H. &. M. H. S. No. 10678 (mounted and sections).

SOURCE OF MATERIAL.—The material upon which this study is chiefly based is part of a collection of parasites sent to Prof. Ch. Wardell Stiles at the Hygienic Laboratory by the Bureau of Fisheries. It consists of specimens from the stomach of *Micropterus salmoides* (P. H. & M. H. S. Nos. 10490, 10494, 10496), from the stomach of *Micropterus dolomieu* (P. H. & M. H. S. Nos. 10495, 10497, 10524) and the stomach of *Amia calva* (P. H. & M. H. S. No. 10527).

The fish from which these were obtained were all from Lake Maxinkuckee, Indiana. I also found one mounted specimen in the MacCallum collection (U. S. N. M. No. 7278). This is from the stomach of *Micropterus* sp., locality not given. The specimens studied by Marshall and Gilbert were for the most part from the stomach, a

few from the mouth or gills of large and small mouthed black bass (*Micropterus salmoides* and *M. dolomieu*) from the lakes around Madison, Wis.

EXTERNAL CHARACTERS.

MEASUREMENTS.—Marshall and Gilbert give the length of the worms studied by them as from 4 to 7 mm. The alcohol specimens at my disposal vary in length from 4.2 to 10 mm., and in greatest width from 2 to 4 mm. A specimen 4.7 mm. in length measures 1.9 mm. in maximum dorso-ventral diameter.

COLOR.—The fresh specimens are described by Marshall and Gilbert as of a peculiar pinkish or yellowish tinge, hardly dark enough to be described as red or yellow.

The specimens (alcoholic) forming the basis of this study were of the color of old ivory with a dark streak on each side running parallel to the lateral margins of the caudal half of the venter. These streaks, representing the vitellaria, extend cephalad almost to the level of the ventral sucker and caudad to a level a little short of the caudal extremity. A dark patch, looking like a shadow, is also present in the cephalic half of that portion of the median field of the venter that is caudad of the ventral acetabulum. This patch which represents the egg-filled coils of the uterus appears relatively sharply delimited caudally by an ivory tinted area; cephalically it fades out gradually and laterally it appears marked off from the lateral longitudinal dark streaks by a narrow ivory tinted strip. The shadow of the uterine coils shows also, though more faintly, on the dorsum.

FORM.—This is described by Marshall and Gilbert as tongue shaped with a blunt anterior and a pointed posterior end. My specimens conform to this general description; some, however, present a median (sagittal) or submedian notch at the caudal pole. The worms are broadest in about their equatorial zone, from which region the body tapers toward both poles, slightly toward the cephalic, considerably toward the caudal. The lateral margins present a gentle curve from pole to pole. In profile the longitudinal axis of the worm is very slightly curved with the convexity dorsad; the outline in this view is somewhat that of a cone with a pointed caudal and a blunt oral extremity. The latter appears inclined obliquely as if beveled at the expense of the ventral aspect. Both ventral and dorsal profile lines are curved, the former slightly, the latter more decidedly. There is no definite line of demarcation between dorsum and venter. The outline of the worm in transverse section is elliptical to circular.

SURFACE.—On this point Marshall and Gilbert simply state that the body is smooth. This I find to be the case in the sense that there are no spines or papillæ. There is, however, some wrinkling with

consequent formation of numerous grooves especially noticeable along the lateral margins. These are probably dependent on the state of contraction of the worm. In the ventro-median line at a point, measured in a reconstruction of a specimen 4.22 mm. long, about two-fifths of the body length from the cephalic margin there presents an irregularly circular aperture that, in sections, is seen to lead into the acetabular cavity. In the same specimen, about 0.28 mm. cephalad of the cephalic margin of the acetabular aperture and obscured in the unsectioned specimen by the surface wrinkles, is the relatively minute (0.02 mm. in diameter) genital pore. At the blunt cephalic extremity in the area which has been described as the beveled portion of the venter is the relatively large irregularly circular oral aperture.

ACETABULUM.—This is described by Marshall and Gilbert as median, a little in front of the center of the body with a diameter of 0.45 mm. The position on the surface of the acetabular aperture in my specimens has already been mentioned. The acetabulum itself is well developed, though smaller than the oral sucker. In the reconstruction it measures 0.60 mm. in the maximum longitudinal and about 0.72 mm. in the maximum transverse diameter. Its margin may or may not project directly on the ventral surface; when it does the projecting ring is marked off by a distinct groove.

INTERNAL ANATOMY.

DIGESTIVE TRACT.—Marshall and Gilbert describe this as follows:

> The oral sucker (in a specimen 5 mm. long) is large 0.75 mm. in diameter and is situated ventral just behind the anterior margin of the body. The pharynx is large, praepharynx and oesophagus absent. The intestinal caeca extend to the posterior part of the body; each caecum at first extends forward for a short distance before passing backward.

Aside from a difference in interpretation with respect to the esophagus the digestive tract, in my preparations, conforms to this general description. As already described, the oral aperture is ventro-subterminal and in the reconstruction, already referred to, measures about 0.26 mm. in sagittal diameter. It gives entrance into the cavity of the oral sucker. This is a well-developed, powerful, muscular organ whose form (fig. 2) in sagittal section resembles somewhat that of *Fasciola hepatica*. In median sagittal section its ventral wall is much shorter than the dorsal. Its maximum dorso-ventral and transverse diameters measure 0.72 mm. and 1 mm., respectively. The oral pole of this sucker projects slightly beyond the embrace of the body parenchyma and forms a well-defined rim on the surface bounding the oral aperture. This projecting rim has an external covering of cuticle continuous with that of the general surface. The base of the sucker appears slightly excavated in some

specimens, as if molded over the adjacent convex pole of the pharynx. The pharynx is attached to the base of the oral sucker without the intervention of a prepharynx. In the reconstructed specimen the pharynx measures 0.29 mm. in maximum dorso-ventral, about 0.30 mm. in maximum longitudinal and about 0.27 mm. in maximum transverse diameter, so that it is approximately globular in form. In transverse section its outline is irregularly circular. A thick nerve cord runs transversely close to its dorsal aspect and at about the level of the base of the oral sucker.

From the base of the pharynx there springs a very short tube (fig. 2) which divides into two lateral stems in the manner of an inverted Y. Inclosing this tube is a ring of sparse cells. This esophagus is lined by a cuticular layer which is continued into its lateral stems. The intestines are continuous with the lateral stems of the esophagus, the change from one to the other being marked by a sphincter-like constriction (fig. 2). Each intestinal tube passes at first dorso-laterad and cephalad then, describing an arch, curves and passes caudad in a slightly zigzag course at a distance of about 0.30 mm. from the lateral margin to terminate cecally at a point somewhat less than the length of the acetabular diameter from the caudal margin, in the vitelline, excretory, and postovarian zones. The intestinal lumen is lined with an epithelial layer, the transition from the cuticular lining of the lateral stem of the esophagus being sharply marked.

Genital system.—*Male organs:* Marshall and Gilbert give the following description of the male genital organs:

> In a ventral view the testes are nearly circular in outline, 0.325 mm. in diameter, and lie nearly in the same transverse plane. One is always slightly in advance of the other, but neither the right nor the left is constant in this respect. Both testes are near the center of the body a little posterior to the acetabulum. The vasa deferentia pass forward dorsal to the acetabulum; they join at the base of the cirrus sac, within which the seminal vesicle is bent upon itself, finally passing into the ductus ejaculatorius, which empties into the genital sinus just anterior to the female opening.

In my preparations, the testes are irregularly globular in form. Measured in the reconstruction of one specimen, the right testis is 0.51 mm. in maximum transverse, 0.30 mm. in maximum longitudinal, and 0.56 mm. in maximum dorso-ventral diameter, while the left is 0.37 mm. in transverse and 0.35 mm. in maximum longitudinal diameter. The left testis is therefore slightly smaller than the right in this particular specimen. They occupy a zone in the intercecal area equal to from one-tenth to one-eighth the total length of the worm (fig. 1). This zone varies somewhat in its relation to that of the acetabulum; in 14 out of 16 specimens it overlapped that of the acetabulum to a greater or less extent, in 1 the testicular and acetabular zones abutted and in 1 only of the 16 specimens were they distinctly separate in

the way described and pictured by Marshall and Gilbert. Considering now the zone occupied by each testis, I find that the relation of one to the other is also subject to some variation. In 7 of 11 sectioned specimens these two zones overlap to a variable degree, though never coinciding, in 2 they abut and in 2 are separate. In 10 of 16 specimens the right testis is the one farther cephalad. The relation of the testicular fields, one to the other, could not be so thoroughly studied as is perhaps desirable. There appears to be a variation from a condition where the fields are separate to one in which they abut or slightly overlap. From the dorso-mesio-cephalic aspect of each testis a thin walled vas efferens arises and passes at first cephalad, then gradually tilts mediad to unite with its fellow at the base of the cirrus sac in the formation of the vas deferens. The vasa efferentia bound, laterally, an area which is filled with coils of the uterus. The vas deferens is a tube which presents differences of structure in its course. The portion immediately succeeding the point of union of of the vasa efferentia is thin walled like the vasa efferentia with a lumen of large but variable caliber filled with spermatozoa. It is coiled and occupies the major portion of the space in the cirrus sac. This is the vesicula seminalis. It is succeeded by a thick muscular walled, quite short, uncoiled part which suggests the pars musculosa of the amphistomes. The change from vesicula to musculosa is marked by a valve-like structure like that described by Stiles and Goldberger (1910) for *Paramphist. crassum.* In form this part of the vas deferens resembles that of a beet, the proximal end is bulbous and tapers rapidly centrifugally. It is succeeded by the pars prostatica which is distinguished by being inclosed in a thick layer of cells with prominent nuclei. The prostatica is short, directed ventrad, and is succeeded by the ductus ejaculatorius which, after continuing for a short distance ventrad, penetrates the base of the genital papilla to discharge at its vertex through a pore in common with the metraterm (fig. 3). The vesicula, musculosa, and prostatica are inclosed in a thin-walled "cirrus" sac. There are in this sac, in addition, a considerable number of cells resembling the prostatic cells, but not staining so deeply. These cells appear to fill the interstices between the coils of the vesicula. The sac, with its contents, lies in the axial region of the worm dorso-cephalad of the acetabulum (figs. 2 and 3).

The genital papilla (fig. 3) is a small, though in most cases a very well defined, somewhat cylindrical structure which projects from the dorsal wall of a fairly roomy, distensible, irregular genital atrium. This chamber (figs. 2 and 3) is at some distance beneath the surface of the venter and in a line directly cephalad of the acetabulum. It discharges by a narrow passage which opens, as already described, on the ventral surface by a small transverse button-hole-like slit as the genital pore.

Female organs: These are described by Marshall and Gilbert as follows:

> The ovary is median, slightly lengthened along the transverse axis of the fluke and is situated midway between the testes and the posterior end of the body. A ventral view shows it lying apparently in the fork of the excretory vesicle. The oviduct passes forward from the ovary for a short distance and joins the receptaculum seminis, receiving Laurer's canal, a long, narrow tube, the external opening of which is dorsal. From this point the tube passes through the shell gland which lies a little to the right of the median line. Specimens containing but few eggs have the uterus confined to a space between the intestinal ceca on the sides, the acetabulum in front and the ovary behind. The vagina enters the genital sinus just posterior to the opening of the ductus ejaculatorius. Each yolk gland consists of a number of follicles on either side, having a slight appearance of being arranged in two or three irregular rows, none of which extends in front of the acetabulum. The yolk duct from each gland passes toward the median line of the body, the two ducts meeting in a yolk reservoir which lies anterior to the ovary.

My preparations show the ovary and the shell gland in the caudal portion of the axial region of the body of the worm. The ovary occupies a position in the axis of the body almost immediately cephalad of the excretory vesicle and between the converging longitudinal excretory canals. It is irregularly globular in form and in the reconstruction measures 0.37 mm. in maximum dorso-ventral, 0.29 mm. in maximum longitudinal, and 0.30 mm. in maximum transverse diameter. The shell gland lies close to the cephalo-dorsal aspect of the ovary (fig. 4), though its position may shift somewhat either to the right or the left and its zone may abut or overlap that of the ovary to a considerable degree. The oviduct springs from the cephalic aspect of the ovary and passes toward the shell gland. On account of the variations in the relations of the gland to the ovary the course of the oviduct is subject to considerable variation, but it usually penetrates the caudal or left latero-caudal aspect of the gland, within which it almost at once unites with the common vitello-duct in the formation of the ootype. Just before the duct penetrates the gland it gives off Laurer's canal (fig. 5). This pursues a sinuous course to the median line of the dorsum where it opens by a minute pore within the zone of the shell gland or slightly cephalad of it (fig. 4). In its course it passes over (cephalad of) the right transverse vitelloduct.

A seminal receptacle such as is described and figured by Marshall and Gilbert was not noted. The structure to which these observers probably refer is, I believe, simply the dilated, fusiform, proximal end of Laurer's canal (fig. 5). This occasionally incloses granules suggestive of vitelline cells and some (a few) spermatozoa. The uterus emerges from the right latero-cephalic aspect of the shell gland and then bends transversely to the left beginning the formation of transverse coils which fill the intercecal area as it passes cephalad. Its

terminal portion (metraterm) eventually gains a position close to the ventral aspect of the cirrus sac and pursues a somewhat sinuous course ventro-cephalad to the base of the genital papilla which it then penetrates in company with the ductus ejaculatorius in the manner already described. The metraterm is longer than the cirrus sac. In some of the specimens masses of spermatozoa were observed in the proximal end of the uterus (receptaculum seminis uterinum of Looss); eggs filling and more or less distending the uterine coils was the rule. Marshall and Gilbert state that when "the eggs have increased greatly in number the outline of the uterus becomes lost and it nearly fills the posterior three-quarters of the fluke." In none of my own specimens do I find this condition, but in two specimens kindly sent by Professor Marshall I find a state of affairs that this description is probably intended to cover. I find in these that in the post ovarian zone and in the zone cephalad of the level of the genital pore a considerable portion of the body parenchyma appears to be replaced by a granular material in which eggs are irregularly scattered. These are particularly numerous in the post ovarian zone in one of the two specimens referred to. There is no indication that the uterus itself has extended into these regions but, rather, one gains the impression that this is a pathological condition, the eggs having burst from the uterus and infiltrated these regions as it were. In one of these specimens, too, I find a Laurer's canal with two pores at the dorsum, one cephalad of the other precisely as observed by Looss (1894, p. 205) in one specimen of *Dist. tereticolle*. The vitellaria consist of two elongate glands which lie substantially parallel to the lateral margins of the body in the postacetabular zone and extend caudally little, if at all, beyond the cecal ends of the intestines; sometimes, indeed, they fall a little short of this level. Each gland is made up of a relatively moderate number of well developed globular follicles which are grouped in irregularly longitudinal rows ventro-laterally of the intestinal ceca. From each gland at a point slightly caudad of its equator a duct departs which passes inward with a tilt caudad in front (ventrally) of the corresponding intestine and dorsally of the longitudinal excretory canals, toward the base of the shell gland, in which region these tranverse ducts unite to form a reservoir distended with vitelline cells. From this reservoir a duct proceeds toward the shell gland which it usually penetrates at the right lateral or latero-caudal aspect and within which it joins the oviduct just as the latter begins to dilate to form the ootype.

Egg: The uterine egg is not described by Marshall and Gilbert. It is elliptical in form, with a thickening of the shell and, as a rule, a low knob-like projection at one of the poles and an appearance strongly suggestive of an operculum at the other (fig. 6). It could not be determined whether the knob is invariably present. In an egg in which

the operculum, if such it be, was partly raised, the margin of the aperture had a serrated appearance. Measurements of eggs taken from the uterus gave 63 μ for the length by 37 μ for the greatest width.

Excretory system.—Marshall and Gilbert describe this as follows:

> The excretory vesicle is Y-shaped, the excretory pore terminal, opening into a short, narrow tube which enters the median vesicle. This latter part is of moderate length, extending forward as far as the ovary, and from its anterior margin the two lateral branches arise and extend forward. We are unable to follow these branches farther than the acetabulum, but from their abrupt ending and thickness at the end we believe that they extend farther into the anterior region of the body.

To this description my preparations conform in a general way. In ventral view that part of the excretory system consisting of the main longitudinal canals, the excretory vesicle and duct, have the form of the letter Y. The excretory vesicle and duct form the median stem of the Y which in this case is relatively very short, while the converging longitudinal canals form the lateral stems or horns. The excretory vesicle is in the axial region of the caudal portion of the body, caudad of the ovary, and between the cecal ends of the intestines. It is a thin-walled tubular structure that discharges at the caudal pole of the body through a relatively long and delicate duct. The duct is lined by a cuticlar layer. The vesicle receives at its dome, or it may be described as being formed by the junction of, two longitudinal excretory canals. These canals begin at about the level of the cephalic margin of the oral aperture. They pass caudad, at first close to the latero-dorsal aspect of the oral sucker, then laterally of the pharynx and ventrally of the arch formed by the first portion of the intestinal ceca to gain a position, which they maintain throughout the remainder of their course, more or less close to the ventro-mesial aspect of the corresponding intestine.

* *

In the collection of parasites of which *Leuceruthrus micropteri* formed a part I found two new forms that belong in the genus *Azygia*.

Genus AZYGIA Looss, 1899.

1904: *Azigia* Stafford, 1904, p. 488, misprint.

Generic diagnosis.—Body elongate, muscular. Cephalic end rounded; caudal end rounded or pointed. Surface unarmed. Oral aperture ventro-terminal or subterminal. Acetabulum slightly to considerably preequatorial. Genital pore slightly preacetabular, median. Oral sucker larger than acetabulum. Pharynx present. Esophagus short. Ceca extend to near caudal margin.

Male organs: Testes postacetabular, post-uterine, post-ovarian, prevesicular, intercecal; surface smooth. Testicular zones separate to slightly overlapping; fields nearly or quite coincide. Cirrus sac present, incloses vesicula, musculosa, and prostatica. Genital papilla present, projects into distensible genital atrium. Protrusile cirrus absent.

Female organs: Ovary postacetabular, post-uterine, pretesticular, and intercecal, median to submedian; surface smooth. Shell gland compact. Receptaculum seminis absent. Laurer's canal present. Uterus transversely coiled, pretesticular, preovarian, intercecal. Vitellaria elongate, extracecal, post-acetabular.

TYPE SPECIES.—*Azygia lucii* (Mueller) Luehe, 1909.

Until 1905 this genus included only the type species. In that year Marshall and Gilbert described a second under the name *Azygia loossii*. With the two new forms here to be described the genus comprises 4 species. They may be distinguished by the following key:

KEY TO THE SPECIES OF AZYGIA.

A¹. Vitellaria do not extend materially posttesticular; they begin at a point, the length of the acetabular diameter, postacetabular.....*A. lucii* (p. 16)

A². Vitellaria extend considerably posttesticular.

B¹. Vitellaria begin at about equator of worm, unbroken; excretory canals discharge independently into roomy vesicle; ceca straight.. *A. loossii* (p. 26)

B². Vitellaria begin considerably preequatorial.

C¹. Vitellaria broken in continuity in ovarian zone; excretory canals unite posttesticular to form median stem; ceca zigzag; genital papilla projects from floor of genital atrium.....*A. bulbosa* (p. 22)

C². Vitellaria unbroken; excretory canals unite in testicular zone; ceca zigzag; genital papilla projects from and forms dorsal wall of genital atrium.....*A. acuminata* (p. 17)

In order to facilitate comparison and for a better understanding of the relationship of the species of the group, it will be convenient to give a diagnosis of the type of the genus before proceeding with the description of the remaining species.

AZYGIA LUCII (Mueller, 1776) Luehe, 1909.

[Fig. 7.]

1776: *Fasciola lucii* Mueller, 1776.
1782: *Planaria lucii* (Mueller) Goeze, 1782a.
1800: *Distoma lucii* (Mueller) Zeder, 1800.
1802: *Fasciola tereticollis* Rudolphi, 1802b.
1809: *Distoma tereticolle* (Rud.) Rudolphi, 1809.
1832: *Distoma rosaceum* Nordmann, 1832a.
1899: *Azygia tereticollis* (Rud.) Looss, 1899b.
1904: *Azigia tereticolle* (Rud.) Stafford, 1904.

SPECIFIC DIAGNOSIS.—The characters of the genus. Body 10–30 mm. long, by scarcely more than 1.5 mm. wide. Cuticle 20 μ thick. Acetabulum at about junction of cephalic with second fourth of body length. Pharynx approximately cylindric; esophagus very short; ceca extend almost to caudal margin.

Male organs: Testes in a zone at junction of middle with caudal third of body length; their zones separate, fields coincide. Cirrus sac very thin walled; ductus ejaculatorius discharges separately from metraterm at vertex of genital papilla. Genital papilla, conical, projects from dorsal wall of a roomy distensible atrium; protrusile cirrus absent.

Female organs: Ovarian zone separate from testicular zone; ovary median, generally smaller than the testes. Uterine coils do not encroach on cecal fields. Vitellaria extend through about two-fifths of the body length, from a point about the acetabular

diameter postacetabular to a point not materially caudad of the plane of the caudal margin of caudal testis, not broken in continuity.

Egg: 45 by 23 μ.

Excretory system: Longitudinal canals unite immediately posttesticular to form median, beaded, tubular, excretory vesicle.

HABITAT.—Esophagus and stomach of *Lucius lucius* J. & E. (type host), *Lucius reticulatus* J. & E., *Lucioperca sandra* Cuv., *Salmo fario* L., *Salmo hucho* L., *Salmo trutta* L., *Salvelinus alpinus* J. & E., *Lota vulgaris* Cuv., *Lota maculosa* J. & E., *Ameiurus lacustris* J. & E., *Perca flavescens* J. & E., *Thymallus vulgaris* Nilss.

AZYGIA ACUMINATA new species.

[Figs. 8-10.]

SPECIFIC DIAGNOSIS.—The characters of the genus. Body 6 to 9.3 mm. long, dorsoventrally slightly compressed, bowed with concavity ventrad, with rounded cephalic and pointed caudal extremity. Cuticle unarmed, on venter about 7.5 μ in thickness and on dorsum about 11.5 μ. Body is constricted in a zone acetabular or slightly preacetabular in position and this marks junction of fusiform cephalic with tongue-like caudal body portion; maximum transverse diameter slightly postacetabular. Acetabular aperture one-fourth to one-third of body length from cephalic margin. Genital pore in mid-ventral line, slightly cephalad of acetabular aperture. Oral sucker larger than acetabulum and with thick layer of meridional fibers in ventral wall; prepharynx absent. Esophagus inverted Y shaped, short, directed dorsad; origin of ceca in pharyngeal zone marked by sphincter-like constriction. Ceca arch into suctorial zone then pass caudad in a zigzag course to end quite near caudal margin.

Male organs: Testicular zone at junction of third with caudal fourth of body length. Testes one obliquely caudad of the other, with zones only slightly separated, abutting or slightly overlapping and with fields overlapping considerably. Caudal testis slightly the larger and more nearly axial in position. Testicular surface without infoldings. Cirrus sac very thin, delicate walled. Ductus ejaculatorius joins with metraterm to form ductus hermaphroditicus; latter discharges at vertex of a conical papilla into distensible genital atrium; protrusile cirrus not present. Genital papilla projects ventrad into and forms dorsal wall of genital atrium.

Female organs: Ovarian zone immediately cephalad, abutting or overlapping slightly zone of cephalic testis. Ovary nearly axial in position, slightly smaller than cephalic testis. Shell gland smaller than ovary and close to dorsal pole of latter. Uterus forms transverse coils, does not encroach on cecal fields. Metraterm longer than cirrus pouch. Pore of Laurer's canal in post-ovarian zone. Vitellaria extracecal between plane of lower margin of acetabulum and a plane about midway between caudal testis and ends of gut, not broken in continuity; caudal ends quite asymetrical as to level. Transverse vitelloducts pass dorsally of excretory canals and ventrally of intestines.

Egg: Uterine egg 64 to 69 μ by 30 to 32 μ.

Excretory system: Excretory system in form of a Y; longitudinal canals unite in testicular zone; median stem tubular; excretory pore caudo-terminal.

HABITAT.—Stomach of *Amia calva* (type host) from Lost Lake, Indiana (type locality).

TYPE.—U. S. P. H. & M. H. S. No. 10500.

SOURCE OF MATERIAL.—The material available for study consists of 13 specimens bearing the U. S. P. H. & M. H. S. No. 10500. The worms were collected in 1906 from the stomach of *Amia calva* from Lost Lake, Indiana, and formed part of the collection of parasites, already mentioned, in which *L. micropteri* was included.

EXTERNAL CHARACTERS.

SIZE.—Three specimens in glycerine-alcohol varied between 6 mm. and 9.3 mm. in length. A specimen 8 mm. in length measures 2 mm. in maximum transverse diameter in frontal sections.

COLOR.—In glycerine-alcohol the specimens are of an olive-green tint.

FORM.—In all the specimens the longitudinal axis is more or less sharply curved with the concavity ventral. Accordingly the venter is concave longitudinally but convex transversely, while the dorsum is convex, both longitudinally and transversely. The worms are elongate bodies with a rounded cephalic and pointed caudal extremity. The body is marked by a slightly constricted zone which is acetabular or slight preacetabular in position. This constriction is particularly noticeable on ventral view, and breaks the continuity of the longitudinal convexity of the lateral margin (fig. 8). The portion of the worm caudad of the constricted zone has a somewhat elongate, pointed tongue-like form on ventral view, and is broadest in the zone immediately postacetabular or, with respect to the worm, in a zone slightly preequatorial. The cephalic portion of the worm forms about one-fourth of the total length; it is somewhat fusiform or cylindrical in outline on ventral view and its maximum width is noticeably less than that of the caudal portion.

SURFACE.—The surface cuticle is unarmed, about 7.5 μ thick on the venter and about 11.5 μ on the dorsum of the worm. It is marked by numerous transverse sulci, particularly numerous and deep on the ventral aspect. These are probably due to contraction of the animal. On the ventral aspect of the cephalic extremity is the oral aperture. In the median line of the venter, at about the junction of the cephalic with the second third or fourth of the body length is the aperture of the acetabulum, and slightly cephalad of the cephalic margin of this aperture is the genital pore. The caudal extremity presents the excretory pore.

ACETABULUM.—The position of the acetabular aperture has been indicated. In a specimen 6 mm. long the longitudinal diameter of the acetabular aperture measures 0.24 mm., while that of the acetabulum itself measures 0.72 mm. In a specimen 8 mm. long the transverse diameter of the aperture is 0.3 mm. and that of the acetabulum 0.825 mm. The acetabulum is smaller than the oral sucker.

INTERNAL ANATOMY.

DIGESTIVE TRACT.—The oral aperture, as has been indicated, is ventro-subterminal in position and gives entrance into the lumen of a well-developed oral sucker (fig. 9). In the specimen 6 mm. long the maximum vertical diameter of the oral sucker measures 0.855

mm., while the thickness of its dorsal wall measures 0.105 mm. In a specimen 8 mm. long, while the maximum vertical diameter is 0.90 mm., the transverse diameter at the level of the caudal margin of the oral aperture is 0.855 mm. In sagittal section the ventral wall is triangular in outline (fig.9) with the base of the triangle directed towards the pharynx. This basal portion in a specimen 6 mm. long is 0.277 mm. in maximum dorso-ventral diameter. This great thickness is due to the great development of the external layer containing abundant meridional muscular fibers. In form the sucker is of the type of that in *L. micropteri*. Attached directly to its base is the pharynx. In the specimen 8 mm. long the pharynx measures about 0.36 mm. in maximum longitudinal, 0.36 mm. in dorso-ventral, and 0.24 mm. in maximum transverse diameter. It is ellispoid in form and gives attachment at its base to a Y-shaped esophagus of the type described for *L. micropteri*, but somewhat longer. The lateral stems of the esophagus are continued as the intestinal ceca, the transition being indicated by a sphincter-like constriction more or less marked and by a change from a cuticular to an epithelial lining membrane (fig. 9). The esophagus from its point of origin is directed dorsad and somewhat cephalad, so that the origin of the intestinal ceca is within the pharyngeal zone. The ceca arch as in *L. micropteri* into the suctorial zone, then pass caudad in a very tortuous zigzag course to terminate about 0.315 mm. from the caudal margin in the specimen 8 mm. long.

Genital system.—*Male organs:* The testes are in a zone at about the junction of the third with the caudal fourth of the body length or slightly nearer the caudal margin than is the acetabular zone to the cephalic margin. They are placed with a slight obliquity caudad one of the other (fig. 8). In press preparations, in ventral view, the caudal testis appears to be the larger, and nearly, if not quite, in the axial region; the cephalic testis is always more markedly excentric, that is, nearer the body margin than the caudal testis. The relation of the testicular zones, one to the other, varies somewhat; they may be slightly separated, abut, or slightly overlapping. The testicular fields always overlap to a considerable extent. In the press preparations the outline of the testes is oval with the major diameter in a transverse diameter of the worm and with the narrower pole of each overlapping that of the other. In one of four specimens, however, the caudal testis was more nearly circular in outline with the cephalic aspect slightly flattened. The surface of each testis is smooth, so far as infoldings are concerned. A vas efferens arises from the cephalic aspect of each testis and passes cephalad on each side in close relation to the ventro-mesial aspect of the corresponding main longitudinal excretory canal. Eventually each vas passes mediodorsad to the axial region of the body and at a point in a plane

slightly caudad of that of the cephalic margin of the acetabulum, unites with the other to form the vas deferens. The vas deferens consists of a long coiled vesicula, a short fairly thick-walled (about 15 μ) musculosa having the valve-like structure at its junction with the vesicula and the beet-like form described for *L. micropteri*, a short pars prostatica and a ductus ejaculatorius. With the exception of the ductus ejaculatorius the vas deferens is inclosed in a very thin delicate-walled pouch (fig. 10) like that in *L. micropteri*. This pouch is elongate, ellipsoidal in form, and lies in close relation to the dorso-cephalic aspect of the acetabulum. The ductus ejaculatorius penetrates a genital papilla to join with the metraterm in the formation of a ductus hermaphroditicus that discharges into a distensible genital atrium (fig. 10). The genital atrium may be regarded as the slightly dilated distensible portion of a relatively short dorso-ventrally directed canal that discharges at the genital pore. This dilated portion is itself apparently subdivided by a circular projection of the inclosing wall into a smaller dorsal and a larger ventral chamber. This is probably due to a folding of the chamber wall. The dorsal chamber is simply a very narrow slit inclosing the conical genital papilla that projects from its dorsal wall. In a series of sections this dorsal wall or vertex of the papilla is 0.12 mm. from the surface.

Female organs: The ovary and the shell gland are in a zone immediately cephalad, abutting or slightly overlapping that of the cephalic testis. Although both the testes on the one hand and the ovary and shell gland on the other may be said to be in the axial body region, one caudad of the other, it should be noted that the former are ventral of and the latter dorsal of an oblique ventro-dorsal plane passing through the main longitudinal excretory canals as these shift dorso-caudad. The ovary in ventral view is elliptical or oval in outline, slightly smaller than the cephalic testis and very nearly if not quite in the median sagittal plane, bearing about the same relation to this plane as the caudal testis. In one of four press preparations the ovary bears exactly the same relation to the testes as that in *Hassallius hassalli;* that is, it is within the zone of the cephalic testis. The shell gland is smaller than the ovary and is placed close to the dorsal pole of the latter; its zone is entirely within that of the ovary. The oviduct springs from the dorso-cephalic aspect of the ovary curves and passes caudad over the right transverse vitello-duct between the ovary and the shell gland. It penetrates the shell gland on its ventral aspect to unite with the common vitello-duct in the formation of the ootype. Laurer's canal leaves the oviduct immediately cephalad of the plane of the transverse vitello-duct and then accompanies the continuation of the oviduct caudad, continuing in this course after the latter plunges into the shell gland. Later it curves dorsad, skirting the caudal aspect of the shell gland; eventually it passes dorso-caudad to

reach the dorsum at a point in a plane a little caudad of that of the caudal margin of the ovary. A receptaculum seminis is absent. The vitellaria consist of a moderate number of well-developed globular follicles in the extracecal fields. They extend from about the level of the caudal margin of the acetabulum to a plane about midway between the caudal margin of the caudal testis and the cecal ends of the intestines. Their cephalic extremities are much more nearly symmetrical than their caudal ends; that is, they are more nearly in the same transverse plane. Their zones may or may not abut that of the acetabulum or one may overlap the acetabuluar zone slightly and the other fall a little short of it. In all of the specimens examined the caudal ends of the vitellaria were in transverse planes considerably separated one from the other. The usual arrangement found is for the gland of the right side to extend a distance about equal to the vertical diameter of the caudal testes post testicular and that of the left side to fall short of reaching the plane of the cecal end of the left gut by about the same distance (fig. 8). There appears to be no definite break in the vertical continuity of either gland such as occurs in *Azygia bulbosa*. From each gland a duct passes inward with a slight tilt caudad between the intestinal ceca and the longitudinal excretory canals to unite with its fellow in the formation of a vitelline reservoir between the ovary and shell gland and slightly to one side of the latter. From the vitelline reservoir a duct passes to the shell gland, skirting its left latero-caudal aspect to penetrate its dorsal aspect, uniting as already mentioned with the oviduct in the formation of the ootype. The uterus emerges from the right latero-cephalic aspect of the shell gland (in one series of sections) and at once forms coils in the area laterally and dorsally of the gland, some loops dipping slightly caudad of the level of emergence but not passing beyond the caudal limit of the zone of the shell gland. The uterus passes cephalad, its coils filling the intercecal area but not trespassing on the cecal fields. As it enters the acetabular zone these coils become less complex and less crowded finally at a point a little caudad of the cephalic margin of the acetabulum and slightly caudad of the base of the cirrus sac it is continued as the metraterm which passes cephaloventrad in a sinuous course between the acetabulum and sac to penetrate the genital papilla and unite with the ductus ejaculatorius, as already described.

Egg: The uterine egg measured at favorable points in sections is about 64 to 69 μ in length by 30 to 32 μ in width.

Excretory system.—The excretory system is well developed. The longitudinal excretory canals begin close to and laterally of the oral sucker in about the plane of the cephalic margin of the latter. The first part of their course caudad is quite like that in *L. micropteri*. In the latter part they take up a position near the ventro-mesial

aspect of the corresponding gut and help to mark the ventro-lateral limits of the uterine area. As they pass through the ovarian zone they gradually shift medio-dorsad, and finally in the dorsal part of the testicular zone unite to form a large median canal. This continues caudad in the axis of the worm to terminate by a relatively short slender duct at the caudal pole. The system is therefore in the form of a Y with a relatively short median stem, which may be regarded as an excretory vesicle, though it is not notably roomy as compared with the terminal portions of the longitudinal canals. The caliber of both the paired canals and median stem is not uniform; there are constrictions and dilations here and there in their continuity, bringing about a beaded effect.

AZYGIA BULBOSA new species.

[Figs 11–14.]

SPECIFIC DIAGNOSIS.—The characters of the genus. Body 4 to 9.6 mm. long, margins approximately parallel, cephalic pole rounded, caudal end bulbous and rounded but less bluntly. Cuticle unarmed, about 7.5 μ thick. Acetabular aperture about one-fifth to one-fourth of body length from cephalic margin; acetabulum smaller than oral sucker and aperture of former smaller than oral aperture. Genital pore in mid-ventral line, slightly cephalad of acetabular aperture. Oral sucker with, at most, moderate development of meridional fibers in ventral wall. Esophagus inverted Y-shaped, directed dorso-cephalad. Origin of ceca in pharyngeal zone. Ceca arch into suctorial zone pursue a tortuous zigzag course, caudad terminating very close to caudal margin.

Male organs: Testicular zone about one-fourth to one-third of body length from caudal margin; testes one directly caudad of the other with slight obliquity, their zones overlapping slightly and fields to a very considerable degree, almost coinciding, caudal testis the larger. Testicular surface without infoldings. Cirrus sac thin, delicate-walled. Ductus ejaculatorius discharges separately from metraterm, or perhaps by a porus hermaphroditicus. Protrusile cirrus absent. Cylindrical to nipple-like genital papilla present, projects cephalad from floor of distensible atrium.

Female organs: Ovarian zone cephalad of and overlapping slightly the testicular zone. Ovary as large or larger than cephalic but smaller than caudal testis. Shell gland smaller than ovary and dorso-cephalad of it. Oviduct may present a short dilated segment—a physiological or pseudo-receptaculum seminis. Vitellaria ventro-lateral of ceca extend from a point about equal to the diameter of acetabulum post-acetabular to a point slightly farther than halfway from testicular zone to caudal margin of worm, present as a rule a bilateral or unilateral break or interval in their continuity in about their equator corresponding in position and more or less in length to vertical diameter of ovarian zone; caudal ends substantially at same level. Pore of Laurer's canal on dorsum at variable level, cephalad of, in zone of, or caudad of shell gland. Uterine coils do not encroach on cecal fields; metraterm longer than cirrus pouch.

Egg: Uterine egg about 56 by 25 μ.

Excretory system: Longitudinal canals unite somewhat post-testicular; median stem discharges into dorso-cephalic aspect of a roomy excretory vesicle. Excretory vesicle distends caudal part and produces transluscent bulbous extremity.

HABITAT.—The stomach of *Amia calva* (type host) at Lake Maxinkuckee, Indiana (type locality).

TYPE.—U. S. P. H. & M. H. S. No. 10502. Cotypes Nos. 10498, 10499, and 10501.

SOURCE OF MATERIAL.—This species is represented by specimens bearing the U. S. P. H. & M. H. S. Nos. 10498, 10499, 10501, and 10502, that were collected from the stomach of *Amia calva* at Lake Maxinkuckee, Indiana, in 1906, and formed part of the collection of parasites already referred to.

EXTERNAL CHARACTERS.

SIZE.—The worms vary in length from 4 to 9.6 mm. A specimen measuring 7.3 mm. in length is 0.975 in maximum width as measured in frontal sections of the worm.

COLOR.—Some of the specimens are of an olive-green tint, others of a grayish-ivory. The position of the vitellaria is indicated by dark lateral lines and that of the uterine coils by a dark area.

FORM.—The worms are elongate bodies with approximately parallel margins and rounded extremities (fig. 11). Some of the worms show a slightly constricted zone in the region immediately preacetabular, but this does not appear to be as common nor as marked a character as in *Azygia acuminata*. Some of the worms instead of parallel margins show a more or less well-marked tendency to diminution in width in the direction of the caudal pole, which, however, is always rounded, though perhaps less bluntly than the cephalic, and contrasts notably with the pointed caudal pole of *Azygia acuminata*. In some specimens the caudal pole is distinctly bulbous from distention of the excretory vesicle. Transverse sections in the equatorial region of the worm are elliptical in outline.

SURFACE.—The surface cuticle measuring about 7.5 μ in thickness is unarmed. The ventral aspect of the cephalic extremity presents the oral aperture. In the median line of the venter about one-fifth to one-fourth of the body length from the cephalic margin is the acetabular aperture, slightly cephalad of which is the genital pore. At the vertex of the caudal extremity is the excretory pore; in some of the specimens this is at the bottom of a more or less marked indentation.

ACETABULUM.—The position of the acetabular aperture has been indicated; it is slightly smaller than the oral. In a specimen 8 mm. long the acetabulum measures about 0.585 mm. in both transverse and longitudinal diameters.

INTERNAL ANATOMY.

DIGESTIVE TRACT.—The ventro-subterminal oral aperture admits to the cavity of the oral sucker. In a specimen 6.6 mm. long the oral sucker measures 0.72 mm. in maximum longitudinal diameter. In a specimen about 8 mm. long the maximum longitudinal diameter of the sucker is about 0.63 mm. These measurements are to some extent

dependent on the degree and direction of contraction of the sucker itself at the time of fixation. In sagittal section the ventral wall of the sucker (fig. 12) resembles that of *Azygia acuminata* in outline, but differs from the latter in the comparatively slight development of the external layer containing the meridional fibers. In the specimen 6.6 mm. long the maximum dorso-ventral diameter of this part of the oral sucker is 0.21 mm. The oral sucker is succeeded by a well-developed muscular pharynx measuring 0.39 mm. in longitudinal, 0.255 mm. in transverse, and 0.30 mm. in dorso-ventral diameter in the specimen 6.6 mm. long. It is succeeded by a short inverted Y-shaped esophagus similar to that in *Azygia acuminata.* The esophagus passes dorso-cephalad, skirting the caudo-dorsal aspect of the pharynx. Its rami are directed cephalo-laterad and are continued as the intestinal ceca. The change from esophagus to gut is marked by a sphincter-like constriction (fig. 12) in the lumen as if due to a sphincter and by a change in the character of the lining membrane as in *Azygia acuminata* The beginning of the ceca is therefore in the pharyngeal zone. The intestinal tubes arch cephalad and outward into the suctorial zone, then pass in a tortuous zigzag course caudad to terminate very close to the caudal margin. The topography of the digestive tract and the course of the ceca is precisely as in *Azygia acuminata;* the cecal ends of the gut, however, are nearer the caudal margin than in the latter. In a specimen 7.3 mm. long the ceca terminated at a point 0.09 mm. from the caudal margin.

Genital system.— *Male organs:* The testes are in a zone slightly farther removed from the caudal margin than is the acetabular aperture from the cephalic margin; that is, about one-fourth to one-third the body length from the caudal margin. The testes are immediately caudad one of the other but with a slight obliquity. This obliquity is decidedly less marked than is the case in *Azygia acuminata.* The caudally placed testis appears in press preparations always to be the larger. The testes may be in close apposition or slightly separated but their zones always overlap to some degree. Their fields do not quite coincide. The surface of the testes is without infoldings. The vasa efferentia must be very delicate structures for in none of the sectioned specimens could they be satisfactorily traced. A cirrus pouch of the type described for *Azygia acuminata* is present. It is located (fig. 12) close to the dorso-cephalic aspect of the acetabulum and incloses a long coiled vesicula, a short beet-shaped musculosa, and a prostatica. The prostatica is continued as a ductus ejaculatorius. This penetrates a prominent genital papilla and discharges independently immediately cephalad of the vaginal pore, or (?) through a porous hermaphroditicus at the vertex of the papilla (fig. 13) into a distensible genital atrium. The genital papilla is a somewhat cylindrical or nipple-like structure (fig. 13) resembling that of

Hassallius hassalli and like the latter rises from the dorsal portion of the floor of the atrium and points cephalad or cephalo-ventrad. When not distended with eggs the atrium walls embrace the papilla and are in contact so that there is only a potential dorso-ventral passage discharging at the genital pore (fig. 12). Measured in sagittal sections the dorsal wall of the collapsed atrium is about 0.345 mm. from the ventral surface, and in one slightly distended it is 0.262 mm. from the surface. A protrusile cirrus is not present in this form.

Female organs: The ovary is cephalad of and in close apposition to the cephalic testis, the zone of the one overlapping to a variable but never to any considerable degree that of the other, their fields nearly coinciding. In size it is intermediate between the two testes and like them it is somewhat polyhedral in form and without infoldings of the surface. Close to its dorso-cephalic aspect sometimes either a little to the right or to the left is the somewhat smaller, compact shell gland. An oviduct springs from the cephalic aspect of the ovary and arches cephalad toward the shell gland then turns caudad between the gland and ovary. As it is about to penetrate the shell gland it gives off Laurer's canal. Laurer's canal describes a variable course in its passage to the dorsum where it terminates at a point immediately cephalad of the shell gland, in the zone of the shell gland, or slightly caudad of it. As in *L. micropteri*, a short segment of the proximal end of the canal is dilated into what may be regarded as a physiological or pseudo receptaculum seminis. The vitellaria are ventro-laterad of the ceca and extend from a point in a transverse plane about the length of or slightly more than the acetabular diameter postacetabular to a point in a plane about or a little farther caudad than midway between the caudal margin of the caudal testis and the caudal margin of the worm. The cephalic and caudal ends are substantially symmetrically placed. In most of the specimens both vitellaria present a well marked break or interval in their continuity at about their equator; that is, at about the level of the ovarian zone. The interval varies in extent. It may equal or slightly exceed the ovarian zone. In some instances the break appears to involve only one of the glands; in a small minority of specimens neither gland presents such an interval. Secondary or minor breaks in the vitellaria especially in the postovarial segments occur in some specimens. The transverse ducts from these glands unite to form a vitelline reservoir close to the shell gland, the relation between the two being somewhat variable. The course of the transverse vitelline ducts could not be traced so that their relation to the excretory canals and ceca can not be stated. A common vitelline duct leaves the reservoir and penetrates the shell gland. The uterus emerges from the cephalic pole of this gland and at once forms coils, some loops of which are tucked down laterally and dorsally of the gland. The distended coils of the

uterus fill the intercecal area between the level of the shell gland and base of the cirrus sac. They do not encroach on the cecal fields. The terminal portion of the uterus or metraterm begins a little caudad of the base of the sac, passes ventro-cephalad between the acetabulum and the pouch to penetrate the genital papilla at the vertex of which it discharges either independently or perhaps (?) through a pore in common with the ductus ejaculatorius.

Egg: The uterine egg as measured at favorable points in sections is somewhat smaller than that of *Azygia acuminata*, being about 56 by 25μ.

Excretory system.—The longitudinal excretory canals can be traced from a point on each side of the oral sucker at about the level of the cephalic margin of the oral aperture. They describe substantially the same course caudad as in *Azygia acuminata* until they reach the ovarian zone. Here, instead of tending obliquely dorsad as in *A. acuminata*, they maintain their direct course caudad in close relation to the inner aspect of the corresponding gut embracing within their interspace the ovary and then the testes. They unite not in the testicular zone but slightly caudad of it (fig. 11). The median canal thus formed passes caudad in about the axial region until it reaches about the level of the caudal ends of the vitellaria where it discharges into the dorso-cephalic aspect of a roomy vesicle (fig. 14) resembling that described for *Azygia loossii* by Marshall and Gilbert. This vesicle when distended causes the bulbous form of the caudal end. The distention affects the ventral aspect particularly, the distended vesicle being nearer the ventral than the dorsal surface (fig. 14), and gives this part of the worm a transluscent blister-like appearance (particularly ventrally). The excretory vesicle discharges by a short duct at the vertex of the caudal pole.

AZYGIA LOOSSII Marshall and Gilbert, 1905.

[Figs. 15–16.]

Specific diagnosis.—The characters of the genus. Body slender, oblong-ovate, 5 to 6. 7 mm. long by 0.5 mm. broad, cephalic pole not so pointed as caudal. Cuticle 15μ thick. Acetabular aperture slightly preequatorial smaller than oral aperture. Esophagus inverted Y-shaped, about as long as pharynx. Ceca substantially straight tubes, begin post-pharyngeal, pass directly caudad, terminate slightly short of caudal margin.

Male organs: Testicular zone about one-seventh to one-eighth of body length from caudal margin. Caudal testis slightly cephalad of dome of excretory vesicle. Testis globular with zones and fields overlapping. Caudal testis more nearly median in position and very near dome of excretory vesicle; surface of testes without infoldings. Cirrus sac with delicate, ill-defined walls. Ductus ejaculatorius discharges separately from metraterm. Protrusile cirrus absent. Large conical genital papilla projects ventro-cephalad into roomy genital atrium.

Female organs: Ovarian zone cephalad of and separate from or overlaps testicular zone. Long (transverse) diameter of ovary exceeds that of either testis. Shell gland dorso-cephalad of ovary. The uterine coils do not encroach on cecal fields; metra-

term longer than cirrus pouch. Vitellaria extracecal extend from a little post-acetabular to about midway between caudal testis and caudal margin of worm.

Egg: Uterine egg measures about 55 by 33.7μ.

Excretory system: Longitudinal canals discharge separately into a roomy excretory vesicle. Dome of excretory vesicle only a little post testicular. Excretory pore caudo-terminal.

HABITAT.—Mouth and stomach of *Micropterus salmoides* (type host), *Lucius lucius*, and *Amia calva* from Madison Lakes and Round Lake, Wisconsin.

COTYPE.—U. S. P. H. & M. H. S. No. 10679 (mounted and sections).

SOURCE OF MATERIAL.—Professor Marshall was good enough to donate several mounted and two sectioned specimens of *Azygia loossii* to the Hygienic Laboratory collection. I have therefore been able to compare this species with *A. acuminata* and *A. bulbosa*. As a result of this comparison I am able to add some points to the rather brief description given by Marshall and Gilbert.

EXTERNAL CHARACTERS.

SIZE AND FORM.—Marshall and Gilbert describe their fluke as oblong-ovate in form, with the cephalic end not so pointed as the caudal; 5 to 6.7 mm. long and 0.5 mm. broad. These measurements are presumably of specimens in alcohol, for one of the mounted and one of the sectioned specimens donated by Professor Marshall both measure 4 mm. in length. In form (fig. 15) it comes nearer to *A. bulbosa* than *A. acuminata*, but is obviously more slender than either, and in ventral view its margins appear more nearly parallel than is the case in *A. bulbosa*.

SURFACE.—The cuticle is unarmed. It measures about 15μ in thickness and is, therefore, somewhat thicker than in either *Azygia bulbosa* or *A. acuminata*. The acetabular aperture is only slightly preequatorial in position differing markedly in this respect not only from *A. lucii*, as noted by Marshall and Gilbert, but also from *A. bulbosa* and *A. acuminata*. The genital pore is slightly cephalad of the cephalic margin of the acetabular aperture, in the same relative position as in *lucii*, *bulbosa*, and *acuminata*.

ACETABULUM.—The position of its aperture on the ventral surface has been indicated. In a sectioned specimen about 4 mm. long the vertical diameter of this sucker is 0.30 mm. and of its aperture about 0.09 mm.

INTERNAL ANATOMY.

DIGESTIVE TRACT.—The oral aperture is terminal in position (fig. 15), but in a plane directed obliquely ventro-caudad, so that at first thought it appears ventro-subterminal.

Marshall and Gilbert state that the oral sucker is 0.425 mm. in diameter. In the series of sections already referred to the oral sucker measures 0.33 mm. in vertical and 0.315 mm. in dorso-ventral diame-

ter and its (oral) aperture 0.195 mm. in dorso-ventral diameter. In sagittal section the ventral wall of this sucker (fig. 16) is oval in outline with the broadly rounded pole at the base; the segment of the oval line limiting the suctorial cavity is slightly flattened. This part of the sucker, in its outline and structure, comes decidedly nearer *A. bulbosa* than *A. acuminata.* The oral sucker is succeeded by a well developed pharynx which Marshall and Gilbert state is 0.15 mm. wide. In the sections of the 4 mm. specimen already mentioned the vertical and dorso-ventral diameters are about equal and measure about 0.14 mm. In their description of this worm, speaking of the esophagus, Marshall and Gilbert state that "the esophagus, if present, is very short and not readily distinguished, the intestinal ceca appearing to arise directly from the pharynx." In the sections before me an inverted Y-shaped esophagus about as long as the pharynx and of the type described for *A. bulbosa* and *acuminata* can be clearly made out (fig. 15), and as in *A. bulbosa* and *acuminata* the transition from esophagus to gut is marked by a constriction as of a sphincter and by the usual change from cuticle to epithelium in the lining membrane. The first portion or median stem of the esophagus passes directly caudad, but the forks or paired stems bend and pass dorsad in slightly separated planes. The ceca begin in a plane immediately post pharyngeal and at first curve latero-caudad, then pass directly caudad without arching into the pharyngeal zone, as is described and pictured by Looss for *A. lucii* (fig. 7), and still less into the suctorial zone as occurs in *bulbosa* and *acuminata.* In a toto preparation 4 mm. long the cecal ends fell short by 0.135 mm. of reaching the caudal margin. In this respect, therefore, this worm is very much nearer *A. acuminata* than *A. bulbosa.* The ceca in this form, as pointed out by Marshall and Gilbert, are much straighter than pictured by Looss for *A. lucii,* and very decidedly more than in *A. acuminata* or *A. bulbosa.* In fact, the ceca in the form under discussion are practically straight tubes.

Genital system.— *Male organs:* The testes are described by Marshall and Gilbert as 0.125 mm. in diameter, placed one behind the other in the posterior part of the body, generally a little diagonally, the posterior being the more constant in its median position and separated from each other by a distance not so great as the diameter of either one. In the toto mounts before me I find that the testes are globular in form without infoldings of the surface and are in a zone about one-seventh to one-eighth the body length from the caudal margin, and, therefore, relatively much farther caudad than in *A. lucii, acuminata,* or *bulbosa.* In none of the five specimens before me are the testes separated to the degree described and pictured by Marshall and Gilbert. In all of these the testes are close one to the other, with zones and fields that overlap to some degree. The

caudal testis is slightly cephalad of the dome of the excretory vesicle between the somewhat converging terminal portions of the excretory canals. The copulatory apparatus conforms to the type described for *A. acuminata* and *A. bulbosa*. The cirrus sac is placed near the dorso-cephalic aspect of the acetabulum. Its wall is quite delicate and ill-defined and seems to inclose all but the terminal segment of the vas deferens. The ductus ejaculatorius penetrates a large conical papilla and discharges into a relatively roomy genital atrium (fig. 16) by a pore that Marshall and Gilbert interpret as just cephalad of the opening of the vagina. The genital papilla projects into the sinus from what may be regarded as the dorsal wall of the latter. Its major axis, however, instead of being directed dorso-ventrally, as in *A. acuminata*, is directed obliquely ventro-cephalad.

Female organs: Marshall and Gilbert describe the ovary as in part or entirely in front of the anterior testis, ovoid in form and slightly larger than either testis, its long diameter lying at a right angle to the long axis of the fluke. "A short oviduct passes anteriorly and is joined by Laurer's canal; then passing forward for a short distance, it turns backward through the shell gland." The shell gland is a compact body that lies close to the dorso-cephalic aspect of the ovary. In the available preparations, the path and termination of Laurer's canal can not be traced. The vitellaria, consisting of a moderate number of follicles, are extracecal and extend from a point a little, about half the acetabular diameter, postacetabular to about midway between the caudal margin of the caudal testis and the caudal margin of the worm. The uterine coils occupy the intercecal area between the plane of the cephalic margin of the ovary and about the equatorial plane of the acetabulum. At about the latter level the metraterm begins; it passes cephalo-ventrad between acetabulum and cirrus sac, penetrates the genital papilla to discharge at its vertex into the genital atrium.

Egg: Measured at what were judged to be favorable points in sections, the uterine egg is about 55 by 33.7μ.

Excretory system.—This is described by Marshall and Gilbert as follows:

> A short narrow duct leads from the terminal excretory pore into the short broad bladder which does not extend in front of the posterior testis. In all the specimens of this fluke, when living, the bladder was swollen and distinctly seen, allowing us to separate this from other flukes by the large, bright swelling which appeared in the posterior end of the body. From the anterior margin of the bladder two long, thin lateral tubes pass forward into the front region of the body. These we have traced almost to the pharynx.

The excretory vesicle is roomy like that in *A. bulbosa*, but differs from the latter chiefly in that the longitudinal canals in this form discharge separately into the vesicle, whereas in *A. bulbosa* the

excretory canals unite to form a median stem and this latter unpaired canal discharges into the vesicle.

RELATED SPECIES.

Besides the foregoing a number of other species belong here or in very closely related genera, namely, *Distomum longum* Leidy, 1851, *Distomum augusticaudum* (Stafford, 1904), *Distomum volgense* (Linstow, 1907) Luehe, 1909, *Distomum veliporum* Creplin, and *Distomum priostophori* Johnston, 1902.

* * *

In the collection of parasites of which *A. bulbosa* and *A. acuminata* formed a part there were three individuals of a species that I believe should be regarded as representative of a new genus.

HASSALLIUS[a] new genus.

GENERIC DIAGNOSIS.—Body small, relatively thick, spindle-shaped with rounded cephalic and pointed caudal end. Cuticle smooth. Acetabular aperture median, preequatorial, acetabulum smaller than oral sucker. Genital pore median, slightly preacetabular. Oral sucker and pharynx present, no prepharynx; esophagus short inverted Y-shaped. Ceca extend to near caudal margin.

Male organs: Testes postequatorial, intercecal, obliquely caudad one of the other; zones overlap slightly, fields to a greater degree. Surface without infoldings. Thin-walled cirrus pouch present; no protrusile cirrus.

Female organs: Ovary by the side and within zone of cephalic testis; ovarian zone cephalad of and abuts zone of caudal testis. Shell gland compact. No receptaculum seminis. Laurer's canal present. Uterus preovarian, pretesticular, and intercecal, in transverse coils. Vitellaria elongate in extracecal areas, postacetabular.

TYPE SPECIES.—*Hassallius hassalli* new species, Indiana, U. S. A.

This new genus aside from its external characters differs from *Azygia* in the position of the ovary, which here is by the side of, that is, in the same transverse plane as the cephalic testis, instead of cephalad of the latter, as in *Azygia*. It is also near to *Ptychogonimus* Luehe, 1900, but differs in the character of the genital atrium and in the topography of the uterus.

HASSALLIUS HASSALLI new species.

[Figs. 17 to 18.]

SPECIFIC DIAGNOSIS.—The characters of the genus. Body muscular, spindle-shaped, 1.94 to 2.31 mm. long by 0.7 to 0.80 mm. in maximum breadth; transverse sections at equator circular to subcircular with slightly flattened venter. Cuticle about 15μ thick. Acetabular aperture two-fifths of body length from extremity in mid-ventral line, smaller than oral aperture. Oral aperture ventro-subterminal; oral sucker 0.435 mm. in transverse and 0.435 to 0.450 mm. in vertical diameter. Pharynx measures about 0.295 mm. in vertical and 0.165 mm. in transverse diameter, no prepharynx. Esophagus is quite short, forked, directed dorsad; esophageal fork at level of base of

[a] Dedicated to Dr. Albert Hassall, to whom, jointly with Professor Stiles, all helminthologists are indebted for that invaluable work, the Index Catalogue of Medical and Veterinary Zoology.

pharynx. Ceca at first arch cephalad into suctorial zone, then pass caudad in a zigzag course approximately parallel to dorso-lateral aspect of body wall, terminate close to caudal margin.

Male organs: Testes polyhedral in intercecal area, in immediate contact and one obliquely caudad of the other; occupy a zone about one-fifth body length from caudal margin; vertical diameter of this zone equal to about one-ninth of body length. Caudally placed testis in axial region, the other decidedly submedian; cirrus pouch, thin-walled, contains vesicula, musculosa, and prostatica. Ductus ejaculatorius succeeds prostatica, penetrates papilla, discharges with metraterm through porus hermaphroditicus into dorso-ventrally elongate genital atrium. Genital papilla relatively large, projects cephalad from dorsal portion of floor of genital atrium.

Female organs: Ovary relatively large, ovoid, in zone of cephalically placed testis in the angle formed by the juxtaposed testes. Shell gland close to dorsal pole of ovary, relatively large but smaller than and entirely within the zone of the latter. Uterus forms coils in transverse plane, passes cephalad filling intercecal area between zone of ovary and shell gland and that of cirrus sac. Metraterm long, begins at level of base of cirrus pouch. Laurer's canal present, pore slightly preovarian. Vitellaria consist of an elongate aggregation of irregularly globular follicles ventro-laterad of intestinal ceca, extend from a little caudad of caudal margin of acetabulum to a little less than midway between caudal testis and caudal margin of worm. Not broken in continuity.

Egg: Uterine egg ovoid, 48 by 26μ.

Excretory system: Main longitudinal canals begin dorso-laterad of oral sucker, slightly caudad of cephalic margin of oral aperture, pass caudad close to inner aspect of corresponding gut, unite slightly caudad of caudal testis to form excretory vesicle. Vesicle discharges by relatively long duct; excretory pore caudo-terminal.

Habitat.—Stomach of *Ambloplites rupestris* (type host) from Lake Maxinkuckee, Indiana (type locality).

Type.—U. S. P. H. & M. H. S. No. 10525 (mounted and sections).

Source of material.—The description of this species is based on three specimens bearing the P. H. & M. H. S. No. 10525. The worms are from the stomach of *Ambloplites rupestris*, and are part of the collection of parasites referred to in previous connection.

EXTERNAL CHARACTERS.

Size.—Two of the specimens were measured in glycerine-alcohol, and were found to be 2.31 mm. and 2.13 mm., respectively, long and 0.77 mm. and 0.80 mm., respectively, in greatest width. Transverse sections of the former specimen gave a maximum of 0.70 mm. in transverse and 0.52 mm. in dorso-ventral diameter. The third specimen, as a press preparation, measures 1.94 mm. in length by 0.74 mm. in maximum width.

Color.—One of the specimens in glycerine-alcohol was of a faint olive green, the other of a brownish olive green tint.

Form.—In ventral view the worms have the appearance of short, plump, somewhat spindle-shaped bodies, with rounded cephalic and more or less bluntly pointed caudal poles. The maximum transverse and dorso-ventral diameters are slightly post-equatorial. The lateral margins of the postacetabular portion of the worm are gently curved, the body of the worm tapering toward both poles, but more particu-

larly toward the aboral. At or immediately cephalad of the acetabular zone the rate of curvature of the lateral margins changes, becoming greatly reduced or almost disappearing, this portion of the body tapering but slightly in the direction of the oral pole. Transverse sections in the equatorial zone are elliptical to subcircular in outline, but with a more marked dorsal than ventral curvature; that is, the venter is relatively slightly flattened.

Surface.—The cuticle is unarmed, and measures 15 μ in thickness. It is marked by numerous transverse sulci, apparently due to contraction of the worm. In one of the specimens these sulci are particularly noticeable as concentric rings around the caudal pole at the center of which in a dimple-like depression is the excretory pore. On the ventral aspect of the cephalic pole there presents the irregularly circular or elliptical oral aperture; caudad of this at about the junction of the second with the third fifth of the body length is the somewhat smaller irregularly circular aperture of the acetabulum. Slightly cephalad of the acetabular aperture in the median line is the minute genital pore.

Acetabulum.—The position of the acetabular aperture has already been indicated. In a sectioned specimen it measures 0.11 mm. in longitudinal and 0.15 mm. in transverse diameter. The acetabulum itself in the same sections measures 0.30 mm. in longitudinal and 0.36 in transverse diameter. It is somewhat smaller than the oral sucker.

INTERNAL ANATOMY.

Digestive tract.—The oral aperture, as already indicated, is ventro-subterminal, and in a series of transverse sections measures 0.225 mm. in transverse and 0.165 mm. in longitudinal diameter, being elliptical to irregularly circular in form and distinctly larger than the acetabular aperture. It gives entrance to the lumen of the oral sucker. Measured in the same series of transverse sections, the oral sucker is 0.435 mm. in maximum transverse and 0.435 to 0.450 mm. in longitudinal diameter. In the press preparation the corresponding diameters measure 0.435 and 0.420 mm., respectively. Its form is ovoid, and at its broadly-rounded base gives attachment to the pharynx without the intervention of a prepharynx. The ventral wall of the sucker is greatly thickened near its base (fig. 18). This portion presents two well-defined layers, an inner, chiefly of radiating with some circular fibers, and an outer of radiating and meridional bundles of fibers. The pharynx is ellipsoid in form, measuring in the transversely-sectioned specimen 0.295 mm. in longitudinal and 0.165 mm. in maximum transverse diameter. In turn, the pharynx is succeeded by a short Y-shaped esophagus like that in *L. micropteri*, which runs directly dorsad, and the lateral stems of which are con-

tinued as the lateral intestinal ceca. The transition from one to the other is marked by a change from a cuticular to an epithelial lining. Because of the direction taken by the esophagus, the press preparation gives one the impression of an entire absence of such a structure. The ceca from their origin pass cephalo-laterad and slightly ventrad, describing an arch, with the concavity caudad, that reaches a little cephalad of the base of the oral sucker. The ceca then pass caudad to terminate about 0.105 mm. from the caudal extremity as measured in the press preparation. The ceca describe a zigzag course approximately parallel to the lateral and dorso-lateral aspect of the body at a distance of from 60 to 75 μ from the surface. The loops are numerous, relatively of considerable amplitude; there is one on either side in the acetabular zone that is especially marked and noticeable in the press preparation.

GENITAL SYSTEM.—*Male organs:* The testes are in the caudal portion of the intercecal area, one obliquely caudad of, though in immediate contact with the other, so that both their zones and their fields overlap, the former slightly, the latter to a little greater extent. They occupy a zone whose longitudinal diameter is equal to about one-ninth the total body length; this zone is a little less than twice its own longitudinal diameter from the caudal margin of the worm. The caudally-placed testis is in about the longitudinal axis of the worm, with its long diameter (as seen in ventral view) at right angles to the latter; the cephalically-placed testis lies immediately either to the right or to the left of the median line, with its long diameter (as seen ventrally) obliquely to the longitudinal axis of the worm and to the long diameter of the other testis. The testes are polyhedral in form, being in contact not only one with the other, but with the ovary, shell gland, and uterine coils. The vasa efferentia could not be made out in the specimens available for study. A cirrus pouch is present. It is a thin-walled sac, placed dorso-cephalad of the acetabulum and incloses a coiled, distended, thin-walled vesicula, a short, slightly coiled, thicker-walled portion, interpreted as the pars musculosa and a short, straight prostatica. The latter is continued as the ductus ejaculatorius which penetrates the genital papilla to discharge at its vertex by a pore in common with the metraterm. A protrusile cirrus is not present.

Female organs: The ovary lies in the angle resulting from the relation of the testes one to the other and in close apposition to both. It is a relatively large ovoid body, but somewhat smaller than either testis. Its zone is entirely within that of the cephalic testis, whose field it overlaps slightly. The oviduct springs from the cephalic aspect of the ovary and passes dorsad to the shell gland which it penetrates on its ventro-cephalic aspect a little to the left. A compact shell gland

considerably smaller than the ovary lies close to the dorsal pole of the latter. The uterus emerges from the cephalic pole of the shell gland and at once forms coils, some loops of which dip slightly caudad laterally (right and left) of this gland. The distended coils of the uterus, as the latter winds its way cephalad, completely fill that portion of the intercecal area between the cephalic limit of the zone occupied by the shell gland and ovary and the plane of the base of the cirrus pouch. At this latter level the uterus ceases to form coils and as the metraterm at first skirts the right lateral then the ventral aspects of the base of the cirrus pouch as it passes ventro-cephalad to assume a position close to the left ventro-lateral aspect of the pouch. It then proceeds cephalad in the angle between the acetabulum and the cirrus pouch until it gains the level of the cephalic margin of the acetabulum. Here it shifts toward the median line to assume a position ventrally of the cirrus pouch and then proceeds ventro-cephalad toward the genital papilla which it penetrates. A receptaculum seminis can not be made out. Laurer's canal leaves the oviduct and passes caudo-dorsad, skirting at first the ventro-caudal aspect, then the caudo-dorsal aspect of the shell gland as it curves dorso-cephalad in its course to the dorsum which it reaches at a point in a plane immediately preovarian. There is a strong suggestion that the proximal segment of the canal is dilated quite like that in *L. micropteri*. The vitellogene glands are well developed and consist of an elongate fairly compact aggregation of irregularly globular follicles ventro-laterad of the corresponding intestinal ceca in a zone equal to about five-thirteenths of the body length. Cephalically they begin a little (about a fourth of the acetabular diameter) caudad of the plane of the caudal margin of the acetabulum and caudally terminate in a plane about three times as far from the caudal margin of the worm as is the caudal margin of the acetabulum from their cephalic ends, that is, a little less than half way between the caudal testis and the caudal margin of the worm. The level of origin and that of termination are substantially symmetrical on the two sides. The course of the vitello-ducts could not be made out. It is clear, however, that they must unite at a point between the adjacent aspects of the ovary and shell gland where one can note a duct distended considerably by vitelline cells. This is probably a vitelline reservoir. The copulatory apparatus does not appear to include a protrusile cirrus, but is of the type of *Leuceruthrus micropteri*. The genital papilla (fig. 18) is a relatively large structure of the form of a truncated cone that measures in dorso-ventral diameter 67μ at the base and 53μ at the summit and in its major axis 19μ from base to summit. It projects cephalad from the floor into and almost completely fills the dorsal portion of an almost slit-like genital atrium. This atrium is a vertically narrow, dorso-ventrally elongate space almost immediately

cephalad of the acetabulum. Its dorsal wall is at about the mid-point between the ventral and dorsal body margin at a level in which the dorso-ventral diameter of the worm measures 0.487 mm. The extreme transverse like the extreme longitudinal diameter is just sufficient to contain the genital papilla. It is probably capable of considerable distention as in *Leuceruthrus* and *Azygia*. The atrium discharges by a minute pore in the ventro-median line at a point but very slightly cephalad of the cephalic margin of the acetabulum.

Egg: The uterine egg appears ovoid in form and in sections at a favorable point measures 48 by 26μ.

Excretory system.—Two main excretory canals begin close to the dorso-lateral aspects of the oral sucker at a level slightly caudad of that of the cephalic margin of the oral aperture. They pass caudad; in their courses they come in relation to the lateral aspects of the pharynx, then the ventral aspects of the arch formed on each side by the corresponding intestine as they pass to a position close to the inner aspect of the latter. This relative position they maintain throughout the remainder of their course. They terminate by uniting at a point slightly caudad of the caudal margin of the caudally placed testis in the formation of an excretory vesicle. The latter is transversely narrow, dorso-ventrally and longitudinally elongate, practically filling the caudal portion of the intercecal area. It discharges by a relatively long duct at the caudal pole.

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ON SOME NEW PARASITIC TREMATODE WORMS OF THE GENUS TELORCHIS.

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(Figs. 19–20.)

The worms here to be described belong in the subgenus *Cercorchis* of Luehe's genus *Telorchis*.

Genus TELORCHIS Luehe, 1899.

GENERIC DIAGNOSIS.—Body elongate, markedly attenuate cephalad of acetabular zone. Cuticle with spines. Acetabular aperture midventral, considerably preequatorial. Genital pore preacetabular, submedian. Oral sucker larger than acetabulum; pharynx present; ceca simple, long.

Male organs: Testes one caudad of the other, intercecal, postuterine near caudal end; surface without in foldings; cirrus pouch very elongate; protrusile cirrus present.

Female organs: Ovary intercecal, in uterine zone; post acetabular; pretesticular at or near base of cirrus pouch. Uterus with descending and ascending limb, intercecal but extending into extracecal areas. Laurer's canal present. Vitellaria in extracecal areas, very elongate.

Excretory system: Excretory bladder, roomy, long, Y shaped.

TYPE SPECIES.—*Telorchis clava* (Diesing) Luehe, 1899.

Subgenus CERCORCHIS Luehe, 1900.

1899: *Telorchis* Looss, 1899*b*.

SUBGENERIC DIAGNOSIS.—The characters of the genus, but uterine coils do not extend extracecal.

TYPE SPECIES.—*Telorchis* (*Cercorhis*) *linstowi* (Stossich) Luehe, 1900.

Several species have been assigned to this genus. These, including those about to be described, may be distinguished by the following key:

KEY TO THE SPECIES OF TELORCHIS.

A¹. Ceca end in intertesticular zone.

B¹. Vitellaria entirely post ovarian; prepharynx absent; esophagus relatively long ..... *T. parvus*

B². Vitellaria not entirely post ovarian, begin a little preovarian; prepharynx present, esophagus relatively very short ..... *T. poirieri*

A². Ceca end farther caudad, in post testicular zone.
B³. Vitellaria entirely postovarian and postacetabular.
C¹. Cirrus pouch begins at cephalic margin of ovary; ovary about at junction of cephalic with middle third of body length or as much postacetabular as acetabulum is postbifurcal..... *T. bifurcus*
C². Cirrus pouch begins considerably preovarian; ovary considerably more than one-third, about two-fifths, of body length from cephalic margin or about half as far post acetabular as acetabulum is postbifurcal..... *T. pleroticus*
B⁴. Vitellaria not entirely postovarian, begin preovarian and preacetabular.
C³. Esophagus absent; well developed prepharynx present; vitellaria extend throughout the length of the body..... *T. clava*
C⁴. Esophagus present; prepharynx absent; vitellaria extend caudally to cecal ends of gut, ending posttesticular..... *T. arrectus*
C⁵. Esophagus present; prepharynx present; vitellaria do not extend to cecal ends of gut, ending pretesticular..... *T. angustus* (p. 47)
B⁵. Vitellaria not entirely postovarian, begin preovarian but postacetabular.
C⁶. Esophagus absent; cirrus pouch begins in ovarian zone; ovary at about body equator and slightly cephalad of vitellarian equator..... *T. robustus* (p. 44)
C⁷. Esophagus present.
D¹. Cirrus pouch begins considerably preovarian.
E¹. Ovary at about equator of vitellaria; prepharynx present; testicular zones abut or overlap..... *T. attenuatus* (p. 38)
E². Ovary distinctly caudad of vitellarian equator; prepharynx absent; testicular zones separated by one-fourth of testicular diameter. *T. stossichi* (p. 38)
D². Cirrus pouch begins in ovarian or postovarian zone.
E³. Vitellaria hardly one-fourth of body length, extend caudad to a point halfway from ovary to cephalic testis; prepharynx present. *T. ercolanii*
E⁴. Vitellaria more than one-fourth of body length, extend caudad more than halfway from ovary to cephalic testis.
F¹. Ovary at about equator of vitellaria; no prepharynx; testes abut, caudal testis its own diameter from caudal margin.... *T. solivagus*
F². Ovary considerably cephalad of vitellarian equator.
G¹. Acetabulum about one-fifth of body length from cephalic margin; prepharynx absent; ovary about two-fifths of body length from cephalic margin; caudal testis but slightly more than its own diameter from caudal margin; egg 46 by 19 μ.. *T. linstowi* (p. 47)
G². Acetabulum about one-fourth of body length from cephalic margin; prepharynx present; ovary only a little less than halfway from cephalic to caudal margin; caudal testis about twice its own diameter from caudal margin; egg 33.4 by 19.4 μ.. *T. nematoides*

Some of the species are as yet insufficiently described and pictured. In 1885 Poirier described and pictured a form from *Cistudo lutraria*, which he identified as *Distomum gelatinosum* Rud. In 1893 Sonsino called attention to differences between the form described by Poirier and specimens of *D. gelatinosum* in his possession and expressed the opinion that Poirier's form represented a new species. Later, apparently on the strength of this, Stossich (1895) changed the name of *Dist. gelatinosum* Poirier to *Dist. poirieri*. In 1904, on the basis of two specimens from the intestine of *Emys orbicularis* belonging to the Monticelli collection, Stossich described and pictured a form that he

identified with Poirier's. At the same time, however, he called attention to certain differences between the form pictured by him and that by Poirier but these he thought were due either to artifacts or to superficial observation by Poirier. A study of Poirier's description and figure and those of Stossich (1904) brings out certain differences, among which may be mentioned the difference with respect to the prepharynx which is present in Poirier's and absent in Stossich's form. The ovary in Poirier's form is distinctly preequatorial; in Stossich's, distinctly postequatorial. In Poirier's form the acetabulum is less than one-fifth of the body length from the cephalic margin, whereas in Stossich's it is slightly more than one-fourth the body length from the cephalic margin. Again in Poirier's form the vitellaria extend through less than one-third the body length and their cephalic ends, though preovarian, are nearer the ovarian than to the acetabular zone, whereas in Stossich's they extend through more than one-third the body length and their cephalic ends, though also preovarian, are nearer the acetabular than to the ovarian zone. Finally the ceca in Poirier's form end in the intertesticular zone while in Stossich's they extend farther caudad ending in the posttesticular zone.

These differences, taken as a whole, appear to me too marked for Stossich's explanation to be convincing. On the contrary it seems to me that they justify the opinion that the form described and depicted by Stossich (1904) is distinct from Poirier's and should be so considered until comparison of Poirier's material with Stossich's shall show them to be identical. Accordingly I would propose for the form described and pictured by Stossich the name *Telorchis stossichi.*

TELORCHIS (CERCORCHIS) STOSSICHI new species.

1904: *Telorchis poirieri* (Stossich, 1895) Stossich, 1904 not *Distomum poirieri* Stossich, 1895.

SPECIFIC DIAGNOSIS.—The characters of the genus. Body 10 to 11 mm. long by 0.9 mm. wide, both poles broadly rounded, margins parallel throughout entire length. Acetabulum slightly more than one-fourth of body length from cephalic margin. Genital pore median, slightly preacetabular; no prepharynx; esophagus quite short; ceca extend posttesticular, ending close to caudal margin.

Male organs: Testes large, spherical, one caudad of the other, with zones separate by about one-fourth of testicular diameter. Cirrus pouch begins at about the ovarian diameter, preovarian.

Female organs: Ovary at junction of equatorial with penultimate fifth of body length; globular, somewhat smaller than testes. Uterine coils entirely intercecal; metratrum long, to right of cirrus pouch. Vitellaria extracecal, extend from a little postacetabular to slightly more than midway from ovary to cephalic testis; vitelline follicles in groups.

TYPE.—The two specimens of the Monticelli collection, forming the basis of Stossich's illustration and description.

TELORCHIS (CERCORCHIS) ATTENUATUS new species.

[Fig. 19.]

SPECIFIC DIAGNOSIS.—The characters of the genus. Body subcylindrical, dorsoventrally flattened; cephalic extremity rounded, caudal bluntly pointed; 7.35 mm. to 11 mm. long by about 0.93 mm. in maximum breadth. Cuticle 11 μ thick, with

delicate spines that are very sparse or absent around caudal pole. Acetabular aperture two-elevenths of body length from oral extremity in mid-ventral line; acetabulum about as large as oral sucker. Oral aperture ventro-subterminal; oral sucker with same dimensions as acetabulum; prepharynx about half as long as, and esophagus of same length as, pharynx; combined length of prepharynx, pharynx, and esophagus about twice the length of oral sucker. Esophageal fork an acute angle; intestinal ceca terminate close to caudal margin.

Male organs: Testes globular to ovoid, near caudal extremity, in intercecal area, zones abut or slightly overlap, fields identical and slightly overlap right intestine. Vasa efferentia cross dorsal aspect of excretory canals. Cirrus sac extends in a loose spiral two-thirds of the way from genital pore to ovary; contains vesicula, prostatica, and cirrus; latter discharges into a genital atrium.

Female organs: Ovary globular to ovoid, slightly smaller than cephalic testis; in intercecal area a little postequatorial, tending dextrad. Shell gland an ill-defined, dorso-ventrally elongate, loose, aggregate of cells close to caudal aspect of ovary, between converging excretory canals. Receptaculum seminis absent. Laurer's canal present, with pore dorsal slightly caudad of plane of caudal margin of ovary. Uterine coils in intercecal area overlap cecal fields and in postvitellarian zone extend into right extracecal area; uterine and testicular zones abut; metraterm, about half as long as cirrus pouch, in a loose spiral sinistral of cirrus sac, discharges into genital atrium. Vitellaria made up of globular follicles externally, ventrally, and dorsally of intestinal ceca; zone extends from a little caudad of acetabular to slightly cephalad of testicular zone; right gland slightly shorter than left, shows one break in continuity; left gland shows two breaks in continuity.

Egg: Uterine egg 30 by 12 to 13 μ.

Excretory system: Excretory canals begin a little caudad of cephalic end of vitellaria, large, unite slightly caudad of shell gland, forming roomy, long vesicle; short excretory duct discharges by caudo-terminal pore. Excretory canals and vesicle together Y-shaped.

HABITAT.—Stomach of *Chrysemys marginata* (type host) at Lake Maxinkuckee, Indiana (type locality).

TYPE.—U. S. P. H. & M. H. S. No. 10523 (mounted and sections).

SOURCE OF MATERIAL.—This species, represented by two worms (U. S. P. H. & M. H. S. No. 10523) from the stomach of *Chrysemys marginata*, was part of a collection of parasites from Lake Maxinkuckee, Indiana, sent in 1908 by the United States Bureau of Fisheries to Professor Stiles for determination. When I took up this study I found one of the specimens already mounted as a press preparation; it presented a good picture of the general topography of the internal structures. The other was in glycerine-alcohol, and was sectioned by me to serve as a check on the first and to permit of more detailed study of the anatomy than was possible from the toto mount alone.

EXTERNAL CHARACTERS.

SIZE.—The specimen in glycerine-alcohol measured 7.35 mm. in length; the mounted specimen is 11 mm. long. This measurement, in both instances, was obtained from a camera lucida outline. After sectioning the former specimen, measurements were made of the transverse diameters at the level of the acetabulum and at that of the

ovary; at the former level the transverse diameter is 0.645 and at the latter 0.735 mm. Measurements of the transverse diameter of the specimen mounted as a press preparation at corresponding levels give 0.57 and 0.825 mm., respectively. The maximum transverse diameter of this form is in a plane slightly caudad of the ovary, and in the press preparation measures 0.93 mm.

Color.—The specimen in glycerine-alcohol was of an olive-green tint.

Form.—The measurements given indicate the elongate form of this species (fig. 19). The body is subcylindrical, the dorso-ventral being shorter than the transverse diameter. Transverse sections in the equatorial zone show an elliptical outline. The cephalic pole is transversely rounded, the caudal bluntly pointed. The worm has its greatest dorso-ventral and transverse diameters in the region slightly caudad of the ovarian zone; from here it tapers gently toward both poles, though more toward the oral. The portion of the worm cephalad of the acetabular zone, as compared with the portion caudad of this zone, is distinctly more flattened.

Surface.—The surface cuticle measures 11 μ in thickness; it is armed with delicate spines that are arranged in transverse and oblique lines. These spines are most numerous in the cephalic region, less so in the middle portion, and seem almost if not quite absent at the caudal extremity caudad of the testicular zone. On the ventral aspect of the ventro-dorsally flattened cephalic pole is the circular oral aperture and about 2 mm. (two-elevenths of the total length) from the cephalic margin the acetabular aperture; the former appears to be slightly the larger in the press preparation. Slightly cephalad of the acetabular aperture and a bit to the left of the median sagittal plane is the genital pore. This is at the vertex of a small but distinctly bulging area. The vertex of the caudal pole presents the excretory pore.

Acetabulum.—The position of its aperture has been indicated. Measured in the press preparation, the acetabulum is 0.165 mm. in transverse and 0.15 mm. in sagittal diameter, or about the same as the oral sucker, except that the oral sucker is longer in the sagittal than in its transverse diameter. As already stated, however, the acetabular aperture appears in the press preparation smaller than the oral and is transversely elliptical in outline.

INTERNAL ANATOMY.

Digestive tract.—As has already been indicated, the oral aperture is ventro-subterminal. It leads into the lumen of an oral sucker. The oral sucker is about as large as the acetabulum. From its base there springs a short prepharynx, which is followed by an ellipsoidal pharynx, measuring in the press preparation about 120 μ in length by

about 90 μ in transverse diameter. The pharynx is succeeded by a short esophagus, the acute angle formed by the forking of the latter being about 120 μ (or the length of the pharynx) from the base of the pharynx. The combined length of the prepharynx, pharynx, and esophagus, in the press preparation, is 0.30 mm., or about twice the longitudinal diameter of the oral sucker. The intestinal ceca are long, straight, unbranched tubes running at 0.12 to 0.15 mm. from and approximately parallel to the lateral margins and terminate about 0.12 mm., that is, about the length of the excretory duct from the caudal margin. They are for the most part flattened in the transverse diameter, though here and there their lumen takes on a more or less circular outline which at some points is considerably dilated. Measured in a transverse section at a point where the lumen is nearly circular and not abnormally dilated the gut is about 65 μ in diameter.

Genital system.— *Male organs:* The testes, two in number, are in the intercecal area near the caudal extremity of the worm. The testicular zones abut or overlap slightly, and combined are equal to about one-eleventh of the body length. The testicular fields substantially coincide. The caudal margin of the caudal testis is separated from the caudal margin of the worm (in the press preparation) by a distance about equal to the longitudinal diameter of this testis. In the press preparation the testes have a somewhat elliptical outline. The cephalically placed testis lies with its long diameter in the transverse diameter of the worm, while the other testis lies so that its long diameter is in the long axis of the worm; the former testis also appears to be a little smaller. In sections the testes appear irregularly globular in form, and their fields slightly overlap the field of the right gut. The point of origin and first portion of the vasa efferentia, of which there are two—right and left—can not be made out in the sections, though it is clear that the right is from the caudal and the left from the cephalic testis. Their subsequent course, however, can be traced. They pass cephalad close to the inner aspect of the corresponding intestine; gradually they tend dorsad and toward the median plane close to the dorso-lateral aspect of each side of the excretory vesicle; finally they cross the dorsal aspect of the excretory canals, just above (cephalad of) the formation of the vesicle to take a position close to the inner aspect of the corresponding excretory canals. They maintain this relation to the end, passing abruptly to the median line, and undoubtedly unite at the base of the cirrus pouch to form the vesicula seminalis interna. The cirrus pouch is a long, relatively thick-walled (7.5 μ) structure, extending in a loose spiral slightly less than two-thirds of the way from the genital pore to the ovary. In the press preparation it measures 0.63 mm. in length, not counting the spiral turns, and 0.18 mm. (0.13 mm. in transverse sections) in greatest transverse diameter. The latter measurement is

near its base, from which region it tapers gently in the direction toward the genital pore, so that at a point just caudad of the acetabulum the transverse diameter is reduced to 0.135 mm. (press preparation) 0.105 mm. (transverse section). Within the pouch is first what is interpreted to be the vesicula interna. This is a compactly coiled, thin-walled duct of considerable relative caliber distended with spermatozoa. It is in intimate contact with the inclosing wall of the pouch. The vesicula is succeeded by a very loose, spirally coiled duct of considerable length and of a greatly reduced diameter and caliber, having a well-developed muscular wall, lined by a well-marked epithelial layer. This portion of the vas deferens is not in direct contact with the cirrus pouch, but is inclosed by a mass of fairly large nucleated cells, which fill the lumen of the corresponding portion of the pouch. This, which perhaps corresponds to the prostatica of other forms, is in its turn abruptly succeeded by and continued as a third or terminal rather long portion, likewise coiled in a rather loose spiral. Its wall is decidedly more muscular and compact than that of the second portion, and in all probability represents the inverted cirrus. This discharges into a sinus or atrium like that of *Fasciola hepatica* in common with the metraterm. Cells like those described as inclosing the prostatica, but more sparse, fill the lumen of the pouch left vacant by the inverted cirrus.

Female organs. The ovary, an irregularly globular body, lies in the intercecal area a little postequatorial. Though approximately in the axial region of the worm, the major portion of its bulk is to the right of the median sagittal plane. It is slightly smaller than the cephalic testis. Immediately caudad of the ovary and in the fork formed by the junction of the excretory canals is the shell gland. It consists of a loose aggregate of cells; this mass is without a definite outline and is rather elongate dorso-ventrally, with a slight obliquity from the median sagittal plane toward the right and ventrad. The oviduct is not clearly traceable. A receptaculum seminis is absent. Laurer's canal is present, but its connection with the oviduct can not be made out in the sections available for study; its pore is in the mid-dorsal line at a point in a plane slightly caudad of that of the caudal margin of the ovary. The uterus emerges from the ventral pole of the shell gland and at once begins to form compact coils. It is distended with eggs, some of which, measured in sections, were 30 μ by about 12 to 13 μ. The course of the uterus can not be traced, but there are indications that make it probable that its coils at first pass caudad, mainly in the right half of the intercecal area, to the plane of the cephalic margin of the cephalic testis; then it turns and winds cephalad to the left of the shell gland and the ovary to the axial region of the preovarian zone, and later ventro-sinistrad of about the caudal half of the cirrus pouch, terminating as a fairly long, spirally

coiled metraterm close to the left aspect of the cirrus pouch. The metraterm discharges into the genital sinus. The form and dimensions of the genital atrium can not be satisfactorily studied in the available sections; there is, however, a suggestive resemblance to the sinus in *Fasciola hepatica;* the atrium communicates with the ventral surface by the genital pore. The uterine coils are distended with eggs, and fill the intercecal area within the indicated longitudinal limits, encroaching more or less on the cecal fields ventrally and to a lesser extent dorsally of the ceca, and at some points caudad of the caudal extremity of the vitellaria extend into the extracecal area. They lie ventrally of the excretory vesicle and between and ventrally of the canals. The vitellaria are in the extracecal areas, extending longitudinally from a little (a distance equal to the length of the caudal testis) caudad of the acetabulum to slightly cephalad of the plane of the cephalic margin of the cephalic testis. The two glands are not of exactly equal length, so that their ends are not in common transverse planes. The right gland does not extend quite as far cephalad as the left. Their continuity appears broken as shown in the press preparation. There are two noticeable breaks in the left and one in the right. The more marked break in the left gland occurs at about the level of the ovary, the less marked a little—about twice the vertical diameter of the ovary above (cephalad of) this. The break in the right gland is at about the same level as the lesser of the two in the left, and is somewhat better defined than the latter. The glands are formed by a moderately close aggregation of fairly numerous globular follicles that envelope the ceca ventrally, externally, and dorsally within the vertical limits already indicated. A transverse duct leaves each gland at a point slightly caudad of the shell gland, and passes toward the latter dorsally of the intestine and the corresponding excretory canal, to unite with its fellow close to the dorso-cephalic aspect of the shell gland. The common duct thus formed penetrates the gland but can not be traced satisfactorily in the available sections.

Excretory system.—The excretory system is enormously developed. Each of the main longitudinal canals begins at point at a little distance from the medio-dorsal aspect of the corresponding intestine in the plane of the cephalic extremity of the shorter (right) vitellogene gland. From the first they are of considerable caliber (60μ), and this gradually increases as the canals pass caudad. They maintain the original relation to the intestines until they terminate by uniting in the formation of an excretory vesicle at a point in a plane slightly caudad of the shell gland. The excretory vesicle is large and extends caudad in the intercecal area dorsally of the uterine coils to the caudal extremity of the worm, where it gives rise to a short relatively stout excretory duct that discharges at the terminal excretory pore.

TELORCHIS (CERCORCHIS) ROBUSTUS new species.

[Fig. 20.]

SPECIFIC DIAGNOSIS.—The characters of the genus. Body, 6 mm. long, 0.945 mm. in maximum width; bluntly pointed cephalic, rounded caudal end. Cuticle 7.5μ thick, with transverse and diagonal rows of spines becoming sparse caudad. Aperture of acetabulum at junction of cephalic with second fifth of body, with genital pore a little cephalad and to left. Acetabulum about 0.202 mm. in diameter. Prepharynx present, esophagus absent. Ceca, simple tubes, terminate posttesticular close to caudal margin.

Male organs: Testicular zone forms about cephalic three-fourths of the caudal sixth of body. Testes caudad one of the other but separated by an interval equal to one-fifth of vertical diameter of caudal testis. Cirrus pouch slightly overlaps the ovarian zone.

Female organs: Ovary at equator smaller than either testis. Shell gland close to sinsistro-caudal aspect of ovary, its zone overlapping that of latter. Uterus with descending limb in left half of post-ovarian and pretesticular portion of intercecal area and ascending limb in the right half of this area. Uterus passes ventrally of ovary, ventrally and to left and then dorsally and to left of cirrus pouch. Uterine coils encroach on cecal fields to some extent. Vitellaria unbroken, extend from a point the length of the vertical diameter postacetabular to a point an equal distance pretesticular.

Excretory system: Large paired longitudinal canals begin at a level of caudal margin of acetabulum, pass caudad near mesial aspect of ceca, converge in post-ovarian zone, unite to form a long median canal or vesicle extending to caudal extremity; vesicle discharges by short, thick duct at caudo-terminal excretory pore.

HABITAT.—Intestine of *Cistudo carolina* (type host), Maryland, U. S. A. (type locality).

TYPE.—U. S. N. M. No. 7079.

SOURCE OF MATERIAL.—The material forming the basis of this description consists of one mounted stained specimen forming part of the Stiles collection and bearing the U. S. N. M. No. 7079. The specimen was collected by Dr. Albert Hassall in 1893 from the intestine of *Cistudo carolina* in Maryland.

EXTERNAL CHARACTERS.

SIZE.—The specimen is 6 mm. long and 0.945 mm. in maximum width.

FORM.—The worm is an elongate body, with a rounded caudal and a bluntly pointed cephalic extremity. It is widest in the zone immediately post-acetabular. From this region the margins converge rather rapidly in the direction of the oral extremity; they converge also in the direction of the caudal pole, but so gradually and so slightly that in the ovarian zone, about at the equator of the animal, the transverse diameter is 0.90 mm. and in the zone of the caudal testis it measures 0.765 mm.

SURFACE.—Measured at the edge of the preparation the cuticle is about 7.5μ in thickness. It is beset with relatively, broad-scale like spines in transverse and diagonal rows. The spines are most abundant in the cephalic region, becoming gradually more sparse toward the caudal pole, while in the immediate vicinity of the excretory pore none can be made out. In the median ventral line at about the junction of the cephalic with the second fifth of the body length is the aperture of the acetabulum; a little cephalad and to the left of the acetabular aperture is the genital pore.

ACETABULUM.—The acetabulum is irregularly circular in ventral view and measures about 0.202 mm. in diameter.

INTERNAL ANATOMY.

DIGESTIVE TRACT.—The oral aperture appears to be ventro-subterminal. A well-developed oral sucker is present and measures about 0.262 mm. in longitudinal and about 0.24 mm. in maximum transverse diameter. It is succeeded by a pharynx. A prepharynx [a] is probably not present, but as in the only preparation available the oral sucker overlies part of the pharynx, the presence of a very short prepharynx can not be excluded. The pharynx appears to be at once succeeded by the intestines without the intermediation of an esophagus. The ceca pass caudad, to terminate in the caudal extremity, close to and on either side of the excretory duct.

GENITAL SYSTEM.—*Male organs:* The testes are in a zone forming about the cephalic three-fourths of the caudal sixth of the body. They are placed one directly caudad of the other in the intercecal area, but separated by an interval equal to about one-fifth the longitudinal diameter of the caudal testis. The cephalic testis is oval in outline, with the major diameter at about a right angle with the long axis of the worm. It measures about 0.48 mm. in transverse and about 0.33 mm. in longitudinal diameter. The caudal testis is more nearly circular in outline, with the cephalic pole, however, slightly flattened. It measures 0.435 mm. in transverse and 0.36 mm. in longitudinal diameter. The vasa efferentia can not be seen. A long cirrus pouch is present. It extends from the ovary cephalad. It is clearly divisible into two portions; the first, forming somewhat less than half the length, tapers from the rounded basal end cephalad and is twisted into a sort of letter S. It has taken the carmine stain deeply and its wall appears to be relatively quite thick. The second much longer portion tapers slightly if at all, appears slightly coiled, and has taken the stain very lightly. It passes dorsally of the acetabulum and ventrally and to the right of the metraterm.

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[a] See note, p. 46.

Female organs: The ovary is in the axial region, midway between the extremities of the worm. It appears oval or nearly elliptical in outline, lies with its major axis transversely, its broader pole to the right, and measures 0.47 mm. in the transverse and 0.225 mm. in the longitudinal diameter. The shell gland is close to the sinistro-caudal aspect of the ovary. The uterus passes caudad from the shell gland, describing transverse loops in about the left half of the intercecal area until it reaches the cephalic testis when it crosses to the right and turns cephalad. The ascending limb likewise forms transverse coils which do not encroach on the area occupied by the descending limb, except for some slight dovetailing of some of the adjacent loops. The coils of both limbs seem to encroach on the corresponding cecal field. When the ascending limb reaches the ovarian zone it passes obliquely cephalo-sinistrad ventrally of the ovary to take up a position to the left and ventrally of the first portion of the cirrus pouch. Here it continues cephalad, its coils becoming less ample, and gradually approaches more nearly the median line but still ventrally and sinistrad of the sac. At a point in a plane slightly caudad of the beginning of the second portion of the cirrus pouch the metraterm begins. It proceeds cephalad, forming some loose coils dorsally and to the left of the second portion of the cirrus pouch. The vitellaria are well developed. They extend in the intercecal areas from a point in a plane about the vertical diameter of the cephalic testis caudad of the caudal margin of the acetabulum to a plane about the same distance cephalad of the cephalic margin of the cephalic testis. They present no break or interval in their continuity, such as described for *T. attenuatus*, though there is a noticeable tendency to a grouping of the follicles (not well shown in fig. 20).

Excretory system.—The excretory pore is caudo-terminal. It leads, by a short thick duct, into a long excretory vesicle that can be traced cephalad, dorsally of the testes and the uterine coils, to the shell gland and ovary. Here it ceases to be distinguishable but, as one can distinguish the large paired longitudinal canals immediately cephalad of the ovary, it is safe to infer that the vesicle receives these in the zone of the ovary and shell gland or just caudad of this zone, in a manner strongly suggestive of that which obtains in *T. attenuatus*. The longitudinal canals extend cephalad, close to the mesial aspect of the intestinal ceca, to about the level of the caudal margin of the acetabulum.

Note.—As this manuscript goes to press I find a bottle (U.S.N.M. No. 5862) containing several specimens of *T. robustus*. The material is hard and cuts unsatisfactorily. Nevertheless some additional points can be made out. A short prepharynx or prepharyngeal pouch is clearly evident. Laurer's canal is present, and its pore opens on the dorsum in about the ovarian zone. A receptaculum

seminis is absent. The longitudinal excretory canals unite in the formation of the vesicle very slightly caudad of the shell gland.

I also find, in Doctor Hassall's collection, three mounted specimens of *Telorchis angustus* from *Chrysemys picta* taken in Maryland. These specimens show, as Stafford (1905) says, that the genital pore is on the dorsal aspect of the left lateral margin; his statement that the cirrus sac and vagina pass dorsally of the left gut I can not confirm. Besides *Telorchis angustus*, Doctor Hassall's collection also contains four mounted specimens resembling *Telorchis linstowi*, from the intestine of *Chelydra serpentina*, in all probability from Maryland. The four specimens vary between 3 and 4 mm. in length and are therefore smaller than the measurements of *T. linstowi*, given by Braun (1901a) or by Stossich (1904). In these specimens the esophagus appears relatively slightly shorter and the ovary relatively slightly larger than in the European specimen pictured by Braun (1901a, fig. 4), but in their form and internal topography they agree with Braun's description and figure.

REFERENCES.

The bibliographic references refer to citations given in Stiles and Hassall's Index. Cat. of Med. and Vet. Zool. (Authors, Bull. 39, U. S. Bureau of Animal Industry; Trematoda, Bull. 37, Hygienic Laboratory U. S. Pub. Health & Mar. Hosp. Serv., Wash., D. C.).

A NEW SPECIES OF ATHESMIA (A. FOXI) FROM A MONKEY.

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(Figs. 21–25.)

The genus *Athesmia* was established by Looss in 1899 for a single species described by Braun early in the same year under the name of *Distomum heterolecithodes*.

Genus ATHESMIA[a] Looss, 1899.

GENERIC DIAGNOSIS.—Body flat, elongate, with attenuate cephalic cone. Cuticle thin, unarmed. Suckers in cephalic fourth of body. Pharynx and esophagus present; ceca simple, slender, long.

Male organs: Testes lobate, intercecal, preequatorial, postacetabular caudad, one of the other with slight obliquity; cirrus pouch with protrusile cirrus, genital pore preacetabular.

Female organs: Ovary lobate, intercecal, submedian, posttesticular; receptaculum seminis present, postovarian; shell gland diffuse, intercecal, submedian, obliquely caudad of ovary; vitellaria, unilateral, single; uterus with descending and ascending adjacent limbs, in transverse coils, intercecal, laterally of ovary and testes dorsally of acetabulum; Laurer's canal present.

Excretory system: Excretory vesicle a sigmioid tube.

HABITAT.—The liver of birds (*Porphyrio porphyrio*, *Gallinula chloropus*) and mammals (*Cebus capucinus*).

TYPE SPECIES.—*Athesmia heterolecithodes* (Braun, 1899) Looss, 1899.

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[a] Looss gives the following diagnosis: "Körper zart, von flacher, verlängerter nach vorn verjüngter, hinten abgerundeter Gestalt. Saugnäpfe einander genähert, Haut glatt. Darm mit kleinem Pharynx, langem Oesophagus und Schenkeln, die etwas vor dem Körperende aufhören. Excretionsblase schlauchförming, S-Förmig gebogen. Keimdrüsen wie bei *Dicrocoelium*, nur ein asymmetrisch gelegener und von der Schalendrüse nach hinten sich erstreckender Dotterstock vorhanden. Schlingen des uterus quer gerichtet, wie bei *Dicrocoelium*, doch laufen die des ab- und aufsteigenden Astes neben einander her und decken sich nur gelegentlich. Eier wie bei *Dicrocoelium*, 0.031 mm. lang, 0.023 mm. breit. In der Leber von Vögeln.

Typus und bislang einzige Art: *Athesmia heterolecithodes* (Braun).

ATHESMIA HETEROLECITHODES (Braun, 1899) Looss, 1899.

1899: *Distomum heterolecithodes* Braun, 1899a, January 16, p. 3.

This species, the type of the genus, was first briefly described by Max Braun (1899a) from the liver of *Porphyrio porphyrio*. This bird was from Africa, Madagascar, and died in the zoological park at Königsberg, Prussia. Later Jacoby (1899a), on the basis of a more abundant material, reported some additional details of its anatomy. In a second paper (1899b) he reported finding it in *Gallinula chloropus* from East Prussia and finally (1899c) pictured and described it quite fully, discussing the variations, amphitypie and relations to other forms. Based on this literature, the following is a diagnosis of this species:

SPECIFIC DIAGNOSIS.—With the characters of the genus. Body 8 to 9 mm. long by 1.5 to 2 mm. broad; width to length 1–5; preacetabular portion conical with rounded cephalic end. Body margins slightly convergent caudad. Surface cuticle 3.64 μ thick. Acetabular aperture midventral, about one-sixth body length from cephalic margin; acetabulum 0.37 mm. in diameter. Oral aperture ventro-subterminal, oral sucker 0.46 mm. in diameter; pharynx 0.096 mm. in diameter; esophagus slender, 0.39 to 0.58 mm. long. Ceca slender, termination postvitellarian, 0.75 mm. from caudal margin. Genital pore immediately postbifurcal.

Male organs: Testes intercecal, within first third of body, approximately of equal size (0.35 to 0.46 mm.) with indented surface. Intertesticular interval about equal to longitudinal diameter of caudal testis. Cirrus sac pear-shaped, preacetabular, 0.29 to 0.38 mm long, contains vesicula and cirrus; cirrus 0.4 mm. long.

Female organs: Ovary posttesticular, on opposite side from vitellarium, zone separate from testicular, major axis transverse, measures 0.36 mm., longitudinal diameter 0.15 to 0.17 mm. Receptaculum seminis postovarian, measures 0.2 mm. by 0.1 mm., zone close to or abuts ovarian. Shell gland diffuse, on same side as vitellarium. Oviduct arises from caudal ovarian margin, shortly thereafter receives duct from receptaculum seminis opposite to which Laurer's canal departs; latter reaches dorsum in ovarian zone. Vitellarium left sided unilateral; shorter cephalic portion intercecal, longer caudal portion extracecal; extends from shell gland into the caudal third of body. Vitello-duct from cephalic end of gland joins ootype. Uterus with transverse coils descends on side of vitellarium, ascending on opposite side; postovarian coils in separate adjacent left and right fields; some coils dovetailing slightly. At cephalic end of vitellarium uterus recrosses to starting side, ascends to left of ovary, then in an S-shaped course between the testes, finally dorsally of acetabulum to genital pore.

Egg: Oval 0.031 to 0.04 mm. by 0.019 to 0.023 mm.

Excretory system: Longitudinal canals begin in esophageal zone, unite in equatorial zone to form sinuous tubular bladder that discharges at caudal pole.

HABITAT.—Liver of birds *Porphyrio porphyrio* (type host), *Gallinula chloropus*.

TYPE.—? Coll. Braun. *Paratype:* U. S. B. A. I. No. 3383.

Up to the present time this has remained the only known species of the genus. In May of this year a capuchin monkey in the Hygienic Laboratory was accidentally killed, and in its liver Doctor Fox found a number of flukes which he very kindly turned over to us. These were all of one kind and very closely resemble *A. heterolecithodes*.

Certain differences, to be discussed later, seem sufficiently distinctive to establish a new species.

ATHESMIA FOXI new species.

[Figs. 21–25.]

SPECIFIC DIAGNOSIS.—The characters of the genus. Body 6.6 to 8 mm. long; maximum width in testicular zone about 0.855 mm. Body with preacetabular relatively attenuate cone-like portion with rounded cephalic margin; caudal portion lancet-shaped with pointed caudal end. Surface unarmed; cuticle 3 to 3.5 μ thick. Acetabulum 0.21 to 0.22 mm. in diameter, one-sixth to one-seventh of body length from cephalic margin, with more or less marked peduncle and very shallow cavity. Genital pore one-third to halfway from acetabulum to cephalic margin. Oral aperture ventro-terminal; oral sucker about 0.262 mm. in longitudinal diameter; pharynx about 0.082 mm. in longitudinal and dorso-ventral diameters; no prepharynx; esophagus about 0.34 mm. long, forks immediately cephalad of genital pore. Ceca terminate in separate planes slightly cephalad of or at about the level of caudal end of vitellarium.

Male organs: Testes in intercecal area in about second fifth of body. Intertesticular interval equal to about one-half or three-fourths the longitudinal diameter of caudal testis. Testes about equal. Elongate pear-shaped cirrus pouch present; incloses a coiled muscular walled vesicula, a prostatica and ductus ejaculatorius; protruded cirrus curved, surface unarmed.

Female organs: Ovary sinistral, about diameter of caudal testes, post-testicular, transversely elongate. Shell gland slightly larger than ovary; as a rule on same side as vitellarium and opposite from ovary. Receptaculum seminis close to caudal aspect of ovary within ovarian field. Oviduct leaves ventro-caudal aspect of ovary, then shortly receives duct from receptaculum, proceeding to shell gland, gives off Laurer's canal just prior to penetrating latter. Laurer's canal reaches dorsum in about zone of shell gland. Vitellarium dextral, may be broken in continuity at its equator with opposing ends overlapping, consists of longitudinal stem with projecting tubuli and alveoli. Gut of same side skirts closely dorso-external aspect of cephalic half of gland, then crosses dorsally to dorsomesial aspect of caudal half. Uterus, emerging from caudal pole of shell gland, passes caudad on same side of body, turns cephalad near caudal margin ascending on other side; adjacent ascending coils dovetail or slightly overlap; ascending limb recrosses obliquely between ovary and shell gland, skirts lateral margin of ovary, fills preovarian zone, then skirts same lateral margin of caudal testis as of ovary, then fills intertesticular zone and as a rule crosses it obliquely to ascend along opposite lateral margin of cephalic testis cephalad of which it approaches median line and ascends dorsally of acetabulum. Metraterm begins slightly preacetabular, ascends dorsally of cirrus sac, discharges immediately cephalad of extruded cirrus.

Egg: Uterine egg 34 by 20 μ.

Excretory system: Longitudinal canals begin in pharyngeal zone, pass caudad close to ventro-mesial aspect of ceca, unite a little caudad of shell gland dorsally of uterine coils, forming a long, sinuous, unpaired canal that discharges by a short duct at caudal pole.

HABITAT.—Liver of *Cebus capucinus* (type host) in Hygienic Laboratory.

TYPE.—U. S. P. H. & M. H. S. No. 10838, mounted and sections.

SOURCE OF MATERIAL.—The material forming the basis of this description was collected by Dr. C. Fox from the liver of a monkey (*Cebus capucinus*) that died by accident in the Hygienic Laboratory.

EXTERNAL CHARACTERS.

SIZE.—Four specimens in alcohol vary from 6.6 to 7.5 mm. in length. A fifth specimen as a toto mount is 8 mm. long and 0.855 mm. in maximum width; that is, the width is to the length about as 1 is to 10.

COLOR.—The fixed worms are of a pale grayish-ivory tint, through which there show in the caudal portion the brownish coils of the uterus, which become darker, almost black, as they pass through the testicular zone. Along one side there shows through also an almost black ragged line; this extends a little more than halfway from the equator to the caudal end of the worm and represents the unilateral vitellarium. The testes and the ovary show as ground-glasslike bodies, as do also the oral and ventral suckers.

FORM.—The worms are elongate, dorso-ventrally flattened bodies, with a relatively attenuate cephalic (preacetabular) portion. They are widest in the testicular zone; from this region in the direction of both poles the width diminishes gradually but progressively. The rate at which the body diminishes in width in the cephalic direction becomes very slight in the acetabular zone, so that the margins of the preacetabular part of the worm, only slightly convergent, gives this portion of the worm a conical appearance in ventral view. The oral end is rounded. The reduction in width of the caudal portion progresses slowly and is very slight until nearly the end is reached, when it becomes marked, so that this end is sharply pointed and gives the caudal part of the worm something of a lancet shape.

SURFACE.—The surface cuticle is unarmed, thin, and measures 3 to 3.5 μ in thickness. Conceiving the cephalic end to be beveled at the expense of the ventral edge, the oral aperture occupies this beveled area and is therefore ventro-terminal. In the median line of the venter at a point about half to two-thirds of the way from the cephalic margin to the acetabulum is the genital pore.

ACETABULUM.—The acetabulum is in the median ventral line at a point about one-sixth to one-seventh the body length from the cephalic margin. It projects more or less prominently on a peduncle of variable length and appears to be with a very shallow or no cavity (fig. 23). In one series of sagittal sections it measures 0.22 mm. in maximum longitudinal diameter; in two series of transverse sections the maximum transverse diameter was the same in each—namely, about 0.21 mm. In structure it is of a decidedly loose-meshed spongy character.

INTERNAL ANATOMY.

DIGESTIVE TRACT.—The oral sucker is well developed and in a series of sagittal sections measures 0.262 mm. in maximum longitudinal diameter. It is succeeded by a pharynx that measures in the same sections 0.082 mm. in longitudinal and dorso-ventral diameters. Both of these structures, though well developed, are, like the acetabulum, composed of delicate, rather loose-meshed fibers (fig. 23). In its turn the pharynx is succeeded by a slender esophagus about 0.34 mm. long, that forks immediately cephalad of the genital pore. The ceca are slender simple tubes that pass caudad near the lateral margins to terminate in slightly different planes slightly cephalad of or at about the level of the caudal end of the vitellarium; in two of three toto mounts the ceca fall a little short of this level (figs. 21, 22); in one, one of the ceca just reaches this plane. In two series of transverse sections, the ceca in one terminate very close to the plane of the caudal end of the vitellarium, in the other they extend about 0.15 mm. caudad of this plane.

GENITAL SYSTEM.—*Male organs:* The testes are in the intercecal area, one caudad of the other with a very slight obliquity. The cephalic testis is about as far postacetabular as the esophageal fork is preacetabular. The intertesticular zone varies in its longitudinal diameter from about one-half to slightly less than the longitudinal diameter of the caudal testis. The testes are about equal in size, irregularly circular to oblong in outline, with marked infoldings of the surface. Measured in two press preparations the cephalic testis in one measured 0.54 mm. in longitudinal by 0.52 mm. in transverse diameter, in the other 0.51 mm. in longitudinal by 0.42 mm. in transverse diameter; the caudal testis in one measured 0.54 mm. in longitudinal by 0.46 mm. in transverse diameter and in the other 0.48 mm. in longitudinal by 0.52 mm. in transverse diameter. The vasa efferentia are not distinguishable in the available sections. A cirrus pouch is present; this extends through the cephalic three-fourths of the zone between the acetabulum and the esophageal fork. The wall of the sac measures about 3.5 μ in thickness. It incloses the vas deferens, the first part of which is coiled, distended with spermatozoa, and its wall but little thinner than that of the sac; this is succeeded by a short segment interpreted as the prostatica, and this, in its turn, by the ductus ejaculatorius, which pierces the cirrus and discharges at its vertex. A well-developed protruded cirrus about 0.135 mm. long and about 0.06 mm. in diameter at its root is present. The cirrus presents a slight curve and a tendency to a spiral twist; its surface is unarmed.

Female organs: The ovary is posttesticular; the interval between the caudal testis and ovary is about equal to the longitudinal diameter

of the caudal testis. It is considerably smaller than either testis, irregular in outline in ventral view, with infoldings of the surface and is transversely elongate. Its position with respect to the axial line is correlated to that of the caudal testis, being nearer to the right or to the left margin of the worm as this testis is nearer to the right or the left margin. In six of the ten specimens the ovary is nearer the left margin as is also the caudal testis. The shell gland, which is a rather loose aggregate of cells, is obliquely caudad of and on the side opposite from the ovary, and as large or slightly larger than the latter. In one specimen it is caudad of the receptaculum seminis, on the same side as the ovary and opposite to that of the vitellarium. The receptaculum seminis, somewhat triangular in outline in ventral view, and considerably smaller than the ovary, is placed close to the caudal aspect of the latter. The oviduct leaves the ventro-caudal aspect of the ovary and passes caudad. After a short progress it gives off a duct of about its own diameter that passes to the receptaculum, after which it proceeds in the direction of the shell gland, the cephalic aspect of which it penetrates. Just before penetrating the shell gland it gives off Laurer's canal; this curves gently dorsad, reaching the dorsum at a point within the zone of the shell gland. As in the case of *Athesmia heterolecithodes*, this worm appears to be with only one vitelline gland. This gland extends along one margin of the worm from the level of the caudal plane of the shell gland for about one-fourth of the worm's length. In five of six specimens the gland was along the right margin and in four of these five specimens on the same side as the shell gland. The gland consists of a longitudinal sinuous stem with projecting tubuli and alveoli of relatively considerable size, somewhat after the fashion of a simple branched tubular or alveolar gland. It presents a rather stiff, rigid appearance. In one of the toto mounts there is a distinct break in the continuity of the gland (fig. 22) at about its equator, the opposing ends overlapping slightly and giving the impression of two glands on one side, one caudad of the other. In the other toto mount (fig. 21) the gland presents a thin (? atrophied) segment in continuity at its equator. A slender duct leaves the gland from near its cephalic end and passes to the shell gland. The relation of the gland to the gut on its own side is of interest. The gut passes caudad close to the outer aspect of the cephalic half of the gland. At the equator of the latter, in the region of the "break" in the specimen referred to, the gut crosses dorsally to the inner aspect of the gland, which relation it maintains to the end. The uterus emerges from the caudal aspect of the shell gland, passes caudad on the same side of the median line as that in which the shell gland lies to within a short distance of the caudal end. Here it turns and ascends on the other side until it reaches the receptaculum, when it passes obliquely between the

receptaculum and ovary on the one hand and the shell gland on the other to the same side in which it started, but cephalad of the shell gland. It now skirts that pole of the ovary directed toward the vitelline gland, then its coils fill that part of the intercecal area between the ovary and caudal testis after which it skirts the same side of the caudal testis as it did of the ovary to reach the intertesticular zone. Having filled this zone, the uterus proceeds cephalad, skirting one or the other lateral aspects of the cephalic testis; in the majority of cases it is the aspect opposite to that of the caudal testis, that is, the uterus in its ascent passes diagonally through the intertesticular zone. Cephalad of the testes it ascends in the median line dorsally of the acetabulum, its coils becoming progressively less ample. At a point slightly preacetabular the metraterm begins and pursues a direct course cephalad, dorsally of and close to the cirrus pouch to discharge cephalically of the protruded cirrus (figs. 24, 25). The uterus in its course forms transverse coils; these fill the post-ovarian part of the intercecal area and the extension of the latter caudad of the cecal zone. The coils of each uterine limb encroach slightly here and there on the area occupied by the other.

Egg: Eggs teased from the terminal portion of the uterus measure 32 μ in length by 20 μ in width.

Excretory system.—Longitudinal excretory canals begin in about the pharyngeal zone, laterally of the pharynx. They pass caudad crossing the intestinal ceca ventrally, a little preacetabular as the latter shift laterad from the esophageal fork. They continue caudad more or less close to the ventro-mesial aspect of the corresponding gut to a point a little caudad of the shell gland when they converge and unite in about the median line dorsally of the uterine coils to form a median unpaired canal that passes caudad in a sinuous course discharging by a short duct at the pointed caudal end.

DIFFERENTIAL CHARACTERS.

From the foregoing description it will be seen that the resemblance of this form to the form described by Braun is quite close. They differ, however, in several respects. *Athesmia foxi* is more slender than *A. heterolecithodes;* the ratio of width to length in the former is about as 1–10, in the latter about as 1–5. The ceca do not extend so far caudad in *A. foxi* as in *A. heterolecithodes;* in the former they do not reach materially, if at all, beyond the vitelline zone; in the latter they extend well beyond. The gut in the new form crosses the vitellogene gland at the equator of the latter whereas in Braun's species the crossing is at a point "not more than one-third of the length of the gland" from its cephalic end. Again *A. foxi* may be

regarded as right handed with respect to the usual position of shell gland and vitellarium whereas *A. heterolecithodes* may properly be considered as left handed in this respect. Finally it may be noted that the type host of *A. foxi* is a South American monkey while that of *A. heterolecithodes* is an African (Madagascar) bird.

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ILLUSTRATIONS.

ABBREVIATIONS.

ac	acetabulum.
c	cirrus.
c. p	cirrus pouch.
d. e	ductus ejaculatorius.
es	esophagus.
ex. c	excretory canal.
ex. p	excretory pore.
ex. v	excretory vesicle.
g. a	genital atrium.
g. pap	genital papilla.
g. p	genital pore.
i	intestine.
L. c	Laurer's canal.
o. s	oral sucker.
ov	ovary.
ov. d	oviduct.
ph	pharynx.
r. s	receptaculum seminis.
s. g	shell gland.
sz	spermatozoa.
t	testis.
ut	uterus.
va	metraterm, vagina.
v. g	vitellogene gland, vitellaria.
v. s	vesicula seminalis.

FIG. 1. *Leuceruthrus micropteri.* Ventral view showing internal topography. After a press preparation. Very slightly diagrammatic. Enlarged. Original.

2. *Leuceruthrus micropteri.* Sagittal section showing oral sucker (*o. s.*) pharynx (*ph.*), constriction marking change from one of the forks of the esophagus (*es.*) to the gut (*i.*); also position and relation of distended genital atrium (*g. a.*) and cirrus pouch (*c. p.*) to the acetabulum (*ac.*). Enlarged. Original.

3. *Leuceruthrus micropteri.* Sagittal section showing terminal copulatory apparatus. Enlarged. Original.

4. *Leuceruthrus micropteri.* Profile projection of caudal portion. Slightly diagrammatic. Enlarged. Original.

5. *Leuceruthrus micropteri.* Sagittal section to show dilated segment (pseudo-receptaculum seminis) of Laurer's canal (*L. c.*) immediately after it takes its departure from the oviduct (*ov. d.*) as the latter is about to plunge into the shell gland (*s. g.*). Enlarged. Original.

FIG. 6. *Leuceruthrus micropteri.* Egg. Enlarged. Original.

7. *Azygia lucii.* Ventral view showing internal topography. Enlarged. (After Looss, 1894, fig. 1.)
8. *Azygia acuminata.* Ventral view showing internal topography. Somewhat diagrammatic. Enlarged. Original.
9. *Azygia acuminata.* Sagittal section showing character of ventral wall of oral sucker (*o. s.*), the pharynx (*ph.*), the sphincter-like constriction marking the change from one of the forks of the esophagus (*es.*) to the gut (*i.*). Enlarged. Original.
10. *Azygia acuminata.* Sagittal section to show terminal copulatory apparatus. Enlarged. Original.
11. *Azygia bulbosa.* Ventral view showing internal topography. From a press preparation. Slightly diagrammatic. Enlarged. Original.
12. *Azygia bulbosa.* Sagittal section showing character of ventral wall of oral sucker (*o. s.*), the pharynx (*ph.*), the sphincter-like constriction marking the change from the right fork of the esophagus (*es.*) to the gut (*i.*). Enlarged. Original.
13. *Azygia bulbosa.* Sagittal section through genital papilla showing its form and position. (See also fig. 12.) Enlarged. Original.
14. *Azygia bulbosa.* Sagittal section through excretory bladder showing its relation to venter and dorsum and point of entrance of median excretory canal (*ex. c.*). Enlarged. Original.
15. *Azygia loossii.* Ventral view showing internal topography. From a press preparation. Enlarged. Original.
16. *Azygia loossii.* Sagittal section showing character of ventral wall of oral sucker (*o. s.*), position of the brain (*e. g.*) with relation to the pharynx (*ph.*s) the constriction marking the change from the left ramus of the esophagu, (*es.*) to the gut (*i.*), the form of the genital atrium and the genital papilla projecting into it (*g. pap.*). Enlarged. Original.
17. *Hassallius hassalli.* Ventral view showing internal topography. Projection from transverse sections; slightly diagrammatic. Enlarged. Original.
18. *Hassallius hassalli.* Sagittal section. Slightly diagrammatic. Enlarged. Original.
19. *Telorchis attenuatus.* Ventral view showing internal topography. Slightly diagrammatic. Enlarged. Original.
20. *Telorchis robustus.* Ventral view showing internal topography. From a press preparation. Enlarged. Original.
21. *Athesmia foxi.* Ventral view showing internal topography. In this specimen the acetabulum is turned on its side and the cephalic testis shows a rupture at its acetabular aspect with extravasation, as it were, of spermatozoa into the parenchyma shown by a horn-like projection cephalo-sinistrad. From a press preparation. Enlarged. Original.
22. *Athesmia foxi.* Ventral view showing internal topography. This specimen appears damaged at the cephalic end and does not show the oral sucker nor pharynx, although in the figure the position of these is indicated. From a press preparation. Enlarged. Original.
23. *Athesmia foxi.* Sagittal section showing character of oral sucker (*o. s.*), pharynx (*ph.*) and acetabulum (*ac.*). Enlarged. Original.
24. *Athesmia foxi.* Sagittal section showing relation of vigina (*va.*) to protruded cirrus (*c.*). Enlarged. Original.
25. *Athesmia foxi.* Transverse section slightly below level of opening of vagina to show relation of this to protruded cirrus. Enlarged. Original.

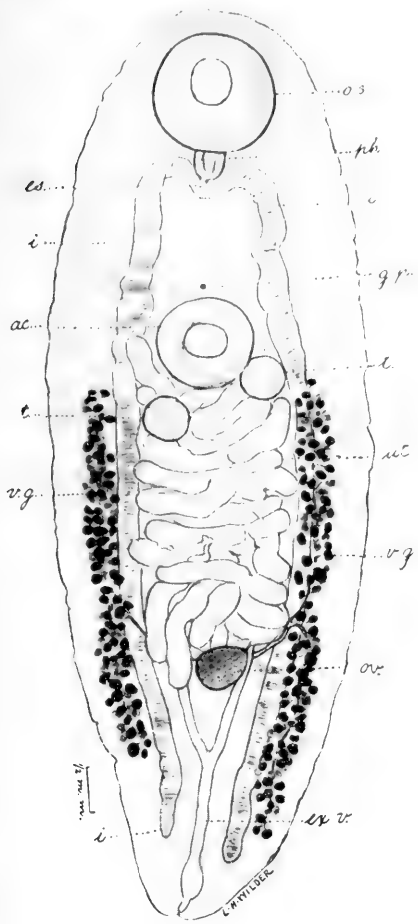


FIG. 1.

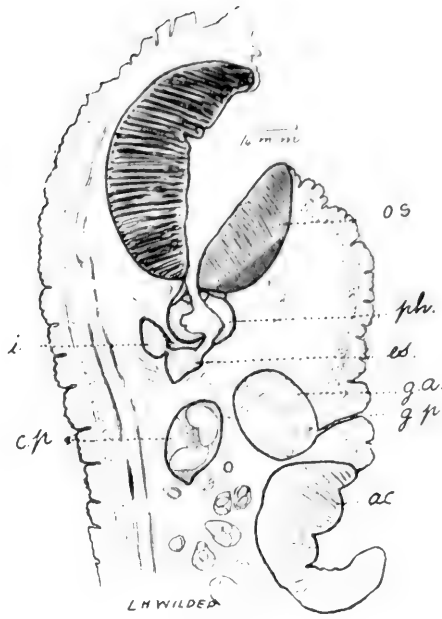


FIG. 2.

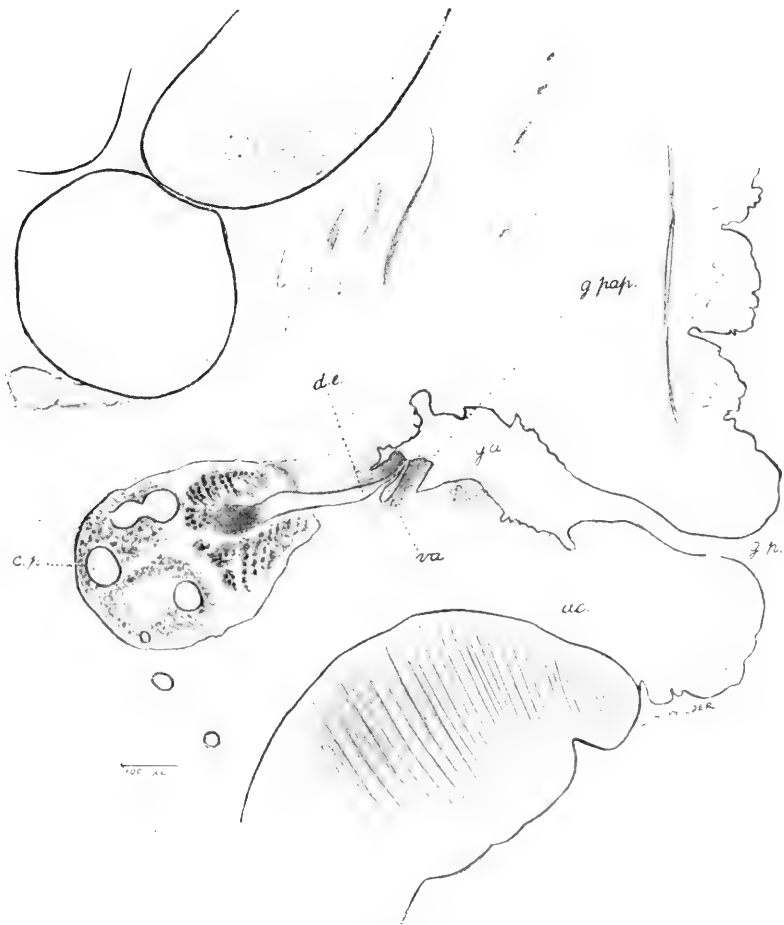


FIG. 3.

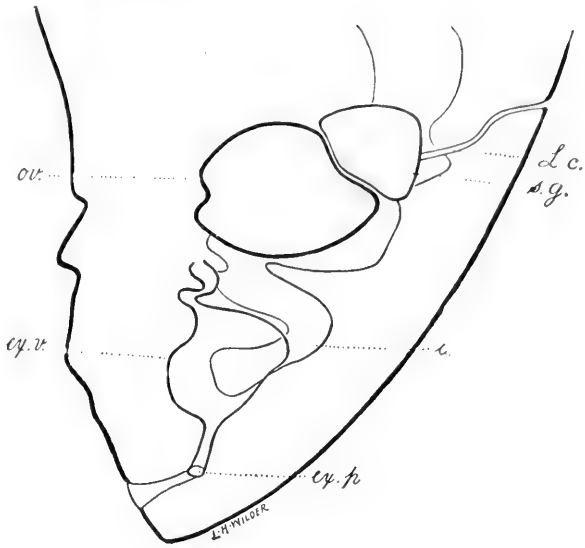
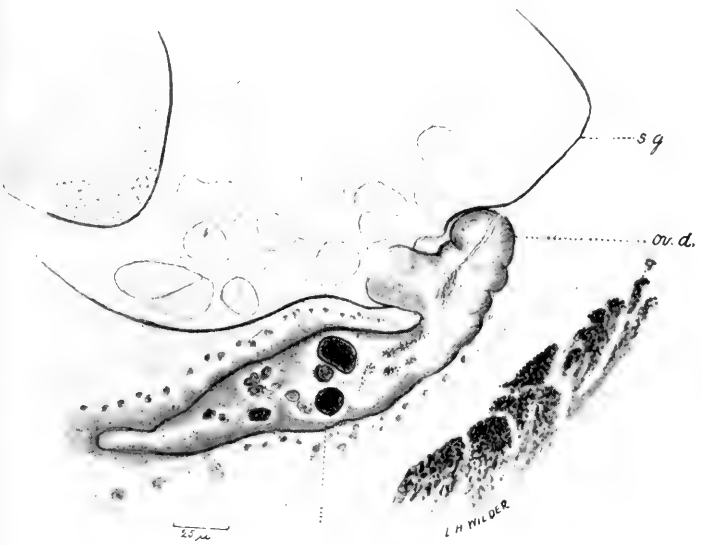


FIG. 4.



L.c.

FIG. 5.

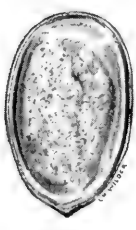


FIG. 6.

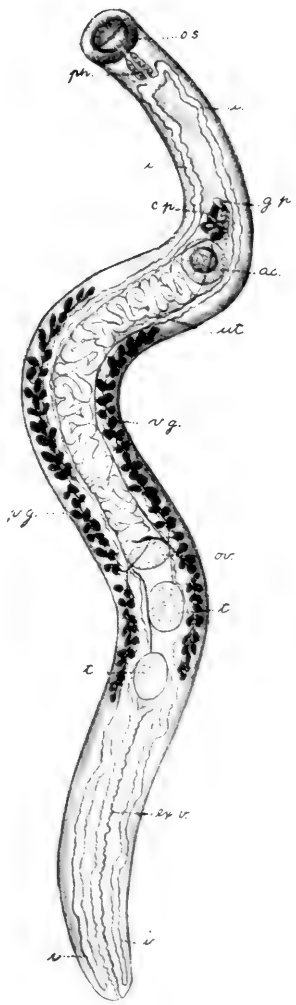


FIG. 7.

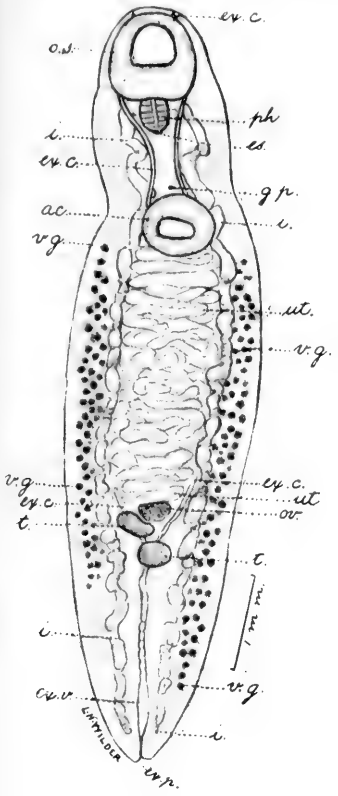


FIG. 8.

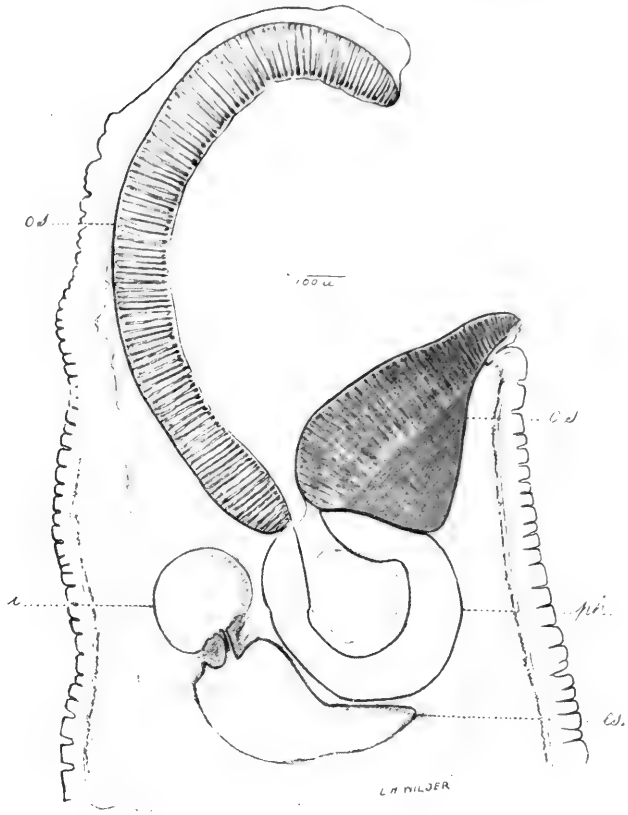


FIG. 9.

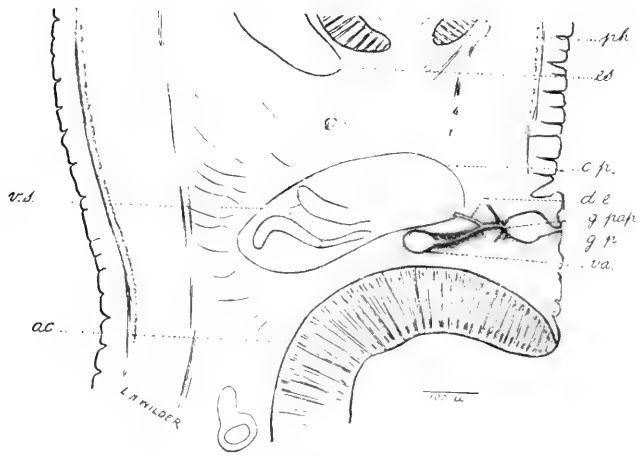


FIG. 10.

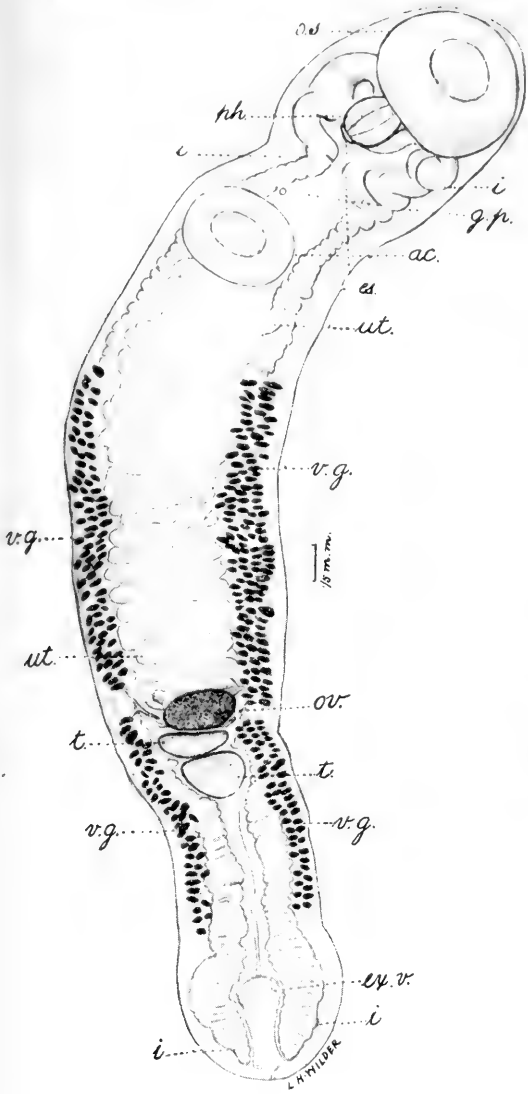


FIG. 11.

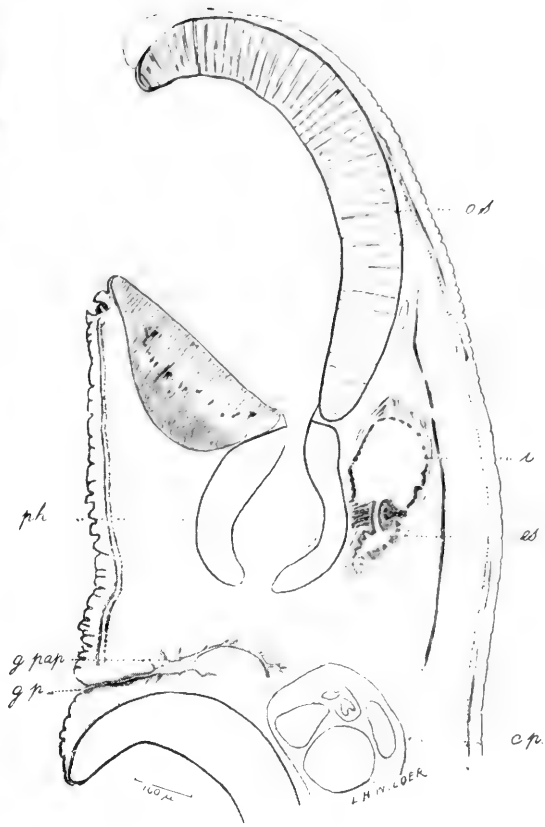


FIG. 12.

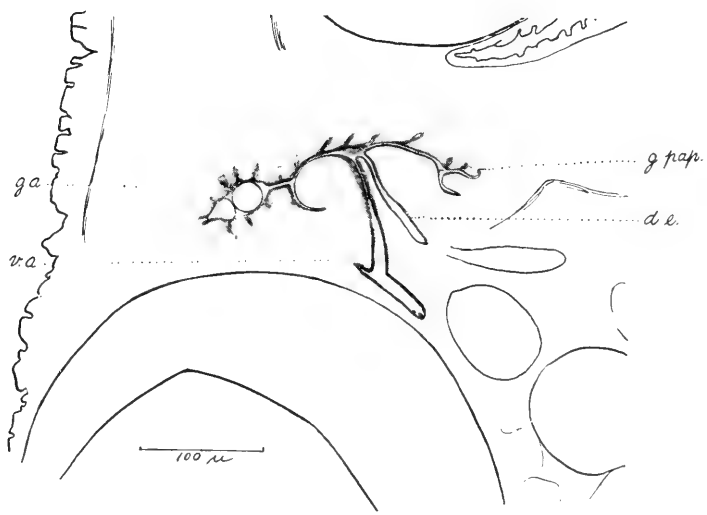


FIG. 13.

PLATE V.

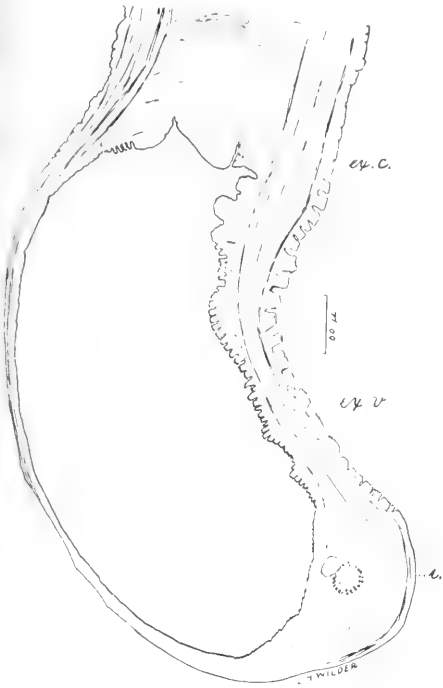


FIG. 14.

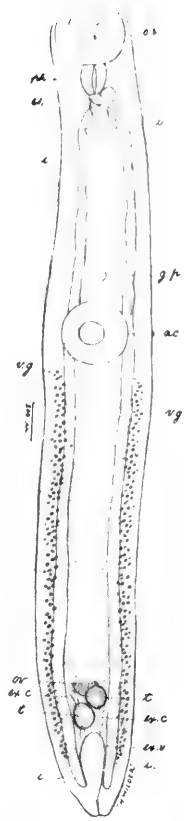


FIG. 15.

FIG. 16.

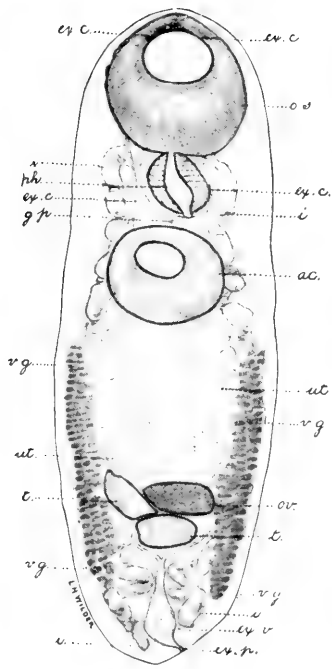


FIG. 17.

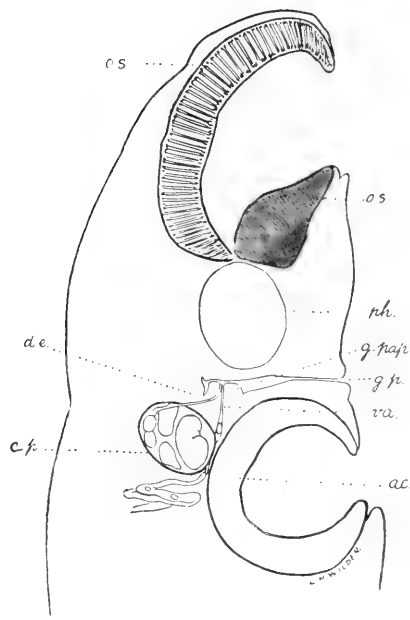


FIG. 18.

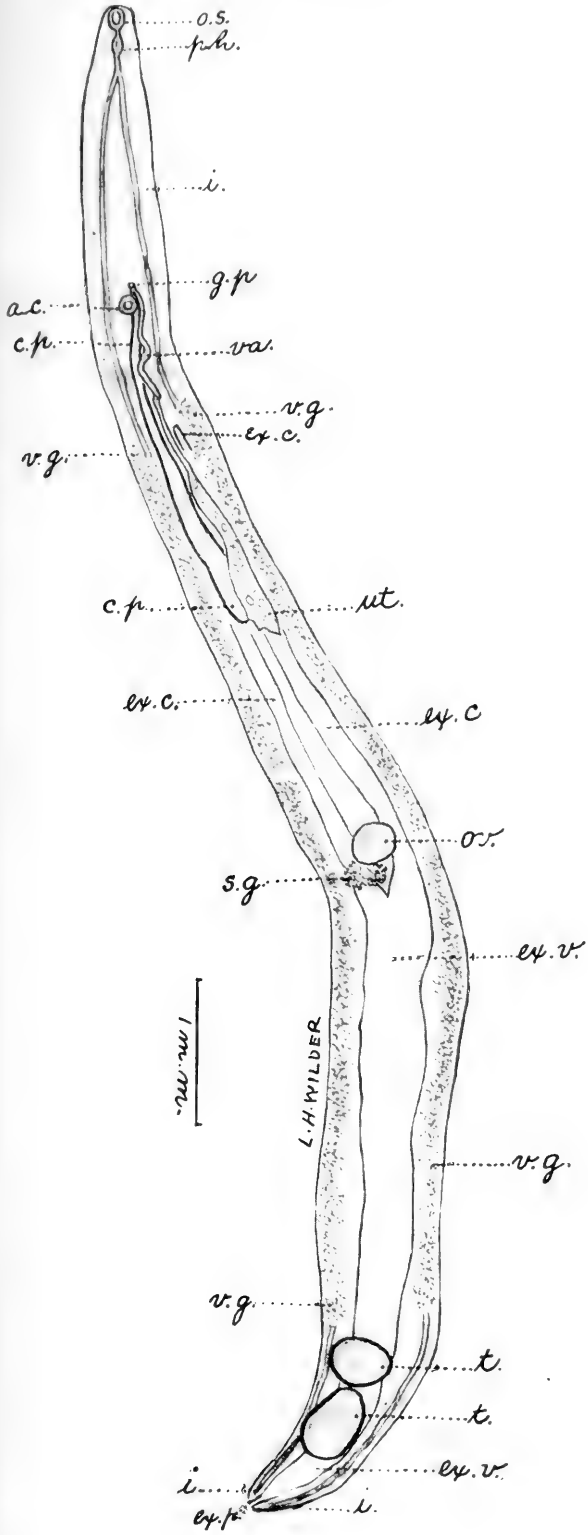

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FIG. 19.

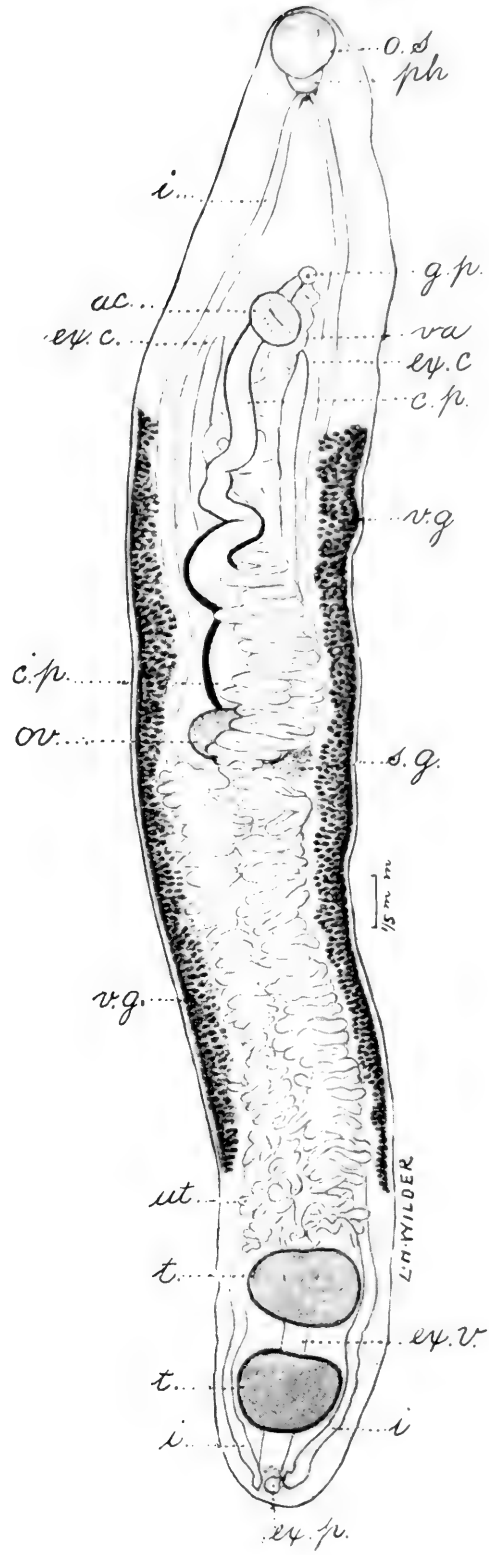
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FIG. 20.

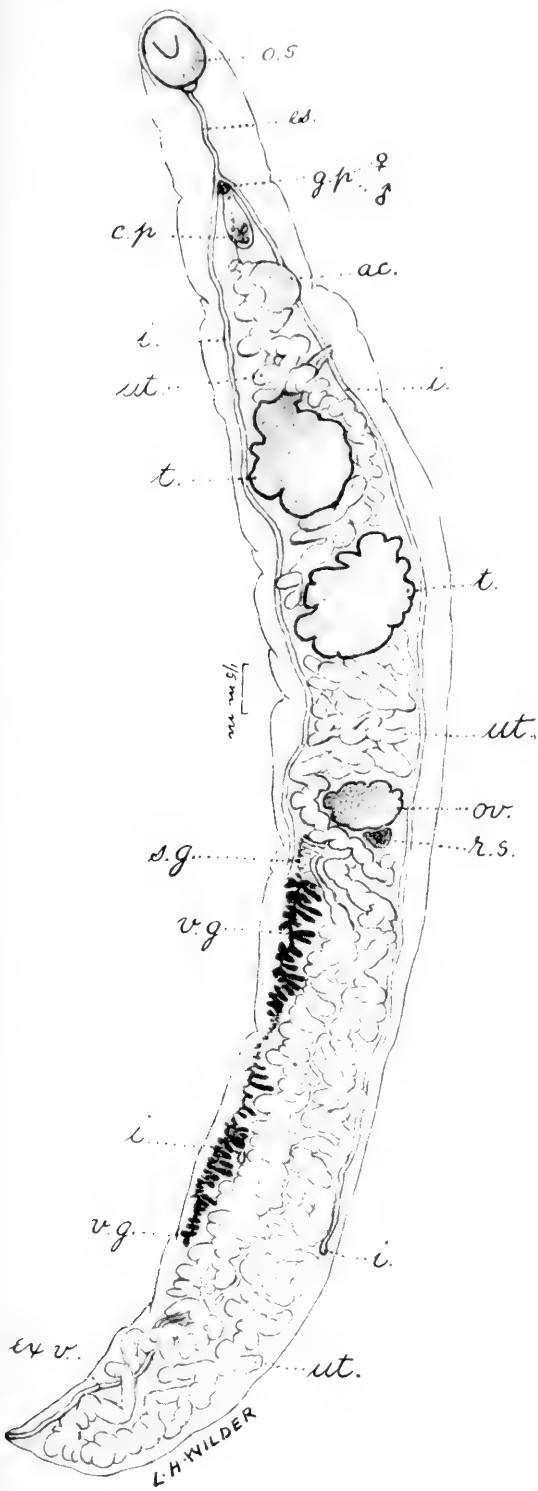
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FIG. 21.

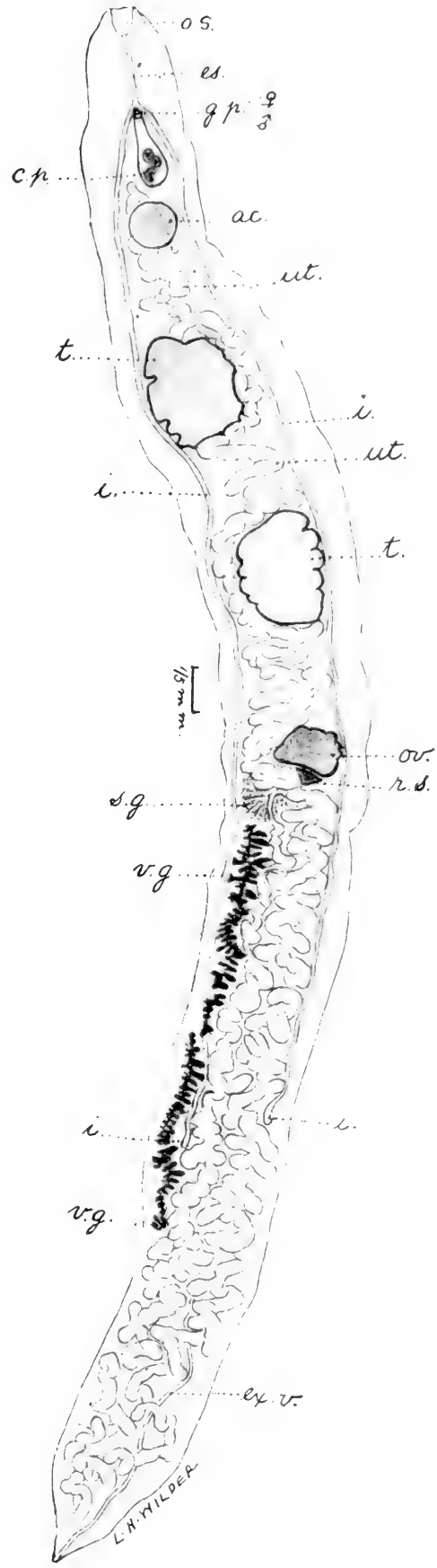
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FIG. 22.

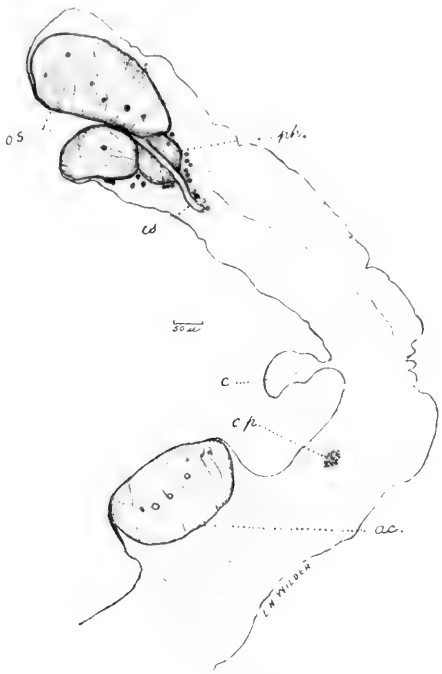

FIG. 23.

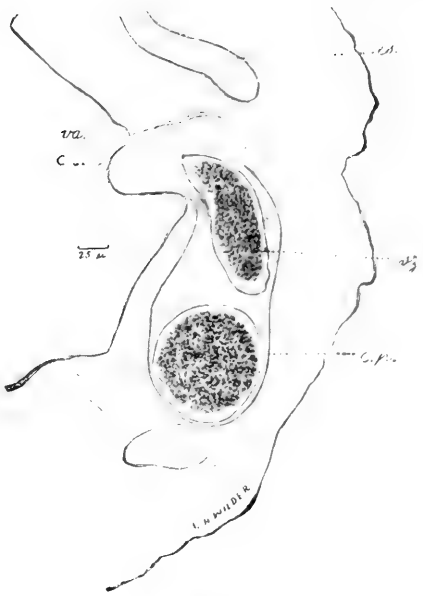

FIG. 24.

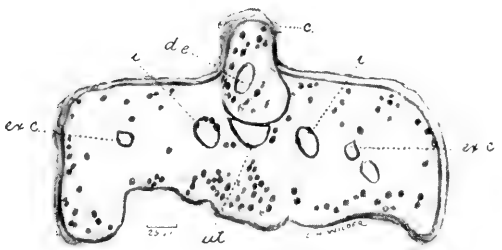

FIG. 25.

INDEX TO ZOOLOGICAL NAMES.

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 ethereal salts. By J. H. Kastle.

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

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

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

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

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

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

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

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

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

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

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

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

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

No. 40.—1. The occurrence of a proliferating cestode larva (*Sparganum proliferum*) in man in Florida, by Ch. Wardell Stiles. 2. A reexamination of the type specimen

of *Filaria restiformis* Leidy, 1880=*Agamomermis restiformis*, by Ch. Wardell Stiles. 3. Observations 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.

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

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

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

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

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

No. 52.—Report No. 3 on the origin and prevalence of typhoid fever in the District of Columbia. 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.

No. 54.—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.

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

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

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

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

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

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

No. 61.—Quantitative pharmacological studies: Relative physiological activity of some commercial solutions of epinephrin. By W. H. Schultz.

No. 62.—The taxonomic value of the microscopic structure of the stigmal plates in the tick genus *Dermacentor*. By Ch. Wardell Stiles.

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

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

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

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

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

HYGIENIC LABORATORY.—BULLETIN No. 72
NOVEMBER, 1910

I. REPORT ON AN OUTBREAK OF TYPHOID FEVER AT OMAHA, NEBR.—(1909-1910)

BY

L. L. LUMSDEN

II. THE WATER SUPPLY OF WILLIAMSON, W. VA., AND ITS RELATION TO AN EPIDEMIC OF TYPHOID FEVER

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United States Public Health and Marine-Hospital Service.

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CHARTS AND MAPS.

I. REPORT ON AN OUTBREAK OF TYPHOID FEVER AT OMAHA, NEBR. (1909–1910).[a]

By L. L. Lumsden,

Passed Assistant Surgeon, United States Public Health and Marine-Hospital Service, Hygienic Laboratory, Washington, D. C.

INTRODUCTION.

The city of Omaha, Nebr., is situated on a series of hills rising gradually from the west bank of the Missouri River. For the greater part of the city the general slope of the land is toward the river, and thus excellent natural drainage is afforded.

The city's present population is estimated at about 138,000, and for the last ten years has varied probably between that number and 100,000. The water supply has been obtained, for the most part, for certainly the last twenty years, from the Missouri River. This river, at numerous points north of Omaha, receives the sewage from a large number of persons, and therefore can be regarded as a somewhat dangerously polluted stream. The treatment of the water before being distributed to the city has not been such as to render it reasonably free from dangerous pollution. Notwithstanding the character of the water supply, Omaha did not have during the twenty years prior to 1909 a typhoid fever rate which could be considered much, if any, above the average for other American cities having comparable climatic and sanitary conditions.

In the latter part of November, 1909, the rate of prevalence of typhoid fever in Omaha became unusually high, and continued so until the latter part of March, 1910. From December 1, 1909, to April 1, 1910, there were reported 582 cases with 59 deaths. The death rate from typhoid during this period was over six times as high as the average rate for the corresponding periods of the five years previous, and, as far as the records show, much higher than it ever had been in this period of any previous years.

Gradually the people awakened to the fact that their city was being visited by an outbreak, or epidemic, of typhoid fever. This

[a] Manuscript submitted for publication June 21, 1910.

awakening was brought about largely by discussions of the subject in the local medical society and by publications in the local newspapers.

Suspicion fell upon the water supply as the source of the infection, and the city commissioner of health advised the people to boil the water before using it for drinking or culinary purposes. There were among the local physicians and the people generally, however, at the time this investigation was begun, many conflicting views expressed as to the cause of the outbreak, and even among those who stated as their belief that the water supply was at fault there were marked differences of opinion as to the source of the infection in the water.

Finally, the Omaha-Douglas County Medical Society sent a petition to the governor of the State requesting that he use his influence to secure a thorough investigation of the situation by an officer of the United States Public Health and Marine-Hospital Service. This petition was signed by the mayor and the commissioner of health of Omaha. Upon the receipt of this petition the governor sent the following letter to the Secretary of the Treasury:

STATE OF NEBRASKA, EXECUTIVE OFFICE,
March 4, 1910.

DEAR MR. SECRETARY: The water supply of the city of Omaha, in the opinion of the health department of that city, has become more or less contaminated with typhoid-fever germs and considerable sickness has resulted therefrom, and at the direction of the mayor of the city and the city physician and other members of the board of health, I am requested to ask you, if possible, to designate a competent officer from the Marine Hospital Corps to go to Omaha and report to Dr. Ralph W. Connell, the city physician, with instructions to make investigations as to the contamination of the water supply and to advise proper means for remedy of existing conditions and prevention of further spread of the disease. What is wanted is an officer who is an expert upon the subject of water supply and purification and a bacteriologist as well.

Trusting that it may be found compatible with the good of the public service to help the city officials of Omaha in this way, I subscribe myself with profound respect,

Yours sincerely,

ASHTON C. SHALLENBERGER.

Hon. FRANKLIN MACVEAGH,
Secretary of the Treasury, Washington, D. C.

The Secretary referred the matter to the Surgeon-General of the United States Public Health and Marine-Hospital Service, and with the approval of the Secretary of the Treasury, the Surgeon-General detailed the undersigned to proceed to Omaha "for a conference with the commissioner of health of that city and investigations of the origin and prevalence of typhoid fever, especially in relation to water supplies."

The investigation of the Omaha situation was begun on March 28 and completed on April 23. The investigation included a sanitary survey of the Missouri River watershed from Omaha to points about 10 miles north of the first intake for the city's water supply; bacteriologic examinations of the water supply; an epidemiologic study

of 105 individual cases of typhoid fever reported between March 1 and April 15 and taken to be fairly representative of all the cases occurring in the outbreak; a clinical examination of about 50 cases reported as typhoid; the making of blood cultures and Widal tests in a number of cases to aid in the determination of the clinical diagnoses; an inspection of the principal dairies; a study of the health-office records; an inquiry into the prevalence of typhoid in neighboring towns and cities; and a careful consideration of the city's sewerage system, and of the supplies of foods and beverages generally.

Upon the completion of the investigation, on April 23, a preliminary report, with conclusions and recommendations which appeared most pertinent to the immediate situation, was submitted to the commissioner of health of Omaha.

In the following report the data collected during the course of the investigation, together with conclusions and recommendations based thereon, are given in detail.

In compliance with instructions received from the Surgeon-General this investigation of typhoid fever in Omaha was made in cooperation with the commissioner of health of that city. Appreciation is hereby expressed of the hearty cooperation of Dr. Ralph W. Connell, commissioner of health of Omaha, and his assistants. From the records of the health office, Doctor Connell furnished numerous transcripts and special compilations. Mr. Claude F. Bossie, one of the sanitary inspectors connected with the health office, was detailed to assist in the investigation, and in every way rendered excellent service.

The bacteriological work was done in the laboratory of the Creighton Medical College, from the authorities of which institution many courtesies were received. In the making of Widal tests, in the preparation of media, etc., at the laboratory, material assistance was received from the city bacteriologist, Dr. Millard Langfeld.

Mr. George W. Craig, the city engineer, and his assistants, rendered valuable assistance in the work. Mr. Craig furnished from his office much important detailed information, including maps and charts, in regard to the city's water supply and sewerage system, and, besides, either went in person or sent an assistant on the surveys of the Missouri River watershed and of the city's water and sewerage systems and in the sanitary inspections of a number of neighboring towns and villages.

Valuable service was rendered by the local newspapers in keeping the public informed as to the nature and results of the investigation.

From the members of the medical profession of Omaha many courtesies and much valuable assistance were received. It was at the instigation of the Omaha-Douglas County Medical Society that this investigation was brought about.

NATURE AND SCOPE OF THE INVESTIGATION.

The immediate purpose of the investigation was to determine the cause of the unusually high rate of prevalence of typhoid, which had begun in the latter part of November, 1909, so that preventive measures might be adopted. The time of occurrence and the character of the outbreak pointed to the water supply, the milk supply, or some other general food supply as the chief source of the infection, and therefore especial attention was given at the beginning of the investigation to these supplies.

A rapid and somewhat superficial survey of the river near by the two points from which the city's water supply was obtained showed that there was dangerous local pollution of the river with sewage. This observation was made within the first two or three days of the investigation, and a statement was then given to the local newspapers and made before the local medical society that, in view of the conditions already discovered and pending the results of a complete investigation of the situation, it was highly advisable for the people to boil the river water before using it for drinking or culinary purposes. A detailed survey of the watershed of the river for some miles above Omaha and a bacteriologic study of the water supply were made as expeditiously as possible, and the results were all confirmatory of the first impression that the water as supplied to the city was dangerously polluted with sewage. Thus the water supply was involved as a possible chief factor in the production of the outbreak. The investigation was made to include a study of all other possible factors which could reasonably be considered to have been operative, in order to determine which of these factors could be involved and which eliminated. To ascertain the conditions liable to be concerned in the production of typhoid fever, to which the persons affected had been exposed prior to illness, an epidemiologic study was made of 105 individual cases. This study comprised a visit to the home of the patient, a sanitary inspection of the premises, and a careful inquiry to collect from the patient or from other members of the household all data called for by the following blank form:

TYPHOID FEVER CASE CARD.

Case No.....
Date reported.....
Date of investigation.....
Name.....Resident in Omaha: years.....; months.....
Age.....Color.....Sex.....Nationality.....
Probable date of onset.....Date of definite symptoms.....
Name and address of physician.....

Residence:

Character of.....
Residence when taken sick.....from.....to.....
Previous residences.....from.....to.....
Subsequent residences.....from.....to.....
Temporary absences from Omaha within thirty days prior.....
Number of occupants.....Ages.....
Number of occupants who have had typhoid fever.....when.....
Newcomers in house within three months prior.....
Newcomers had typhoid?.....
Servants:
White: Resident.....
Name.....
Address.....
Typhoid.....
Nonresident.....
Name.....
Address.....
Typhoid.....
Colored: Resident.....
Name.....
Address.....
Typhoid.....
Nonresident.....
Name.....
Address.....
Typhoid.....
Typhoid at home of servants.....when.....
W. C. in house.....W. C. in yard.....
Privy.....location.....
General sanitary condition of residence.....
House screened?.....Flies?.....Other insects?.....

Occupation:

Place.....from.....to.....
Drinking water.....
Other cases.....

Water within thirty days prior:

Solely.....Principally.....Occasionally.....
Source of ice used in or for drinking water.....

Food within thirty days prior:

Where taken.....
Milk (how used).....From.....
Boiled?.....Pasteurized?.....
Ice cream.....Where?.....
Uncooked fruits and vegetables.....Lettuce.....Onions.....
Celery.....Radishes.....Strawberries.....
Raw 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 patient.....
Other precautions.....
Remarks .....
Summary.....

Signature .....

The 105 cases so canvassed were reported from March 15 to April 15, and had definite onsets of illness between January 14 and April 9. They were taken serially in the order in which they were reported to the health office and are believed to be fairly representative of all the cases which occurred in the outbreak.

OCCURRENCE AND EXTENT OF THE OUTBREAK.

Judging by the rate of report of cases to the health office, the outbreak of typhoid fever at Omaha began about November 25, 1909, and continued until about March 25, 1910. From December 1, 1909, to April 1, 1910, 582 cases were reported. The following table shows the number of cases and deaths reported, by days, from December 1, 1909, to June 1, 1910:

TABLE No. 1.—*Giving typhoid fever cases and deaths reported, by days, in Omaha.*

Day of month.	1909.		1910.									
	December.		January.		February.		March.		April.		May.	
	Cases.	Deaths.	Cases.	Deaths.	Cases.	Deaths.	Cases.	Deaths.	Cases.	Deaths.	Cases.	Deaths.
1					1		8	2	14			
2		2	4		7		8	1		1		
3	5		2		5		3	1			3	
4	2	1			7		9		4	2	2	
5			2	1	29		2	2	1	1		
6	2		2	1		1		2	1	1		
7	2				7	1	9	2	3			
8	3			1	8	1	8	2	4			
9	10				10		9	2			1	
10	20		1		21		10			1		1
11			1		17		4		2			
12					11	2	2	1	2		1	
13	5	1				1			1		1	
14	8	1	2		20		9		1			
15	3		1		10		3			1		
16	6	1			12	1	6			2		
17	1		4		4	2	4	3				
18	5		2		8	1	4	1			1	
19		1	1		11		1				1	
20	4		2	1				1	1			
21	10	1	5		4	1	6	1			1	
22	1		2		6		6					
23					13	1	4			1	1	
24	8	1	6		11	1	1				2	
25			3		11	1	7	1	3			
26			4		7	2	5				1	
27	2		8					2			1	
28	4		9		6		7	1	2			
29			2				10	1	1			
30	3						3	1	5			
31	10	2	5				6	2			2	
	114	11	68	4	246	16	154	28	45	10	18	1

The records show that the report of cases previous to the latter half of 1909 was very incomplete. Therefore it is impossible to use the report of cases as a basis for a comparison of the rate of prevalence during the period of the outbreak with the rates for corresponding periods of previous years. The report of deaths, however, appears to have been quite complete for some years past, and may be used as a fair basis for comparison.

From December 1, 1909, to April 1, 1910, the number of deaths from typhoid fever reported was 59. For the four corresponding periods of the years immediately preceding those in which the outbreak occurred the numbers were as follows:

Number of deaths from typhoid fever reported:

December 1, 1905, to April 1, 1906	8
December 1, 1906, to April 1, 1907	8
December 1, 1907, to April 1, 1908	9
December 1, 1908, to April 1, 1909	12

The average for these four periods is nine, which is less than one-sixth the number reported in the period extending from December 1, 1909, to April 1, 1910.

Table No. 2 and Chart No. 1 show the number of deaths from typhoid fever reported, by months, during the calendar years from 1905 to 1910.

TABLE NO. 2.—*Showing number of deaths from typhoid fever reported, by months, in Omaha.*

Month.	1905.	1906.	1907.	1908.	1909.	1910.
January	1	2	3	1	1	4
February	2	3	.....	2	1	16
March	4	2	1	2	1	28
April	1	.....	4	.....	4	10
May	.....	2	1	.....	.....	1
June	3	3	1	1	3	.....
July	1	1	1	1	2	.....
August	1	.....	1	2	3	.....
September	3	2	2	5	6	.....
October	2	5	4	1	3	.....
November	2	4	3	2	2	.....
December	1	4	4	9	11	.....
Total	21	28	25	26	37	.....

Chart No. 2 shows the aggregate number of deaths from typhoid fever reported, by months, during the four years 1905 to 1908, inclusive. Judging by these charts (Nos. 1 and 2) it appears that the

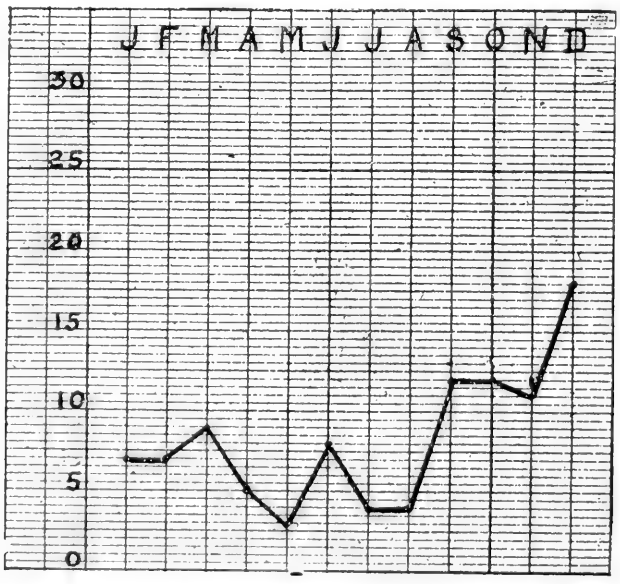


CHART NO. 2.—Showing the number of deaths from typhoid fever reported in Omaha during the four years 1905 to 1908, inclusive, in monthly aggregates.

prevalence of typhoid fever (by occurrence of cases) in Omaha usually is greatest during the summer and fall, and, therefore, that the factors in the production of typhoid fever there usually are operative

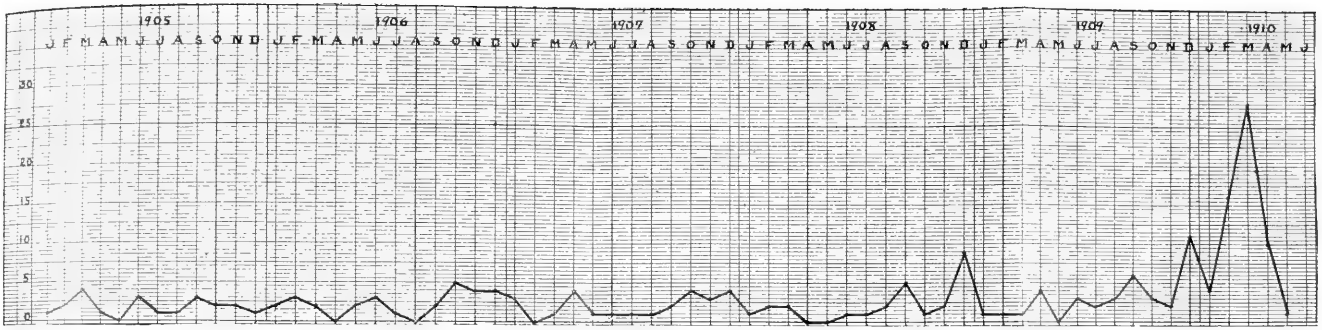


CHART No. 1.—Showing number of deaths reported from typhoid fever in Omaha, from January 1, 1905, to June 1, 1910.

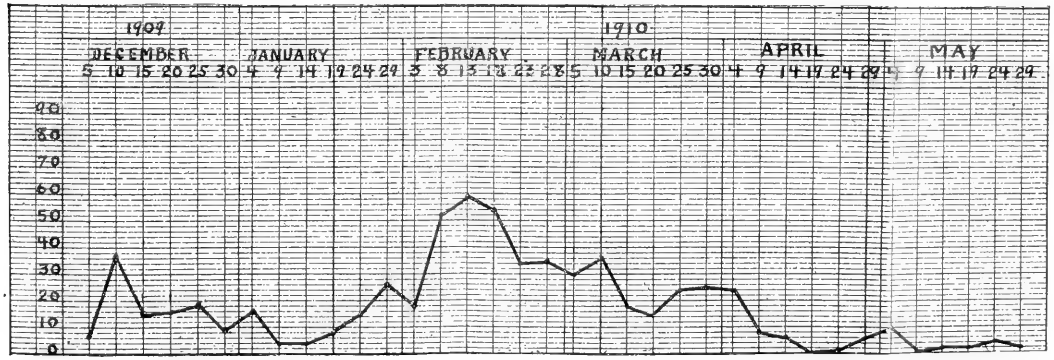
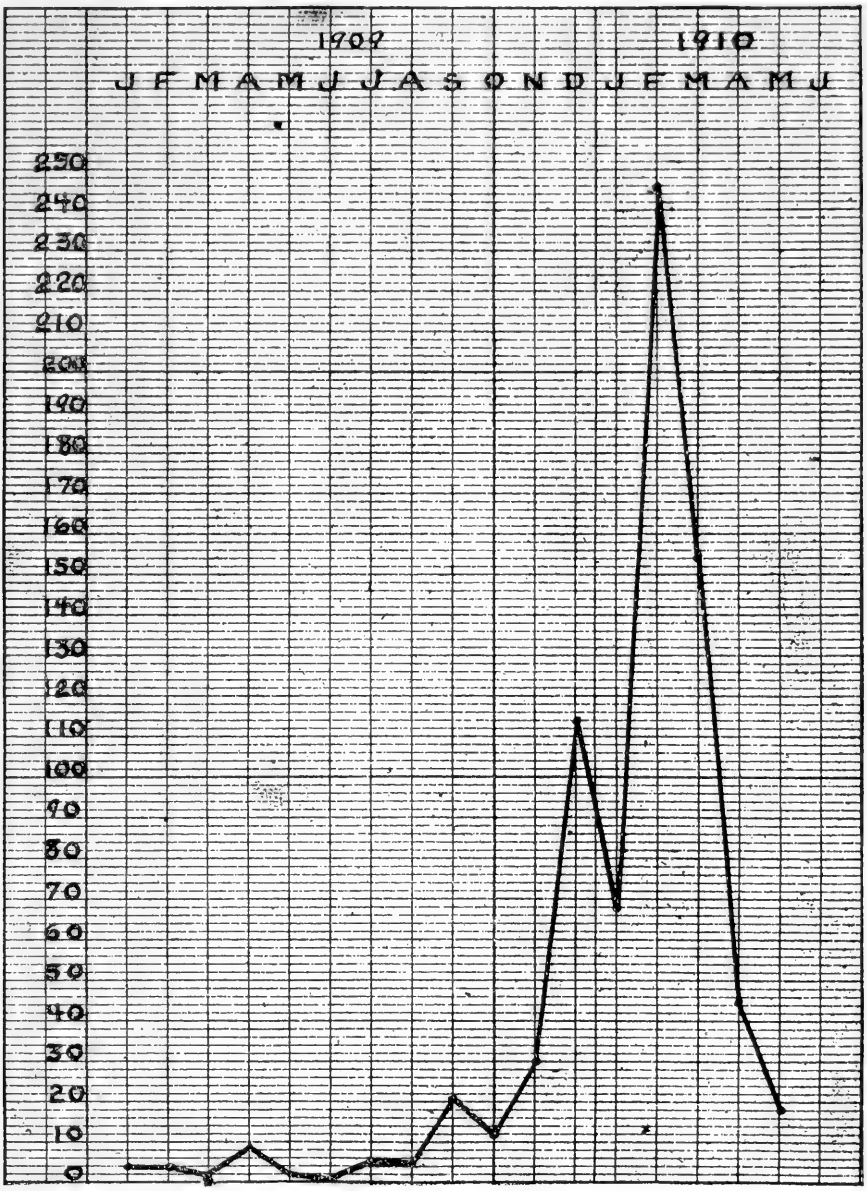


CHART No. 4.—Showing number of cases of typhoid fever reported in Omaha from December 1, 1909, to June 1, 1910, in five-day periods.

) the greatest extent during the months of July, August, September, nd October.

The time of occurrence of the outbreak of 1909–10, as is shown charts Nos. 1, 3, and 4, therefore, was not the seasonal period in hich typhoid in Omaha usually is most prevalent. Judging by the te of report of cases the outbreak had its causation in conditions



ART No. 3.—Showing typhoid cases, as reported by months, in Omaha before and during the period of the outbreak.

hich became operative about November 15, 1909, diminished somehat in December and the early part of January, then markedly creased to reach the maximum about January 25, from which time ey continued operative at a diminishing but still high rate until out March 15, 1910, when they rapidly declined. (See Chart o. 4.)

Some idea of the extent of the outbreak may be obtained by a nsideration of the following table, showing the number of deaths

from typhoid fever in Omaha for the fifteen calendar years preceding those of the outbreak:

TABLE No. 3.—*Giving the number of deaths from typhoid fever in Omaha for the calendar years 1895 to 1909 and for the first three months of 1910.*

Year.	Number of deaths reported from typhoid fever, according to records of health office.	Population of Omaha according to United States census reports or estimates.	Year.	Number of deaths reported from typhoid fever, according to records of health office.	Population of Omaha according to United States census reports or estimates.
1895	29	(a)	1904	19	.....
1896	19	.....	1905	21	120,56
1897	25	.....	1906	28	.....
1898	32	.....	1907	25	.....
1899	26	.....	1908	26	.....
1900	24	102,555	1909	37	.....
1901	23	.....	January, February, and March, 1910	48	138,57
1902	21	.....			
1903	11	.....			

a Official estimate of population in 1895 is not obtainable, but the actual population then was probably about 95,000.

It is evident that the number of deaths (48) in the first quarter of 1910 exceeded considerably the number recorded in the whole of any one of the previous years.

AGES OF PERSONS AFFECTED.

As far as could be ascertained from the report of cases recorded at the health office, the ages of the persons affected during the period of the outbreak were as follows:

TABLE No. 4.—*Giving ages of persons affected.*

	Number of cases reported.				
	December, 1909.	January, 1910.	February, 1910.	March, 1910.	Total.
0 to 4 years	8	1	7	7	2
5 to 9 years	15	7	20	21	6
10 to 14 years	8	6	32	15	6
15 to 19 years	13	10	33	23	7
20 to 24 years	15	13	46	21	9
25 to 29 years	22	2	42	19	8
30 to 34 years	11	12	18	6	4
35 to 39 years	5	3	9	5	2
40 to 44 years	2	.....	5	2	
45 to 49 years	2	2	4	5	1
50 to 54 years	1	1	1	1	
55 to 59 years	.....	1	3	1	
60 to 64 years	.....	.....	1	.....	
Not stated	12	10	25	28	7
Total	114	68	246	154	58

From Table No. 4 and Chart No. 5 it appears that the disease was distributed quite generally through the population. Persons of the age generally considered to be that of greatest susceptibility to typhoid infection—viz, from 10 to 30 years—furnished the majority of the cases.

The proportion of cases among persons under 15 years of age was not strikingly large. That there was no strikingly disproportionate incidence of the disease among children is one point in the evidence

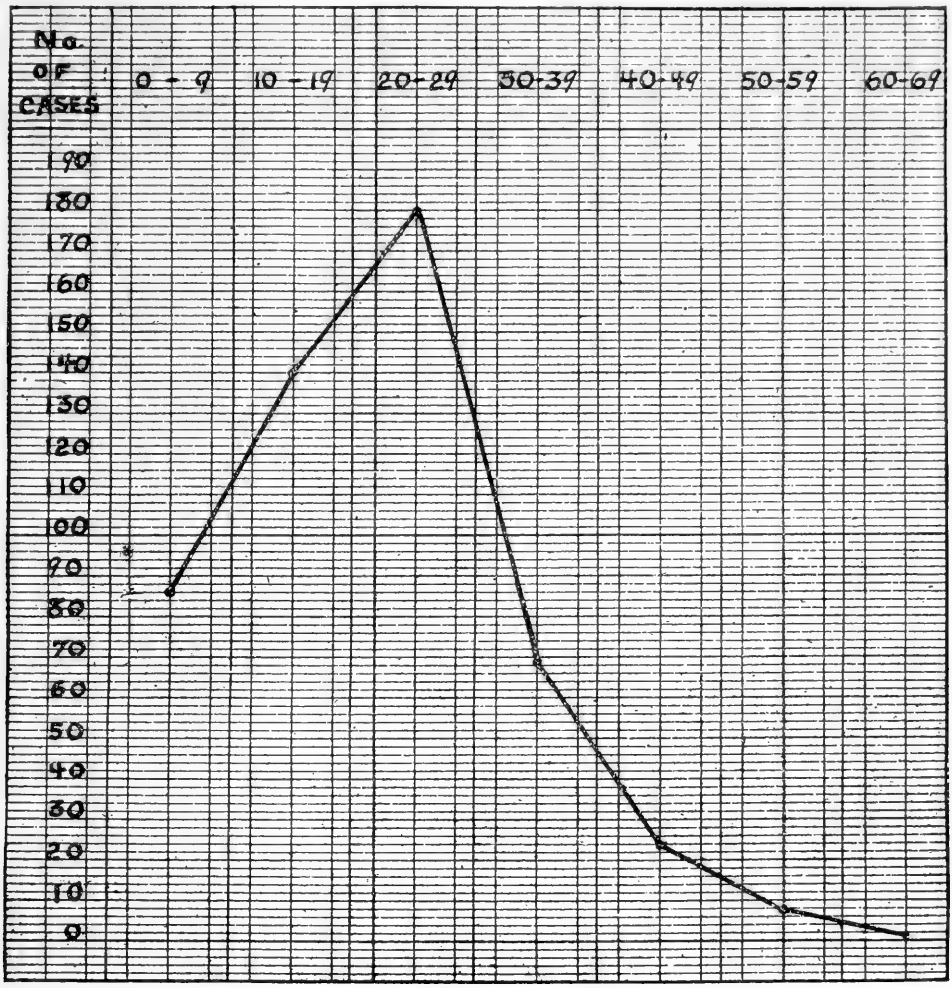


CHART No. 5.—Showing incidence of cases among persons in different decades of life.

against the milk supply having been the chief factor in the production of the outbreak.

In this connection it is instructive to compare the proportion of cases among children in Omaha with the proportion observed to occur in Washington, D. C., where milk and contact are considered the chief factors in the spread of typhoid fever infection. In Omaha persons under 15 years of age furnished about 29 per cent of the cases occurring during the outbreak, while in Washington persons under

15 years of age furnished about 37 per cent of the cases occurring during the typhoid seasons (May 1 to Nov. 1) of 1907 [a] and 1908 [b].

GEOGRAPHICAL DISTRIBUTION.

Maps Nos. 1, 2, and 3 show the location of residences of persons affected according to addresses given in report of cases to the health office during January, February, and March, 1910.

It appears from these maps that the disease was quite generally distributed over the city. Comparatively few cases developed among persons whose residences were not in the area supplied with the city's water supply from the Missouri River. A large proportion of the persons whose residences are not connected with the city water supply no doubt use the river water either at their places of occupation or at places visited for business or social purposes.

The results of a detailed study of 105 cases indicated that the incidence of the disease was disproportionately high among persons who resided in or who had their places of occupation in an eastern section of the city bounded on the north by Lake street, on the west by Sixteenth street, and on the south by Leavenworth street. A discussion of the significance of the high prevalence among persons habitually exposed to the conditions in this section of the city is given on page 37 under the head of "Water."

DIAGNOSIS.

After attention has been sharply attracted to the existence of an outbreak of typhoid fever in a locality, there is always a likelihood that a number of cases will be reported by the local physicians as typhoid fever which are really cases of other diseases. At the meeting of the Omaha-Douglas County Medical Society on April 2, it was suggested by some of the physicians present that the large number of cases reported might be explained, in part at least, by erroneous diagnoses.

During the investigation a special effort was made to determine, as accurately as was then possible, the percentage of error in diagnosis. About 50 cases, still in the febrile stage of the disease, were examined clinically. The vast majority of these presented the characteristic clinical features of typhoid fever. Widal tests of the blood of about 20 patients were made and the majority gave a positive reaction. Cultures were made from the blood of seven patients and the typhoid bacillus was obtained from the blood of five. The blood serums of the two patients from whose blood the typhoid bacillus was not obtained by culture gave positive Widal reactions.

The technique followed in making the blood cultures were as follows: Five cubic centimeters of blood were taken with a syringe

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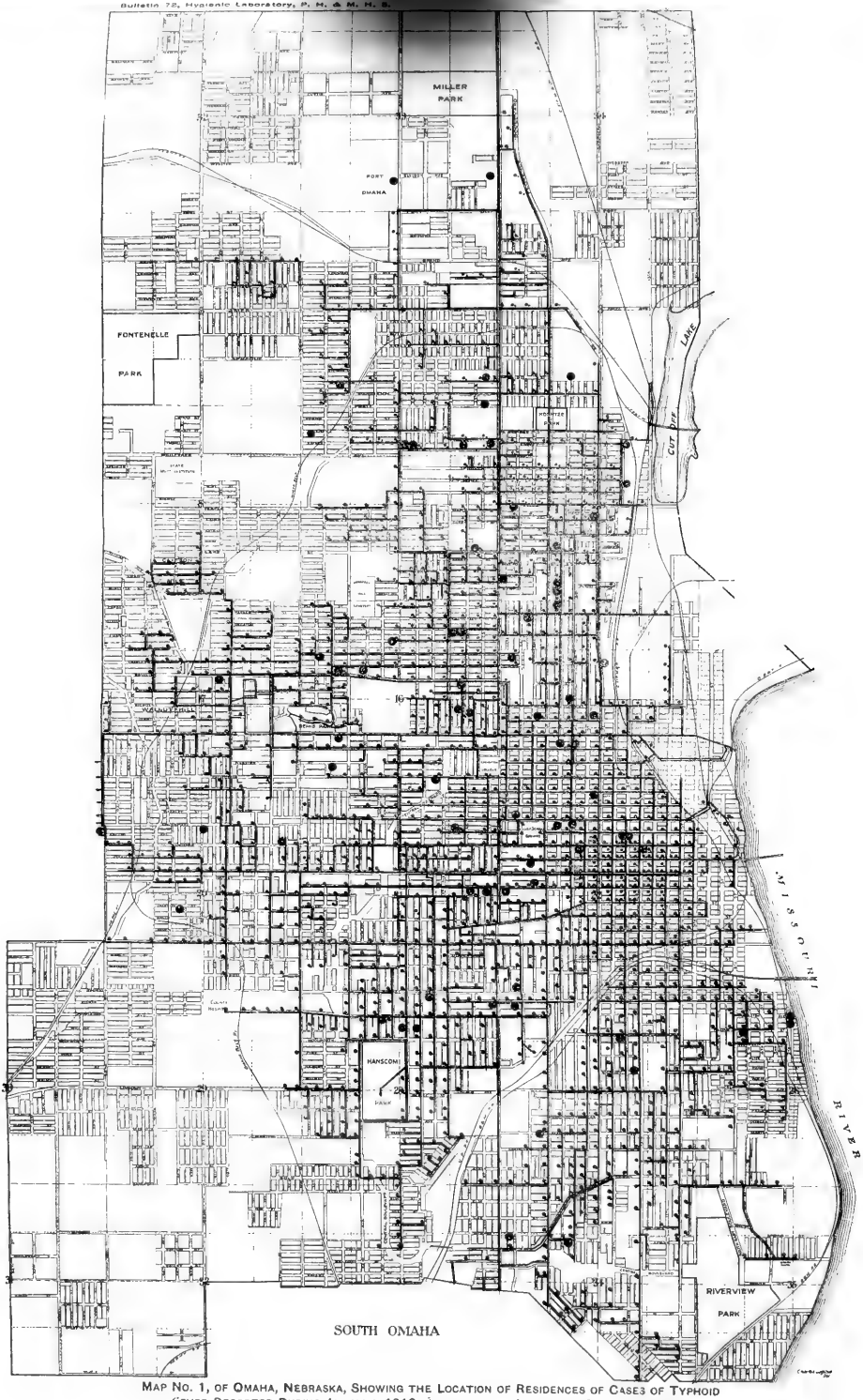
[a] Hygienic Laboratory Bulletin No. 44, Report No. 2, on the "Origin and Prevalence of Typhoid Fever in the District of Columbia (1907)," pp. 15–17.

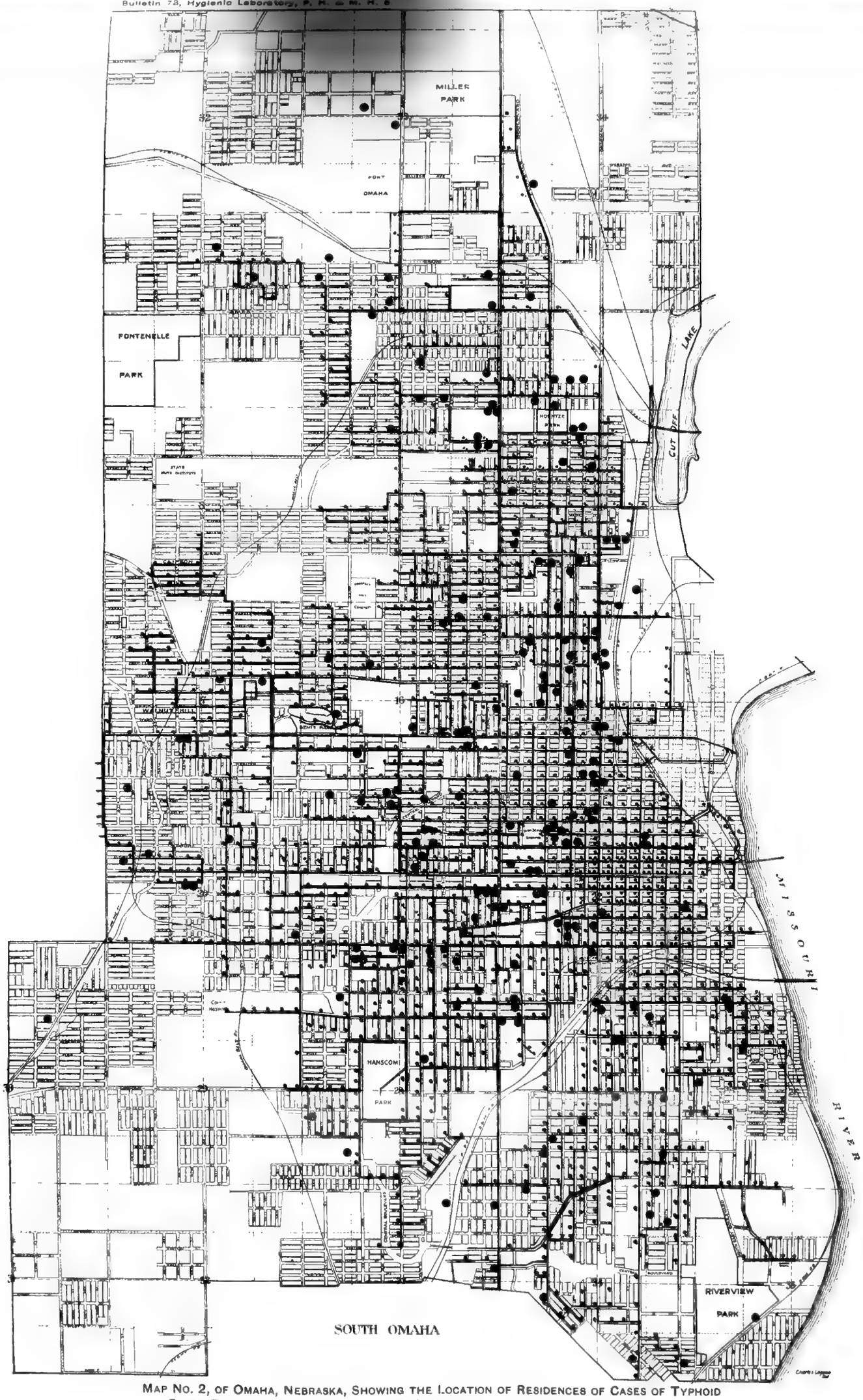
[b] Hygienic Laboratory Bulletin No. 52, Report No. 3, on the "Origin and Prevalence of Typhoid Fever in the District of Columbia (1908)," pp. 20–21.



MAP NO. 1, OF OMAHA, NEBRASKA, SHOWING THE LOCATION OF RESIDENCES OF CASES OF TYPHOID FEVER REPORTED DURING JANUARY, 1910, ACCORDING TO ADDRESSES GIVEN ON REPORT CARDS, IN RELATION TO DISTRIBUTION OF WATER-SUPPLY FROM THE MISSOURI RIVER.

● = TYPHOID FEVER CASES; TWO OR MORE DOTS WITHIN A CIRCLE INDICATE TWO OR MORE CASES IN SAME HOUSE.

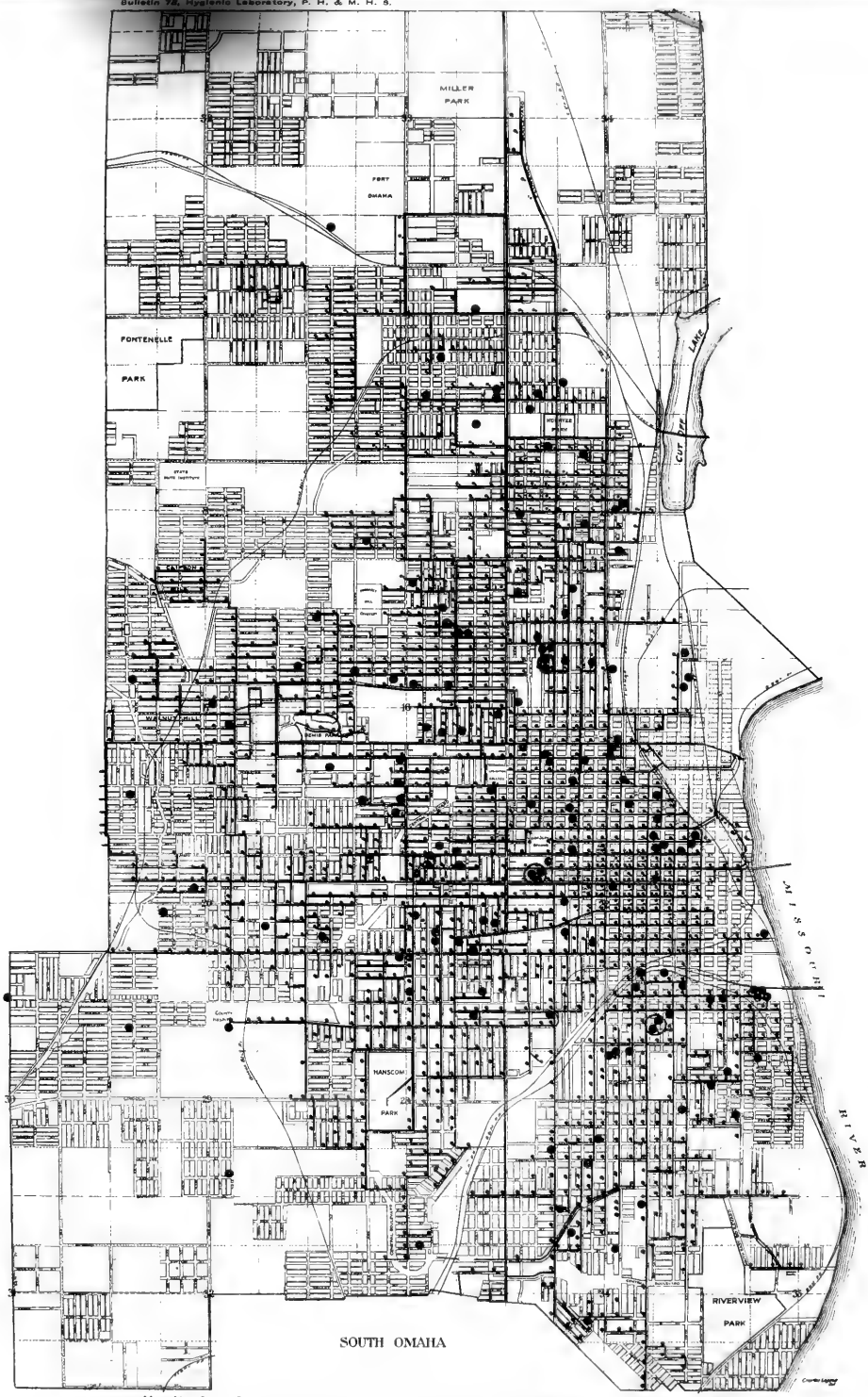
= CITY WATER SUPPLY (= PIPE LINE; = WATER HYDRANTS.)



MAP NO. 2, OF OMAHA, NEBRASKA, SHOWING THE LOCATION OF RESIDENCES OF CASES OF TYPHOID FEVER REPORTED DURING FEBRUARY, 1910, ACCORDING TO ADDRESSES GIVEN ON REPORT CARDS, AND IN RELATION TO DISTRIBUTION OF WATER-SUPPLY FROM THE MISSOURI RIVER.

● = TYPHOID FEVER CASES; TWO OR MORE DOTS WITHIN A CIRCLE INDICATE TWO OR MORE CASES IN SAME HOUSE.

= CITY WATER SUPPLY (= PIPE LINE; = WATER HYDRANTS.)



MAP NO. 3, OF OMAHA, NEBRASKA, SHOWING THE LOCATION OF RESIDENCES OF CASES OF TYPHOID FEVER REPORTED DURING MARCH, 1910, ACCORDING TO ADDRESSES GIVEN ON REPORT CARDS, IN RELATION TO DISTRIBUTION OF WATER-SUPPLY FROM THE MISSOURI RIVER.

● = TYPHOID FEVER CASES; TWO OR MORE DOTS WITHIN A CIRCLE INDICATE TWO OR MORE CASES IN SAME HOUSE.

= CITY WATER SUPPLY (= PIPE LINE; = WATER HYDRANTS.)

nder aseptic conditions by puncture of a vein at the elbow. The lood was transferred at once to a tube containing 20 cubic centiieters of sterilized ox-bile. The blood-bile mixture was incubated t 37° C. for from fifteen to twenty-four hours. Then four or five rops of the blood-bile mixture were pipetted onto a plate of lactose-itmus agar or plain agar.[a] The drops were spread over the first late with a glass rod bent at a right angle. From the first plate he rod was rubbed over the surfaces of a second and a third plate, hus securing dilution. The plates then were incubated for eighteen o twenty-four hours and typhoid-like colonies were "fished" and lanted in tubes of bouillon. Tubes which showed a typhoid-like rowth were tested with antityphoid serum. If agglutination ccurred, the tube was shaken to break up clumps and some of the ontents plated out in order to obtain the organism in pure culture. fter the organism was obtained in presumably pure culture it was arried through the various media on which the typhoid bacillus ives differentiative cultural characteristics.

The following table gives the results of the blood cultures:

TABLE No. 5.—*Showing results of blood cultures for B. typhosus.*

o.	Patient.	Definite onset of illness.	Clinical course.	Temperature when blood was taken (°F.).	Quantity of blood taken (c. c.).	Date of taking blood.	Result of culture.	Remarks.
1	E. W. (M., W., adult).	Mar. 19	Mild; no rose spots observed.	99.2	10	Apr. 1	—	Widal +; temperature curve descending for several days before blood was taken.
2	S. H. (M., W., aet. 29).	...do.....	Typical.....	101.4	5	Apr. 7	+	Widal +; temperature descending.
3	J. R. (M., W., aet. 22).	Mar. 29	Mild; rose spots (?).	101.0	5	...do.....	—	Do.
4	K. C. (M., Syrian; aet. 10).	Apr. 1	Typical.....	103.5	5	...do.....	+	Widal +.
5	J. R. (M., W., adult).	Feb. 20	Typical and long duration.	102.0	5	Apr. 9	+	Widal —.
6	T. B. (M., W., adult).	Mar. 20	Typical.....	100.6	5	...do.....	+	Widal +; temperature descending.
7	R. S. (M., W., adult).	Apr. 4	.....do.....	103.3	5	Apr. 8	+	Widal —.

[a] It is a considerable advantage, but by no means essential, to use for this plating Endo or some other edium selective for the typhoid group of organisms because by so doing the confusion and trouble occaonally resulting from contaminations with skin cocci or other organisms can be obviated to a large tent.

Judging by the results of the clinical observation of cases and of the laboratory tests, it seems probable that at least 85 or 90 per cent of the cases reported as typhoid fever during the outbreak were correctly reported as such.

In the canvass of about 100 homes, 8 or 10 cases were found which were clinically typhoid but which had not been reported. Therefore it appears reasonable to believe that fully as many cases of typhoid actually occurred as the record of reported cases showed. This view is supported by the fact that the proportion of deaths to cases was as high as is usual in extensive outbreaks of this character. The records show a report from December 1, 1909, to April 1, 1910, of 582 cases and 59 deaths, thus showing a case fatality rate of about 10 per cent.

IMPORTED CASES.

One of the many suggestions offered to explain in whole or in part the high rate of prevalence of the disease was that a large proportion of the cases reported had occurred in persons who had contracted the infection elsewhere and had come to Omaha after or a short while before onset of illness. Effort was made, therefore, to determine as accurately as possible the place of infection of the cases. The whereabouts of the persons affected during the thirty days prior to onset of illness and the probability of exposure to infection in each place were considered.

Of the 105 cases investigated only six gave a history of having been away from Omaha within thirty days prior to onset of illness. Of these six, one had not been in Omaha prior to onset of illness and undoubtedly contracted the infection elsewhere; one had been away from the city, nursing a case of typhoid fever for a period of six weeks ending eleven days before definite onset of illness, and probably contracted infection while away; and four had been away from Omaha for periods of less than one-fourth of the thirty days prior to illness and possibly contracted the infection while away, but more probably contracted the infection in Omaha.

The 105 cases may be classed as follows:

	Number of cases
(1) Infection undoubtedly contracted in Omaha	9
(2) Infection probably (chances over 75 per cent) contracted in Omaha	
(3) Infection probably (chances over 75 per cent) contracted away from Omaha	
(4) Infection undoubtedly contracted away from Omaha	
Total	10

Judging from these data it appears that over 95 per cent of the persons affected during the outbreak contracted the infection in the city of Omaha.

POSSIBLE SOURCES OF INFECTION.

The time of occurrence, the extent and the distribution of the outbreak indicated (1) that the infection had been spread under conditions of cold winter weather; (2) that some condition or set of conditions had arisen which were remarkably, and certainly very unusually, favorable to the causation of typhoid fever in that season of the year; and (3) that the city's population quite generally had been exposed to the infection. The following possible immediate sources of infection, therefore, were to be considered: (1) Water supply, (2) milk supply, (3) raw vegetables and fruits, (4) raw shellfish, (5) other food supplies, including ice cream, bakery products, etc., (6) ice supply, and (7) air (dust).

Personal contact, sewage disposal, and local sanitary conditions, also, were to be carefully considered as factors operating through the above-mentioned media or otherwise.

Flies as a considerable factor in the spread of the infection could be definitely excluded, because during the period in which the outbreak was caused there were certainly very few flies in Omaha, and these could not have been very active during the cold weather. Considered together, the season and the distribution of the disease preclude the likelihood that insects or any vermin could have constituted a very considerable factor.

ICE.

As the outbreak was caused for the most part during cold winter weather, it is unlikely that many of the persons affected had used ice in drinking water or in other ways by which organisms contained in the ice would have been swallowed.

Of the 103 cases considered to have contracted the infection in Omaha, which were investigated, only two gave a history of having habitually used ice in drinking water during the thirty days prior to onset of illness. One of these had used only the manufactured ice and the other was not certain whether the ice used was manufactured or natural.

A large part—possibly as much as 40 or 50 per cent—of the ice used in Omaha is natural ice. Some of this is harvested from nearby

lakes, but the greater part of it is harvested from the Missouri River. The ice harvested from the river, in all probability, contains fewer disease-producing organisms than did the water from which it was derived. Although ice may be eliminated beyond reasonable doubt as having been a considerable factor in the production of the outbreak, it should be borne in mind that ice harvested from polluted waters, as was most of Omaha's natural supply during the winter of 1909–10, may become, upon the advent of warm weather and consequent increased use of ice in beverages and foods, an important source of infection.

MILK.

The general features of the outbreak were not those usual to an extensive outbreak caused by milk-borne infection. There was no disproportionately large number of cases among children, and there appeared to be no unusually large number of instances in which two or more cases developed at about the same time in the same household. Yet some of the local physicians had become impressed with the fact that a large proportion of their typhoid patients were among persons who had used milk supplied by a certain dairy company which conducts a large retail business in Omaha, and expressed the view that the outbreak was caused by infection in the milk supplied by this company.

Special attention was given, in the course of the investigation, to the milk supply as being possibly the immediate source of the infection. In order to determine if there was any disproportionately large number of typhoid cases among the customers of any particular dairymen, a list of all the dairies in Omaha was made, giving, as accurately as could be ascertained, the amount of milk sold from each during the months of January, February, and March, 1910. Then, in the investigation of cases, careful inquiry was made to determine the sources of milk used during the thirty days prior to illness by the persons affected.

Of the 103 cases investigated, satisfactorily definite data as to use of milk during the thirty days prior to onset of illness were obtained from 99. Of these, 18 gave a history of not having used milk in any way, 4 of having used only milk which was boiled subsequent to purchase, 3 of having used milk only as ice cream, 5 of having used milk only from their own or a neighbor's cows, and the remaining 69 cases were distributed among the customers of 30 different dairymen.

The 103 cases gave the following history as to the way in which milk was used prior to illness:

How used:	Number of cases.
As a beverage	[a] 28
On fruits or cereals, but not as beverage	33
In hot coffee or tea only	18
As ice cream only	3
Not in any way	18
Not determined	3
Total	103

Of the cases which used milk as a beverage, 4 used boiled milk exclusively.

The milk supply of Omaha is distributed from about 70 dairies located in or near the city. Two dairies, A and B, are controlled by companies and do a large business. About 50 per cent of the milk consumed in Omaha is distributed from these two dairies. Most of the milk from dairy A is retailed, about 5,000 households being supplied directly from this dairy; while most of the milk from dairy B goes into the wholesale trade, being distributed to hotels, restaurants, groceries, bakeries, etc. At both dairies (A and B) milk cans and bottles are sterilized. About 95 per cent of the milk distributed from dairy A is pasteurized; the remaining 5 per cent is obtained from special farms and is "certified." The whole output from dairy B is pasteurized.

The work at both these dairies is supervised as thoroughly as practicable by the health officer. An inspector from the health office makes frequent visits, usually daily and sometimes on several occasions within a day, to each of the plants.

The health officer appears to have been commendably energetic in his effort to get a safe milk supply for Omaha. Besides supervising the work at these two large dairies, he is apparently using to the best practicable advantage his too limited force of inspectors to

[a] It is instructive to compare this percentage (27) with the percentages of cases which gave a history of having used milk as a beverage among those investigated in Washington, D. C., in 1906, 1907, and 1908. Thus:

Year.	Number of cases investigated.	Percentage used milk as beverage.
1906	747	65
1907	523	55
1908	542	42
Average		54

See Hygienic Laboratory Bulletins: No. 35, p. 61, for 1906; No. 44, p. 46, for 1907; No. 52, p. 100, for 1908.

bring about improved sanitary conditions and safer methods of handling milk at the farms and small dairies from which the rest of the city's milk supply comes.

Of the 103 cases of typhoid fever investigated, the number of cases among the customers of the different dairymen was roughly proportionate to the amount of milk sold by the dairymen. No one of the dairymen had a number of cases among his customers sufficiently out of proportion to amount of milk sold to warrant a reasonable suspicion that the milk sold by him was the source of the infection.

Twenty-four of the 103 cases gave a history of having used regularly or occasionally within the thirty days prior to illness milk supplied from dairy A. This number, at first glance, seems large, and it was the output from this dairy that some of the local physicians, as referred to above, had regarded as the source of infection. But when this number, composing about 23 per cent of the cases investigated, is considered in connection with the fact that about 35 per cent of the milk retailed in Omaha is distributed directly or indirectly from this dairy, it becomes evident that the number was not even large enough to show that the incidence of the disease was as great among persons supplied with milk from this dairy as it was among the general population of the city.

In this connection, it should be borne in mind that the proper sterilization of milk bottles and the pasteurization of milk may be something of a safeguard to a community when visited by an outbreak of typhoid fever even caused solely by water-borne infection, because if the infectious organisms in the water find their way into milk, as they are almost sure to do from time to time if the cans and bottles are washed in the polluted water and not sterilized before being used as containers for the milk, they may undergo there tremendous multiplication and so be conveyed to the people in much larger doses than they probably ever would be conveyed by the water directly.[a]

From the foregoing data it appears that the milk supply can be definitely eliminated as having been the chief and primary or even a considerable factor in the production of the outbreak.

ICE CREAM.

A large proportion of the ice cream used in Omaha is made from pasteurized cream. The raw cream used for making ice cream is supplied almost, if not quite, wholly from dairies from which milk is distributed in the city.

Of the 103 cases investigated only 12 gave a history of having eaten ice cream within the period of thirty days prior to illness. These 12

[a] Hygienic Laboratory Bulletin No. 56, "Milk and Its Relation to the Public Health," p. 157.

cases were distributed among the customers of several ice-cream producers.

It is evident from these data that ice cream was not a considerable factor.

RAW SHELLFISH.

The following table gives the history of the 103 cases in regard to the eating of raw oysters and clams within the thirty days prior to illness:

Shellfish.	Yes.	No.	Not determined.	Total cases.
Oysters	7	91	5	103
Clams	0	98	5	103

The small proportion of cases giving a history of having eaten oysters or clams shows that raw shellfish could not have been an important factor in the production of the outbreak.

RAW VEGETABLES.

The following table gives the history of the 103 cases in regard to the eating of celery and lettuce within the thirty days prior to illness:

Raw vegetables.	Yes.	No.	Not determined.	Total cases.
Celery	41	57	5	103
Lettuce	24	74	5	103

These figures show that the majority of the cases certainly could not have been caused by infection in either the celery supply or the lettuce supply.

Most of the celery sold in Omaha during the winter was obtained from truck farms in sections of Michigan and of Southern States. From these same sections no doubt other American cities, some of which did not have during the past winter any unusual occurrence of typhoid fever, also obtained a part of their supply of celery. Most of the lettuce sold in Omaha during the winter was leaf lettuce raised in hothouses, located in Iowa and Nebraska. Some—the head lettuce—was obtained from truck patches in the South. Some of the other cities and towns using lettuce from the same sections of the country did not have typhoid outbreaks. From a consideration of the foregoing, it does not appear reasonable to believe that celery and lettuce could have been a very important factor in the production of the outbreak in Omaha.

BAKERY PRODUCTS AND OTHER GENERAL FOOD SUPPLIES.

In view of the complex conditions under which the general food supplies of the average city are prepared and distributed, the number of persons through whose hands they pass, and the readiness with which typhoid infection may be carried from the bedsides of patients and from the excreta of bacillus carriers, it is almost inconceivable that these food supplies do not play some part in the spread of infection after the infection has been introduced into a city and widely disseminated among the people. But considering the general features of the outbreak and the fact that the conditions under which the food supplies were handled during the period in which the outbreak was caused, were, so far as could be ascertained, either better or certainly no worse than in previous years, it does not seem reasonable to suppose that bakery products and other general food supplies could have played more than a secondary and relatively small part in the production of the outbreak in Omaha.

DUST.

As the ground at Omaha was covered with snow from December 3, 1909, to February 1, 1910—that is, during the greater part of the time in which the outbreak was caused—it does not seem possible that much of the infection could have been disseminated in air-borne dust. Furthermore, if dust had been abundant it would still be difficult to conceive of any source from which infection sufficient to cause such an outbreak could have been carried in the dust.

SEWAGE DISPOSAL AND GENERAL SANITARY CONDITIONS.

The greater part of the city is provided with a water-carriage sewerage system. Exact data on the subject were not obtainable, but it is estimated that about two-thirds of the houses in the city are connected with the city sewerage system. The main sewers empty the sewage entering the water-carriage system into the Missouri River. Three of these main sewers discharge into the river at points along the city's water front. One discharges into Papillion Creek and thence into the river below (south of) the city, and one discharges into the river at a point above (north of) the city and about 8 miles upstream from one of the intakes for the city's water supply. (See Map No. 4, and p. 36 under head of "Water.") Thus Omaha, consistent with the practice followed by many other cities on the Missouri River, uses this river both as a sewer and as a source of water supply, even going so far, by having one of the intakes for its water supply below the outlet of one of its main sewers, as to partake in its own drinking-water supply some of the sewage which it contributes to the general pollution of the river.

In view of the rapidly increasing population along the great water courses of the United States, and the frequently demonstrated fallacy of the belief that running water adequately purifies itself of dangerous sewage pollution, it certainly seems time for action to be taken by interurban or interstate agreement to have the different streams used either as sources of water supply or as sewers, and not for both purposes.

The Missouri-Mississippi River and the other great interstate water courses seem to present, at the present time, a large and very important field of action for the health forces of the National Government. The investigation of these water courses in respect to sewage pollution and to their use as sources of water supply certainly could be conducted by the agents of the national health organization in such a way as to obviate any reasonable criticism that the methods followed and the conclusions reached were being influenced by local (or state) prejudice.

Furthermore, if the different laboratories necessary to the investigation were equipped from the same national appropriation and their personnel were responsible to the same central head it is obvious that the work could be done more economically, more consistently, and, so far as expense to the different States affected would be concerned, more equitably than it could be done by the health organizations of the different States working either independently or cooperatively.

As the pollution of these water courses affects the commercial welfare of the communities using these water courses as sources of water supply—particularly and specifically by affecting the health of personnel of vehicles operated by common carriers—and as the pollution in water supplies will certainly from time to time find its way into and dangerously contaminate and render impure many of the various beverages and foods (dairy products particularly) such as are usually handled in the course of interstate traffic, the investigation and control of the sewage pollution of these water courses would appear to come thoroughly within the scope of the constitutional powers of the Congress of the United States to regulate interstate commerce.

All of the residences inspected in Omaha which were not connected with the water-carriage sewerage system were found to be provided with either cesspools or privies, cesspools (or "privy vaults") being vastly in the majority. Very few of the cesspools were found to be water-tight, most of them being merely holes dug in the ground to a depth of about 6 feet. In a few instances there appeared to be a likelihood of seepage from the cesspool to nearby wells, but evidence was lacking that such seepage contributed much to the spread of infection during the outbreak.

The incidence of the disease appeared to be as great among persons living at residences connected with the city's sewerage system as among those living at residences provided with cesspools. Of the 103 cases investigated 68 were among persons living at residences connected with the city sewerage system and at which there were water-closets, 34 at residences not connected with the city sewerage system and at which there were cesspools or privies, and 1 at a residence where the method of sewage disposal was not noted. Of the 68 cases at residences connected with the city sewerage system, 58 were at residences having water-closets in the house only, and 10 at residences having water-closets in the yard only.

In the investigation of the 103 cases, nine instances were met with in which two or more cases had developed in the same residence. Of these nine instances, six were at homes connected with the city sewerage system and three at houses provided with cesspools or privies; so it appears that secondary cases were not much, if any, more frequent among persons living at houses provided with cesspools or privies than among those living at homes connected with the city sewerage system.

In the following table is given the general sanitary condition of the residences at which the persons who furnished the 103 cases investigated had lived at the time when the infection was presumably contracted:

Condition of residence.[a]	Number of cases.	Per cent of cases.
Good	22	21.4
Fairly good	37	36.0
Rather bad	29	28.1
Bad	14	13.6
Not determined	1	.9
Total	103	100.0

[a] The definitions of the terms used in this table correspond to those used in Hygienic Laboratory Bulletin No. 35, Report No. 1, on the "Origin and Prevalence of Typhoid Fever in the District of Columbia," p. 46.

From the foregoing data it is evident that the chief factor in the production of the outbreak was not dependent for its operation upon insanitary conditions at places of residence of persons affected.

CONTACT.

Of the 103 cases investigated, 14 gave a history of association during the thirty days prior to onset of illness with previous cases in the febrile stage of the disease, and were attributable to infection by personal contact.

As the cases investigated were reported subsequent to February 28 it is probable that the percentage of secondary or contact cases

was higher among them than it was among those occurring in the early part of the outbreak.

Few facts in the epidemiology of infectious diseases have been more definitely established than has the fact that when typhoid infection is introduced into a community, no matter through what channels, and the disease continues to prevail extensively for some time, personal contact or contagion sooner or later will become, unless specific and rigid precautions be taken to prevent it, an important factor in the spread of the infection.

The outbreak at Omaha was not exceptional in this respect and personal contact beyond reasonable doubt did operate to some extent as a factor in the spread of the infection; but in view of the extent and the explosive character of the outbreak, the percentage of cases among those occurring even toward the end of the outbreak which gave a history of association with previous cases, and the fact that without any extraordinary precautions having been taken to prevent infection by personal contact, the disease, after becoming widely disseminated over the community, suddenly began to decline and soon reached a point which may be called "normal" for the community at that season, it is evident that in the production of this outbreak personal contact operated only as a factor secondary to some other factor which was chief and primary.

WATER.

The general water supply of Omaha is obtained from the Missouri River. It is estimated that about four-fifths of the houses in the city proper are connected with this water supply and that probably about 90 per cent of the population use the river water as the sole or principal supply for drinking and culinary purposes. There are a number of wells and a few springs still in use, but these are for the most part in the outskirts of the city, and all of them, so far as was ascertained, are on private property.

Of the 103 cases investigated, 98, or about 95 per cent, gave a definite history of having used the unboiled Missouri 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 onset of illness. The following table gives the sources of water used for drinking during the thirty days prior to illness by the 103 persons affected and whose cases were investigated:

Water:	Number of cases.
Raw tap—	
Solely	51
Principally	21
Occasionally	26
Occasionally(?)	3
Boiled tap—	
Solely	1
Principally	21
Occasionally	15
Private wells or springs in Omaha—	
Solely	1
Principally	7
Occasionally	5
Bottled and vended—	
Solely	0
Principally	1
Occasionally	0
Various sources out of Omaha—	
Solely	0
Principally	0
Occasionally	4

Of the 30 cases which gave a history of having used solely or principally for drinking the boiled tap, well or spring, or bottled waters, 9, or 30 per cent, were in persons who had been in the thirty days prior to illness in free association with previous cases in the febrile

stage of the disease and were attributable to infection by personal contact. The relatively large percentage of contact histories in this group of cases is somewhat significant, in that it suggests that when the raw tap water as a possible factor in the distribution of the infection could be to some extent eliminated, other factors took a more prominent place.

Of the cases listed as having used principally for drinking water other than the raw tap water, the vast majority were in persons who did use in considerable amount the unboiled tap water for drinking, many of them being school children and other persons who used for drinking boiled tap, well or spring, or bottled water at their homes, but who drank the raw tap water at the public schools or at their places of occupation.

The school board did not see fit to take action at any time during the outbreak to provide water other than the raw tap for use in the public schools. If this water were the source of the infection which caused the outbreak it is almost certain that some of the cases in school children resulted from the use of unboiled water at the public schools. Furthermore it may be expected that certain families were influenced to discontinue or not to begin boiling the water for use at their homes by reason of the fact that their children were given the unboiled water to drink at the public schools. Several instances of this kind were learned of definitely in the course of visits to homes for the investigation of cases.

The public water supply of the city has been owned and controlled for the past twenty years or more by a private company known as the "Omaha Water Company." A year or so ago the city authorities took steps to take over from the water company the control and ownership of the water-supply system, but the terms of assessment of the property could not be agreed upon and the matter was taken before the courts for adjudication, and was still pending before the courts at the time of the outbreak. This circumstance added somewhat to the complexity of the situation, particularly in respect to the adoption of measures for the improvement of the water supply.[a]

The city's water supply is taken from the river at two points (see Map No. 4), one, the north intake, being a few feet from the bank of the river at Florence, and the other, the south intake, being about 250 feet out in the river nearly on a line leading east from the foot of Burt street. About 20,000,000 gallons of water from the river are supplied to the city daily; of this amount about 16,000,000 gallons are pumped from the north or Florence intake, and about 4,000,000 gallons from the south or Burt street intake. At each station the water passes through several settling reservoirs, the capacity of these reservoirs and the rate of flow through them being

[a] See Appendix, p. 50.

such as to give the water storage for about six hours (estimated). In the second reservoir of the series at each station a coagulant (alum) is applied to the water. The river water is very turbid, but the coagulant and the storage, even for such a short period, effect a marked reduction in the turbidity and also a considerable reduction in the bacterial content of the water. The process can not be regarded, however, in view of the results of the bacteriologic examinations given below, as being sufficient to render the water reasonably free from whatever dangerous pollution it may contain.

From the Florence reservoirs the water is pumped to the Walnut Hill reservoir (see Map No. 4), which is located at a relatively high elevation in the western part of Omaha and serves as a standpipe from which pressure is supplied for distributing the water to the city. The main from the Burt street reservoirs extends to the Walnut Hill reservoirs. There appeared to be some difference of opinion among the local engineers as to whether any of the water from the Burt street station was pumped during the period of the outbreak as far as the Walnut Hill reservoir, but the officials of the water company, who were certainly in a position to be correctly informed on that subject, positively asserted that none of the water from the Burt street station had reached the Walnut Hill reservoir at any time within the ten years preceding the investigation in April, 1910. These officials state that all the water taken in at the Burt street station is limited in its distribution to the relatively unelevated and eastern section of the city bounded on the north by Lake street, on the west by Sixteenth street, and on the south by Leavenworth street. When the pump at the Burt street station is stopped, as it frequently is at night—this Burt street supply being used only as an auxiliary to the principal supply from the Florence station—the mains in this section of the city are supplied with water from the Walnut Hill reservoir. Therefore, according to information received from the manager of the water company it is clear that while the water from the Florence intake is distributed over all parts of the city connected with the water system, the water from the Burt street intake is distributed only to the eastern, relatively unelevated section of the city, referred to above.

BACTERIOLOGIC EXAMINATION OF THE MISSOURI RIVER WATER SUPPLY.

The technique followed in making the bacteriologic examinations of the water was that recommended by the committee on standard methods of water analysis of the American Public Health Association on January 9, 1905.

The laboratory of the Creighton Medical College, where the bacteriologic work was done, did not have facilities for the use of gelatin plates, so that in making the bacterial counts agar plates were used.

The colonies were counted after the plates had been kept at 37° C. for forty-eight hours from the time of seeding. For most waters it is understood that agar plates kept at 37° C. will average considerably lower counts than will gelatine plates kept at 20° C. The press of other work made it impossible at the time to conduct a very exhaustive bacteriologic study of the water, but enough was done to form a satisfactorily definite idea of the bacterial content of the water and the degree of improvement of the water effected by the storage and use of coagulant at the two systems of reservoirs. The samples of water examined were taken from (*a*) the inlet to the first storage reservoir at the Florence station; this representing the water from the river as it entered the Florence intake; (*b*) the outlet from the last in the series of storage reservoirs at Florence; this representing the water after storage and treatment with a coagulant in the Florence system of reservoirs and just as it started to the Walnut Hill reservoir for distribution to the city; (*c*) the inlet to the first storage reservoir at the Burt street station; this representing the water from the river as it entered the Burt street intake; (*d*) the outlet from the last in the series of storage reservoirs at the Burt street station; this representing the water after storage and treatment with a coagulant in the system of reservoirs at the Burt street station and as it was delivered to the mains for distribution to the city; (*e*) the tap at the bacteriological laboratory of the Creighton Medical College located on the northeast corner of Fourteenth and Davenport streets; this representing a mixture of the effluents from both the Florence and Burt street reservoirs after the water had traversed the city mains for some distance.

In the following tables, A, B, C, D, and E, are given the results of the bacteriologic examinations of these samples of water:

TABLE A.—*Samples of (river water) influent to Florence system of reservoirs.*

Date of examination.	Number of bacteria in 1 c. c.	Fermentation in lactose bouillon.		B. coli in—	
		10 c. c.	1 c. c.	10 c. c.	1 c. c.
1910.					
April 5	3,840	+	+	(?)	(?)
April 6	4,600	+	+	(?)	(?)
April 7	3,600	+	+	(?)	(?)
April 8	9,200				
April 15	5,400	+	+	+	+
April 18	11,400	+	+	+	+
Average	6,340	100%+	100%+	100%+	100%+

TABLE B.—*Samples of effluent from Florence system of reservoirs.*

Date of examination.	Number of bacteria in 1 c. c.	Fermentation in lactose bouillon.		B. coli in—	
		10 c. c.	1 c. c.	10 c. c.	1 c. c.
1910.					
April 5	260	+	+	+	+
April 6	300	+	+	(?)	(?)
April 7	(a)	+	−	(?)	−
April 8	200	+	Bubble.	(?)	−
April 15	220	+	+	(?)	−
April 18	540	+	+	+	+
Average	304	100%+	66%+	100%+	40%+

a Plates overgrown; "spreaders."

TABLE C.—*Samples of (river water) influent to Burt street system of reservoirs.*

Date of examination.	Number of bacteria in 1 c. c.	Fermentation in lactose bouillon.		B. coli in—	
		10 c. c.	1 c. c.	10 c. c.	1 c. c.
1910.					
April 5	4,600	+	+	(?)	(?)
April 6	5,800	+	+	(?)	(?)
April 7	(a)	+	+	(?)	(?)
April 8	(a)				
April 15	4,600	+	+	+	+
April 18	11,600	+	+	(?)	(?)
Average	6,650	100%	100%+	100%+	100%+

a Plates overgrown from 0.005 c. c.

TABLE D.—*Samples of effluent from Burt street system of reservoirs.*

Date of examination.	Number of bacteria in 1 c. c.	Fermentation in lactose bouillon.		B. coli in—	
		10 c. c.	1 c. c.	10 c. c.	1 c. c.
1910.					
April 5	(?)	+	−	+	−
April 6	240	+	+	(?)	(?)
April 7	240	+	−	(?)	−
April 8	(a)	+	+	(?)	(?)
April 15	280	+	+	+	+
April 18	1,700	+	+	+	+
Average	615	100%+	66%+	100%+	50%+

a Plates overgrown; "spreaders."

TABLE E.—*Samples of water from tap at laboratory on corner of Fourteenth and Davenport streets.*

Date of examination.	Number of bacteria in 1 c. c.	Fermentation in lactose bouillon.			B. coli in—		
		10 c. c.	1 c. c.	0.1 c. c.	10 c. c.	1 c. c.	0.1 c. c.
1910.							
April 1	160	+	−	.....	(?)	−	.....
April 2	220	+	−	.....	+	−	.....
April 16	320	+	+	−	+	+	−
April 19	520	+	+	.....	(?)	(?)	.....
Average	305	100%+	50%+	0%+	100%+	33%+	0%

It should be noted that these examinations were made on and after April 1, which was subsequent to the period of causation of the typhoid outbreak. A number of samples of the water examined during January and February, when the outbreak was at its height, showed, according to the results of Doctor Langfeld, the city bacteriologist, a considerably higher bacterial count than the average for the above.

THE MISSOURI RIVER WATER SUPPLY AND ITS RELATION TO THE TYPHOID OUTBREAK.

The Missouri River at numerous points north of Omaha receives the sewage from a large number of persons, and therefore can be reasonably regarded as a somewhat dangerously polluted stream. The nearest large city to the north of Omaha which discharges its sewage directly into the river is Sioux City, Iowa. Sioux City is about 90 miles upstream from Omaha and has a population of about 50,000. Along the watershed of the Missouri between Sioux City and Omaha there are a number of towns and villages, from many of which sewage finds its way into the river, either directly from sewers or overhanging privies or indirectly by surface washings or through streams tributary to the Missouri River. The water of the Missouri presents a decidedly turbid appearance, and in popular parlance the river is frequently referred to as "Old Muddy." The suspended matter appears to be made up largely of loam, clay, and fine sand. It is comparatively heavy, and when the water is allowed to stand it settles rapidly. Accurate turbidity readings were not made, but in a liter flask filled with the water and violently agitated and then kept at rest for five to ten minutes about 90 per cent, at a rough estimate, of the suspended mud will settle to the bottom. The high turbidity of the water, though objectionable from an esthetic standpoint, is probably, from a strictly sanitary standpoint, a considerable advantage, because it seems reasonable to believe that the mud in streaming constantly through the water must carry down with it a considerable proportion

of whatever pathogenic organisms chance to be suspended in the water. The process may be regarded as one of natural filtration; the sand in this instance streams through the water, and so is the reverse of the process in artificial filtration, where the water streams through the sand.

LOCAL SEWAGE POLLUTION OF THE OMAHA WATER SUPPLY.

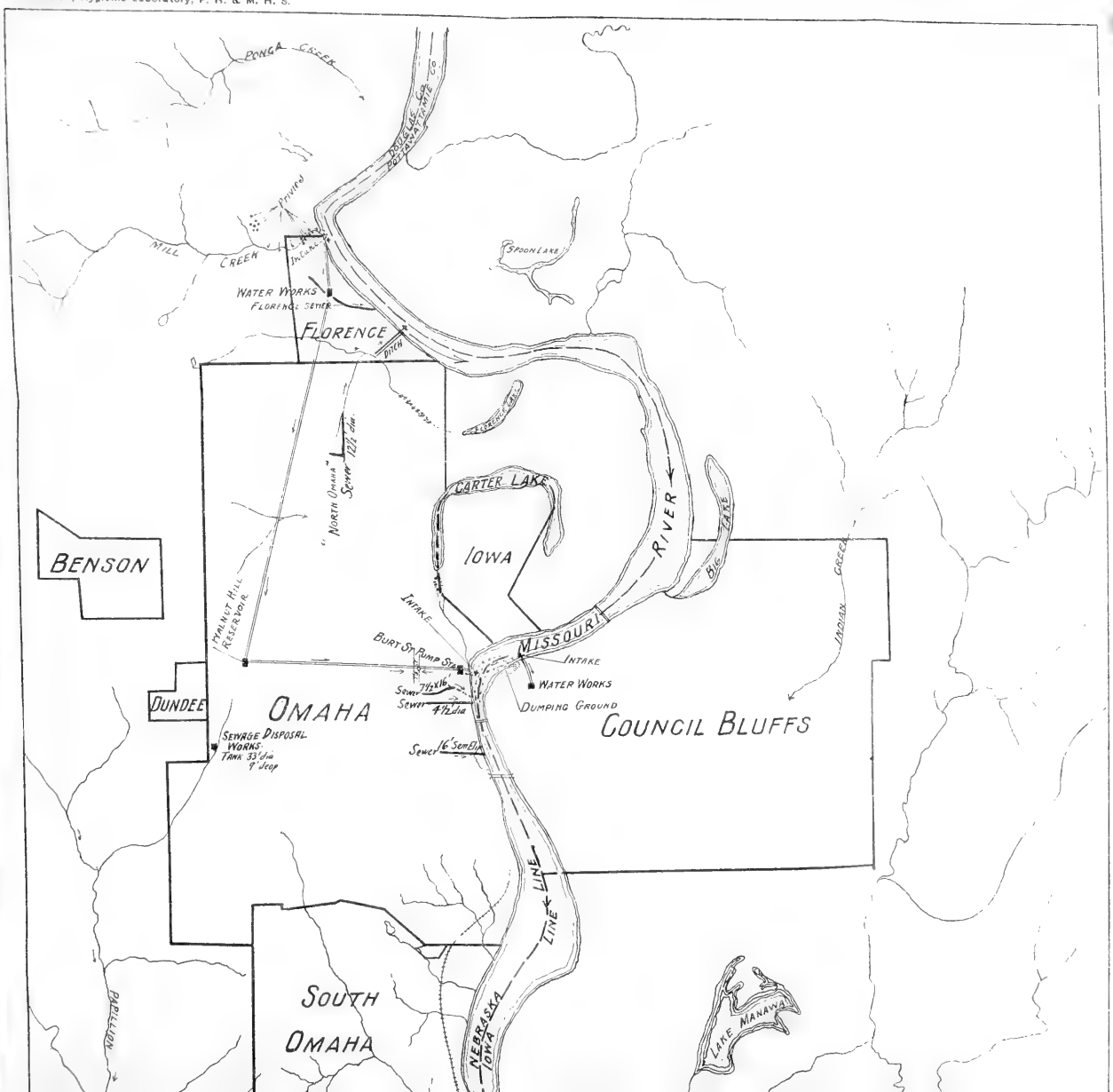
Besides the large volumes of sewage known to enter the river at points farther upstream, the investigation revealed evident and gross pollution of the water with human sewage at points within 8 miles above each intake for the city's water supply. The mouth of Mill Creek empties into the river at a point about 500 feet above the Florence intake. This creek carries into the river the drainage from a number of cesspools or privies and from a number of stable-manure dumps, etc. (See Map No. 4.) About 12 of the cesspools or privies are located on the banks of the creek within half a mile—1 within 40 feet—of the creek's mouth. Considering the proximity of the creek's mouth to the intake and the evident direction of the currents in that part of the river, the contents of this creek undoubtedly constitute a dangerous source of contamination for the water entering the Florence intake.

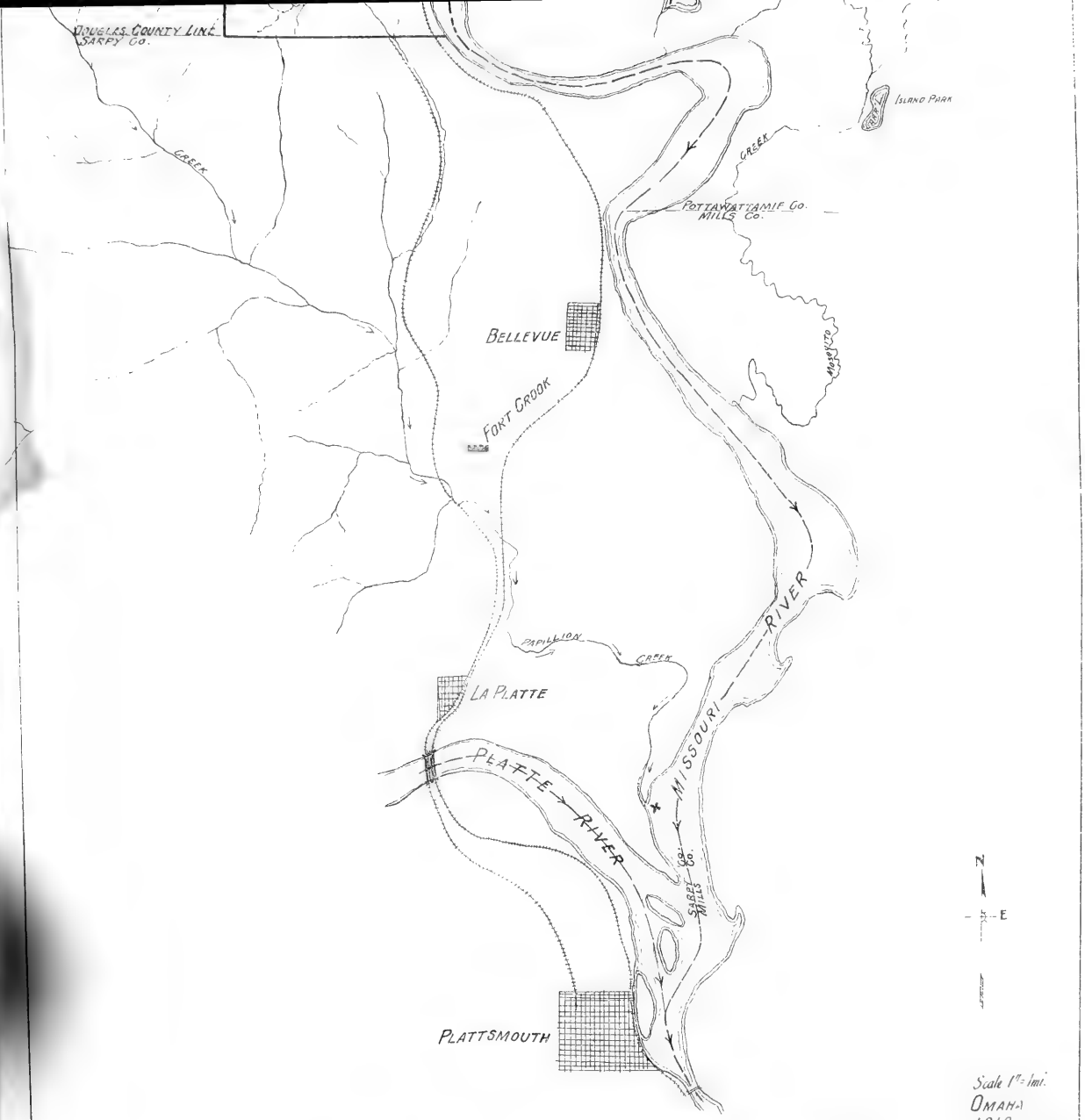
Below the Florence intake and above (upstream from) the Burt street intake the river was found to receive gross sewage pollution from several different sources. (See Map No. 4.)

(1) The main sewer from Florence passes under the Florence storage basins and discharges the sewage of several hundred persons into the river at a point about 1 mile below the Florence and about 9 miles above the Burt street intake.

(2) The North Omaha sewer, conveying the sewage from about 5,000 persons, including the occupants of a hospital, living in the northwest section of the city of Omaha, discharges into the river at a point about 8 miles above the Burt street intake. Previous to June 8, 1909, this North Omaha sewer discharged into Florence Lake. On that date a ditch (see Map No. 4) was completed, and since then the sewage from this sewer has been conveyed through the ditch directly into the river. Hence the winter of 1909–10 was the first winter in which the water at the Burt street intake had been polluted with the discharge from this large sewer.

(3) An extensive dumping ground of Coucil Bluffs, Iowa, on the east side of the river, drains certainly to some extent, and at times of heavy rains to a very considerable extent, into the river at points from a half to three-quarters of a mile upstream from the Burt street intake. This ground receives the usual dumpings from a city, such as ashes, rubbish, etc., and also dead animals and the night-soil from a large number of privies. At one place in the dumping ground there


PONCA CREEK
DOUGLAS POINT PIPE LINE CO.
PIPING
MILL CREEK
INTAKE
WATER WORKS
FLORENCE SEWER
FLORENCE
DITCH
SPOON LAKE
FLORENCE LAKE
NORTH OMAHA SEWER 12½ Dia.
CARTER LAKE
IOWA
RIVER
BIG LAKE
BENSON
WALNUT HILL RESERVOIR
INTAKE
BURT ST. PUMP STA.
MISSOURI
INTAKE
WATER WORKS
DUMPING GROUND
INDIAN CREEK
DUNDEE
OMAHA
SEWAGE DISPOSAL WORKS: TANK 33' dia 9' deep
SEWER 7½x16
SEWER 4½ Dia
SEWER 16' Semi Elip
COUNCIL BLUFFS
NEBRASKA IOWA LINE
LINE
SOUTH OMAHA
PAPILLION
LAKE MANAWA



MAP NO. 4, OF OMAHA, NEBRASKA, AND VICINITY, OUTLINING WATER-SUPPLY AND SEWERAGE SYSTEMS

was observed a large heap which partially hung over the bank of the river and which was composed in considerable part of unmistakable privy contents. In order to determine definitely if the drainage from this particular heap was carried to any extent to the point in the river at which is located the Burt street intake, floats, some on the surface, some partially submerged, and some entirely submerged, were placed in the river just under the dump heap and followed downstream by several of us in a boat. Most all of the floats were carried by the current, with all the definiteness of a laboratory experiment, immediately over or within 100 feet of the Burt street intake.

(4) A number of privies, about 10 or 12, are located on the Omaha side of the river within a few hundred feet of the Burt street intake. In times of high water it is very probable that the contents of some of these privies contribute to the contamination of the water entering the intake.

From these observations it was evident that the water received from the Burt street intake was at the time of the outbreak exposed to much greater sewage pollution from comparatively near-by sources than was the water received from the Florence intake. In this connection the fact that the disease appeared to be particularly highly prevalent among persons who habitually used for drinking the water in the section of the city to which the distribution of the Burt street supply was confined becomes of striking significance. According to information received from the manager of the Omaha Water Company, practically none of the water from the Burt street station was supplied to any part of the city outside of the section, previously referred to, bounded on the north by Lake street, on the west by Sixteenth street, and on the south by Leavenworth street. This section contains a number of industrial establishments, but has less than one-tenth of the city's population. Of the 103 cases investigated, 65, or over 63 per cent, were among persons who resided in, had their place of occupation in, or both resided and had their place of occupation in this section. Thus the 10 to 20 per cent or less of the population habitually using for drinking purposes the water supplied in this section of the city furnished over 63 per cent of 103 cases investigated, in the order in which they were reported from the whole city.

CONDITIONS IN THE RIVER WHICH MAY HAVE AFFECTED THE AMOUNT AND DEGREE OF INFECTION IN THE WATER.

If the outbreak in Omaha was caused by the pollution of the Missouri River water, the question which naturally arises, since the people had been using the polluted river water for years, is, Why had not similar extensive outbreaks occurred before? The answer to this question may be found in one, or, perhaps to some extent, in all of the following hypothetical conditions:

(1) The specific organisms which cause typhoid fever may have been introduced into the water in unusually large numbers.

(2) The organisms when introduced into the water may have been of an unusually high degree of infectiveness, or they may have become so after getting into the water.

(3) The river may have afforded conditions which were unusually favorable to concentration in the water of such organisms as were introduced.

(4) Conditions in the river water may have been unusually favorable to the viability of the organisms.

In support of the view that the organisms were introduced in unusually large numbers is the fact that a part of the city's water supply (the Burt street station supply) was exposed during the winter of 1909–10 to more sewage contamination (that from the North Omaha sewer having been added) than it had been in any other winter for certainly a good many years.

In support of the view that the conditions in the river water were unusually favorable to the concentration and perhaps also the life of the causative organisms are the following facts:

(1) The condition of the river during the winter of 1909–10 was very unusual. A heavy rain beginning on November 12 continued for three or four days; the fall during this period was over 4 inches. These heavy rains must have washed into the current of the river a large amount of sewage and other matter which had accumulated on the watershed of the river above Omaha during the summer and fall. Judging by the report of cases, the beginning of the unusually high prevalence of typhoid fever was (by onset of illness of cases) about November 25, or about twelve days (the usual incubation period) after the occurrence of the heavy rains.

Soon after the heavy rains the cold weather set in. By December 10 the river at Omaha was practically covered with ice from side to side. The river continued frozen over until March 4, 1910, when the breaking up of the ice at Omaha began. According to statements obtained from a number of observers, this was the first winter within the past fifteen or twenty years that the river at Omaha had remained frozen over for any considerable period. Tremendous volumes of floating ice were going down the river from March 4 to about March 10, when the river became practically clear of running ice.

During the freeze the volume of water coming down the river must have been reduced very considerably below the normal, so that whatever sewage entered the current caused more concentrated pollution than it would have caused in the normal volume of water. As the thawing began, the volume of water in the river of course became increased. The flooding and overflow of the river banks began about March 15, and reached the maximum on March 22. By April 1 the

river was again well within its banks and had resumed what may be called its usual condition for the season. It is readily conceivable that during this marked increase in the volume of the river, whatever dangerous pollution was in the water became markedly diluted—even allowing for what was washed from without the banks by the overflow—and was carried rapidly down the river. It is therefore reasonable to expect that with the beginning of the thaw and the rise in the river, there was a lessened concentration of dangerous pollution in the water. When the river subsided it may be readily conceived that in the reduced volume of water the pollution did not again become concentrated to the same extent as it had been before the thaw, because there was then less pollution to meet the reduced volume of water, the vast bulk of polluting matter having been washed away by the freshets.

Judged by the report of cases, the typhoid outbreak in Omaha rapidly declined from about March 25. Allowing for an incubation period of ten days or two weeks, this means that the chief cause of the outbreak became markedly diminished in its operation about March 10 or 15, which was coincident with the floodings of the river. The unusual condition of the river being followed so remarkably by an unusual occurrence of typhoid fever among persons drinking the water strongly suggests cause and effect.

(2) The reports showed that in other communities using water supplied from the Missouri River the typhoid fever rate was unusually high in the winter of 1909–10, while in communities neighboring these, but using water from other sources such as lakes, springs, wells, etc., the typhoid fever rate generally was not unusually high.

In the following table is summarized the information received from the different health departments of the several towns and cities:

TABLE No. 6.—*Typhoid fever in towns and cities in the vicinity of Omaha.*

Town or city.	Estimated population.	Source of water supply.	Month.	Typhoid fever reported—												Remarks.
				1905.		1906.		1907.		1908.		1909.		1910.		
				Cases.	Deaths.	Cases.	Deaths.	Cases.	Deaths.	Cases.	Deaths.	Cases.	Deaths.	Cases.	Deaths.	
Council Bluffs, Iowa	38,000	Missouri River	January	....	....	....	....	....	1	....	....	....	....	(?)	3	Water undergoes short storage and treatment with coagulant (alum). Intake for supply about three-fourths of a mile upstream from line on which is Burt street intake for Omaha's supply. Typhoid fever in Council Bluffs said by health officer to have been much more prevalent in winter of 1909–10 than in any other winter within last fifteen or twenty years.
			February	....	....	....	....	....	2	....	....	....	....	52	3	
			March	....	....	....	....	....	1	....	....	....	....	21	2	
			April	....	1	....	....	....	....	....	....	....	1	....	....	
			May	....	....	....	....	....	....	....	....	....	1	....	....	
			June	....	....	....	....	....	....	....	....	....	....	....	....	
			July	....	....	....	....	....	....	....	....	....	1	....	....	
			August	....	....	....	....	....	....	....	....	....	1	....	....	
			September	....	2	....	1	....	....	....	2	....	....	....	....	
			October	....	....	....	....	....	....	....	....	....	....	....	....	
			November	....	2	....	....	....	....	....	1	....	1	....	....	
			December	....	....	....	....	....	2	....	1	....	2	....	....	
Total				....	5	....	1	....	6	....	4	....	7	....	....	
Nebraska City, Nebr.	7,380	Missouri River		(a)	(a)	(a)	(a)	(a)	(a)	(a)	(a)	(a)	(a)	(a)	(a)	No record of cases and deaths for previous years, but Health Officer Dr. F. S. Marnell, in a letter dated March 31, 1910, stated as follows: "In the months of January, February, and March (1910) there were (in Nebraska City) 24 cases of typhoid fever, with 2 deaths. * * * This is the largest number of cases in any one year for the last six to my knowledge. No cases occurred after the ice went out of the river. The city water supply comes from the (Missouri).

																	River, and while no definite investigations were made 1 case occurred in a family where all but the one taken sick drank boiled water."
Kansas City, Mo.	340,000	do	January	(a)	(a)	3	2	5	4	6	2	15	1	138	17	Typhoid fever evidently was unusually highly prevalent in January, February, and March, 1910.
			February	(a)	(a)	7	1	6	3	9	4	26	2	112	21	
			March	(a)	(a)	6	2	13	1	10	2	25	2	52	12	
			April	(a)	(a)	7	3	6	4	7	4	15	1	....	....	
			May	(a)	(a)	....	....	6	3	8	....	9	1	....	....	
			June	(a)	(a)	4	....	4	4	2	....	8	2	....	....	
			July	(a)	(a)	8	6	....	7	8	2	6	3	....	....	
			August	(a)	(a)	19	6	9	6	7	1	14	6	....	....	
			September	(a)	(a)	41	5	19	7	14	1	24	4	....	....	
			October	(a)	(a)	21	5	13	3	3	1	32	2	....	....	
			November	(a)	(a)	15	1	17	5	20	2	32	3	....	....	
			December	(a)	(a)	5	3	4	4	5	1	24	3	....	....	
Total				....	....	136	34	102	51	99	20	230	30	....	....	
St. Joseph, Mo.	125,000	Missouri River	January	....	1	(a)	1	(a)	....	....	2	....	....	1	2	Water supply from river passed through sedimentation basins and mechanical filter; notwithstanding this treatment of the water typhoid fever appears to have been considerably more prevalent than usual for the winter season in the winter of 1909–10.
			February	....	1	(a)	....	(a)	....	....	....	....	....	1	1	
			March	....	....	(a)	1	(a)	2	....	1	....	....	1	2	
			April	....	1	(a)	1	(a)	2	....	1	2	2	....	....	
			May	....	....	(a)	....	(a)	1	....	....	2	2	....	....	
			June	....	....	(a)	1	(a)	2	....	1	1	1	....	....	
			July	....	....	(a)	1	(a)	1	9	1	....	....	....	....	
			August	....	2	(a)	....	(a)	1	9	3	2	....	....	....	
			September	....	2	(a)	2	(a)	1	4	4	....	1	....	....	
			October	....	2	(a)	3	(a)	1	2	1	1	2	....	....	
			November	....	2	(a)	3	(a)	2	1	....	1	4	....	....	
			December	....	....	(a)	1	(a)	1	2	....	1	2	....	....	
Total				....	11	....	14	....	14	27	14	10	14	....	....	

a No record.

TABLE NO. 6.—*Typhoid fever in towns and cities in the vicinty of Omaha*—Continued.

Town or city	Estimated population.	Source of water supply.	Typhoid fever reported— Month.	1905.		1906.		1907.		1908.		1909.		1910.		Remarks.
				Cases.	Deaths.	Cases.	Deaths.	Cases.	Deaths.	Cases.	Deaths.	Cases.	Deaths.	Cases.	Deaths.	
St. Louis, Mo.	735,000	Mississippi River, but a large proportion of the water in that part of the Mississippi River comes from the Missouri.	January	20	6	26	7	4	5	23	5	24	1	35	9	Water supply passed through large storage basins and treated with a coagulant (lime and sulphate of iron). This treatment is said to effect a very marked improvement in the water. It appears that typhoid in 1910 was somewhat more prevalent in January, February, and March than usual for that season.
			February	14	6	17	5	15	6	19	3	14	3	17	3	
			March	19	4	20	6	14	2	11	2	17	7	23	9	
			April	....	6	17	5	15	3	11	6	12	1	....	....	
			May	12	9	12	3	19	2	15	1	15	6	....	....	
			June	15	....	14	3	25	4	33	5	30	5	....	....	
			July	59	18	50	8	36	8	161	14	63	9	....	....	
			August	130	24	154	16	119	12	152	16	140	10	....	....	
			September	163	17	125	29	130	17	130	11	130	27	....	....	
			October	103	23	101	14	92	24	69	12	108	13	....	....	
			November	63	8	50	10	56	12	33	9	63	9	....	....	
			December	36	6	24	5	35	7	27	10	45	10	....	....	
Total				634	127	610	111	560	102	684	94	661	101	....	....	
Sioux City, Iowa	50,000	Driven wells		(a)	(a)	(a)	(a)	(a)	(a)	(a)	(a)	(a)	(a)	(a)	(a)	No water from the Missouri River used; water supply for twenty years or more practically all from driven wells. According to information from the mayor there occurred in the city within the six months ending April 1, 1910, only 2 cases of typhoid fever, and within the year ending on that date not more than a dozen cases.

Lincoln, Nebr.	**65,000**	**Artesian wells**	January	....	....	1	....	....	1	....	....	....	....	....	....	**Water supply practically all from artesian wells; very few private wells in use in recent years. The low typhoid rate, particularly for January, February, and March, 1910, is striking.**
			February	....	....	....	....	1	....	1	....	1	....	....	....	
			March	....	....	2	....	....	....	....	....	2	2	1	....	
			April	....	....	1	1	....	....	....	....	....	....	....	....	
			May	....	....	3	....	....	....	1	....	....	....	....	....	
			June	....	....	....	1	....	1	....	1	....	....	....	....	
			July	....	....	2	....	2	....	....	1	....	....	....	....	
			August	4	1	....	....	2	4	....	1	....	1	....	....	
			September	....	5	4	2	4	4	5	1	4	....	....	....	
			October	13	2	....	....	8	....	2	....	1	....	....	....	
			November	2	1	1	....	1	....	....	....	3	1	....	....	
			December	1	....	....	3	....	....	....	....	2	....	....	....	
Total				20	9	14	7	18	10	9	4	13	4	....	....	
Benson, Nebr	1,500	Artesian wells		(b)	(b)	(b)	(b)	(b)	(b)	(b)	(b)	(b)	(b)	(b)	(b)	This town is practically continuous with the northwest part of Omaha. According to information from local physicians, no cases of typhoid fever developed among the residents of Benson during the four months ending April 1, 1910. In April 2 cases occurred, both in persons who made frequent visits to Omaha.

[a] Records incomplete.

[b] No record.

From the data in this table it appears that the disease in the winter of 1909–10 had an especially high prevalence in those towns and cities which used the insufficiently purified Missouri River water, but not, so far as could be ascertained, in those which used other water. This fact alone points strongly to the Missouri River as the source of the infection which caused the outbreak in Omaha, and suggests that during the winter of 1909–10 the germs of typhoid fever existed in the water of the Missouri River for a considerable part of its course either in unusually large numbers or with an unusually high degree of infectiveness.

That the past winter was an unusually favorable one for typhoid infection in river waters somewhat generally is suggested by the fact that in the extensive territory to the north and east of Omaha a number of cities using water supplies from rivers other than the Missouri had pronounced outbreaks—Minneapolis,[a] Minn., using water from the Mississippi River, and Montreal,[b] Canada, using water from the St. Lawrence River, being striking examples.

CONCLUSIONS.

Prevalence.—In the period from November 25, 1909, to March 25, 1910, typhoid fever prevailed in Omaha in epidemic form. The rate of occurrence during this period was 1 case to about every 225 inhabitants, and, so far as the records show, was over six times as high as the average rate for corresponding periods of previous years.

Age.—The disease was distributed quite generally through the population. Persons between 10 and 30 years of age furnished the majority of the cases. The disease was not especially prevalent among children.

Geographical distribution.—The disease was generally distributed over the city. The rate of prevalence was, so far as could be ascertained, somewhat higher among persons whose residences were connected with the city water, supplied from the Missouri River, than among those whose residences were supplied from other sources, and was particularly high among persons habitually exposed to conditions in an eastern section of the city in which practically all of the water from the Burt street station was distributed.

Diagnosis.—The evidence is convincing that at least 85 or 90 per cent of the cases reported as typhoid fever during the outbreak were correctly reported as such, and that the number of actual cases not reported was fully as large as the number reported as typhoid fever under erroneous diagnoses.

Imported cases.—Not over 5 per cent of the persons affected contracted the infection while away from the city of Omaha. This

[a] Engineering News, 1910, vol. 63, No. 14, April 7, p. 392. [b] Loc. cit.

number was probably more than offset by the number of cases in persons who contracted the infection in Omaha and developed the disease elsewhere.

Ice.—Very few of the persons affected during the period of the outbreak gave a history of having used ice in foods or beverages during the thirty days prior to onset of illness, and ice may therefore be eliminated as having been a considerable factor in the production of the outbreak. But as a considerable proportion of the ice to be used in Omaha during the summer is that which was harvested from the river and other polluted water, this ice may become, upon the advent of warm weather and the consequent increased use of ice in foods and beverages, an important source of infection.

Milk and ice cream.—The evidence is convincing that milk, ice cream, and other dairy products were not a considerable factor in the production of the outbreak.

Raw shellfish.—As only a small proportion of the cases gave a history of having eaten raw oysters or clams within the thirty days prior to onset of illness, these shellfish could have played but a small part, if any, in the spread of the infection.

Raw vegetables.—The evidence is convincing that neither lettuce nor celery could have played a major part in the production of the outbreak.

Bakery products and other general food supplies.—In view of all the evidence, it is not reasonable to believe that bakery products and other general food supplies played more than a secondary and relatively small part in the production of the outbreak.

Dust.—In view of the fact that the ground at Omaha was covered with snow during the greater part of the period in which the outbreak was caused and in view of the general epidemiologic features of the outbreak, it is not reasonably conceivable that such an outbreak could have been caused by infection disseminated in air-borne dust.

Sewage disposal and general sanitary conditions.—As the majority of the cases investigated were found to be among persons living at residences connected with the city sewerage system and at which the general sanitary conditions were good or fairly good, it appears that whatever factors were concerned in the production of the outbreak were not dependent for their operation upon insanitary conditions at place of residence of persons affected.

Contact.—About 13 per cent of the cases investigated gave a history of association during the thirty days prior to onset of illness with previous cases in the febrile stage of the disease and were attributable to infection by personal contact. It appears, therefore, that in Omaha, as is generally true for other places where typhoid fever prevails, personal contact was an important factor in the spread of the infection, but the evidence is abundant and convincing that in this

outbreak personal contact operated as a factor secondary to some other factor which was chief and primary.

Water.—The unusually high rate of prevalence—or outbreak—of typhoid fever in Omaha during the period extending from about November 25, 1909, to about March 25, 1910, was beyond reasonable doubt caused by infection in the water supply obtained from the Missouri River. Some of the points in the evidence on which this conclusion is based are as follows:

(*a*) The river water obtained at both intakes was polluted to a dangerous extent with sewage.

(*b*) The results of the bacteriologic examinations show that during the period in which the outbreak was caused the treatment of the water, previous to its distribution to the city, by storage and by the use of a coagulant was not efficient to render this water reasonably free from dangerous pollution.

(*c*) The vast majority, over 95 per cent of the 103 cases particularly investigated, were in persons who during the thirty days prior to onset of illness used as the sole, principal, or occasional source of water for drinking purposes the unboiled and unfiltered tap water as supplied from the river through the city water system, and besides this water there was no factor common to the majority of the cases which could reasonably be considered as having been concerned in the production of the disease.

(*d*) There was a parallelism between the occurrence of certain unusual climatic conditions which particularly affected the river water and the unusual prevalence of typhoid fever, which very strongly suggests a relationship of cause and effect.

(*e*) Reports from a number of other cities for the period in which the outbreak at Omaha occurred showed that in those cities which were using water from the Missouri River the typhoid fever rates generally were unusually high, while in cities neighboring these but using water from other sources such as wells, springs, lakes, etc., the typhoid fever rates generally were not unusually high.

(*f*) Among persons who habitually used for drinking the water distributed from the Burt street station—which water in the winter of 1909–10 was exposed to greater sewage pollution than it had been in any other winter for certainly many previous years and to relatively more sewage pollution from near-by sources than was the water distributed from the Florence intake—the disease appeared to prevail at a rate which was disproportionately high.

(*g*) The time of occurrence and the extent of the outbreak point to the water supply as the source of the infection.

(*h*) The results of the investigation eliminate, beyond reasonable doubt, all possible sources of infection other than the water supply which could have been responsible for an outbreak of such character.

RECOMMENDATIONS.

1. *The improvement of the water supply obtained from the Missouri River.*—Of the measures required to make this water reasonably free from dangerous pollution, the following are indicated:

(*a*) The abandonment of the intake at the Burt street station as soon as practicable. This is particularly advisable, even if the water supply is to be subjected to purification processes much more efficient than those which have been and are now in operation, because the water at this intake is polluted not only with sewage in the river from more distant sources, but also with the sewage from Florence and from a northwest section of the city of Omaha, having a population of several thousand, and at times of high water with the contents of privies located within a few hundred feet of the intake. There is also contamination of the water at this point by drainage from the dumping ground of Council Bluffs, Iowa, on the east side of the river. None of the processes usually adopted as practicable for the improvement on a large scale of polluted surface river water should be relied upon to remove absolutely all disease-producing organisms. Therefore the water to be treated should be protected as thoroughly as practicable from pollution, particularly near-by pollution, with sewage. If it is not practicable to abandon the intake at the Burt street station, measures should be taken to prevent dangerous pollution of the river between the Burt street and the Florence intakes. Among the measures necessary to accomplish this would be the changing of the course of the Omaha and the Florence sewers so that their sewage would not empty into the river above the Burt street intake.

(*b*) The protection of the Florence intake from pollution entering the river through Mill Creek. This could be accomplished either by moving the intake to a point above the mouth of the creek or by changing the course of the creek so that it would empty below the intake. If it is not feasible at present to secure protection of the water at the Florence intake against the contents of Mill Creek by changing the present relative positions of the creek's mouth and the intake, a considerable safeguard could be accomplished by proper disposal of the sewage on the watershed of the creek for 2 or 3 miles upstream, so that the contents of privies and cesspools would not empty or drain into the creek.

(*c*) The treatment of the water supply by some purification process or processes which will render it free from dangerous pollution. Judging from an inspection of the watershed on each side of the river for a distance of about 10 miles above Florence and from reports as to amounts of sewage entering the river at points farther north, it appears probable that water taken from the Missouri River at any point between 200 and 1,000 feet north of (or upstream from) the

present mouth of Mill Creek would average little, if any, higher in content of disease-producing organisms than would the water taken from the river at any points north of Omaha and south of Sioux City. The self-purification of a river of the character of and being polluted as is the Missouri should not be depended upon. This is particularly true in the time of thaws, when floating ice may carry for long distances organisms, which when so carried are not exposed to the same conditions of sedimentation, etc., as when free in the flowing water. Therefore if the Missouri River is to be continued as the source of water supply for the city of Omaha, this water should be treated in such a way as to make it at all times wholesome and safe before it is delivered to the city.

In this connection the contemplated application to the water of the hypochlorite of lime treatment is to be commended as a step in the right direction. If it be found that the hypochlorite treatment will not effect an improvement to a point which may reasonably be considered one of safety, other processes of purification in the place of or in addition to the hypochlorite treatment should be applied. The efficiency of the hypochlorite treatment should be determined by a thorough bacteriologic study of the water before and after treatment.

The other processes recommended for consideration are (1) increased storage by installation of additional sedimentation reservoirs and (2) filtration.

What processes are best suited to meet the local conditions can be definitely determined only by experimentation. It is suggested that steps be taken as soon as possible to have such experiments conducted under the supervision of persons skilled in engineering and in the bacteriologic and chemical examination of water. On general principles and from such detailed study as has been made, a combination of the following processes for the improvement of the water supply of Omaha is recommended for particular consideration:

(1) Protection, as thorough as may be practicable, of the watershed of the Missouri River against sewage pollution from sources—particularly near-by sources—upstream from the intakes.

(2) Installation of additional storage reservoirs.

(3) Use of adequate amount of coagulant.

(4) Mechanical filtration.

(5) Treatment of effluent from filters with hypochlorite of lime.

(*d*) Until some method or combination of methods of demonstrated efficiency for the purification of the water supply is in operation, the boiling by the people generally of all river water to be used for drinking purposes, or in any other way liable to result in swallowing by persons of organisms contained in the water. The boiling of the water for use in public schools and other public institutions is particularly advisable.

2. *Ice.*—The adoption of measures which will prevent, so far as practicable, the use of natural ice collected from the Missouri River or other polluted sources in drinking water or any foods or beverages subsequently to be consumed without cooking.

3. *Cesspools and privies.*—The immediate abolishment of all faulty cesspools and privies. All cesspools and privies should be abolished as rapidly as the extension of the sewerage system will permit. On premises not having sewer connections, cesspools which are not known to be water-tight, and which are not so maintained as to obviate any reasonable likelihood of leakage of contents occurring, with resulting pollution of surroundings, should be replaced with privies having water-tight tubs, pails, or boxes, placed above ground, for the reception of sewage. The privies should be screened so that flies will be prevented from having access to the contents. The tubs or boxes should be emptied before they become more than two-thirds full of sewage. The use of an efficient germicide, such as a solution of carbolic acid or chloride of lime, so as to keep the privy contents disinfected, is advisable at all times, and particularly in the summer time. The disposal of the privy contents through septic tanks connected with the sewerage system is suggested. All cesspools, whether to be maintained or abolished, should be disinfected as thoroughly as possible. This is particularly indicated for those which have received the dejecta from typhoid-fever patients.

4. *The exercise of rigid precautions at the bedside to prevent the spread of infection from typhoid-fever patients.*—As soon as a case is reported a representative of the health office should visit the residence of the patient and make an investigation to determine if possible how the infection was contracted and to see that the proper precautions are being exercised to prevent the spread of infection from the patient. The employment of visiting nurses to aid in the carrying out of precautionary measures at homes of typhoid-fever patients is strongly advised. Provision should be made for the free distribution of disinfectants to families unable to purchase them.

5. *The improving of general sanitary conditions and the exercise of as rigid sanitary supervision as possible over all places where foods or beverages are prepared for sale or offered for sale.*—These general sanitary measures are, of course, always advisable, but they are particularly so in Omaha at the present time, when the city has just passed through an extensive outbreak of typhoid fever and has, in consequence, an unusually large number of foci of infection in typhoid-fever patients and probably in typhoid-bacillus carriers, and with the warm weather season approaching, when the conditions are usually most favorable for the spread of prosodemic typhoid fever.

APPENDIX.

An ordinance relating to the water supply of Omaha, passed April 26, 1910, and approved April 28, 1910:

ORDINANCE No. 7113.

An ordinance providing for the preservation of the public health of the residents of the city of Omaha, to prevent disease, to guard against epidemics of disease, to fix a standard of purity and quality of water brought in the city through pipes or other means and delivered, sold, or distributed to the residents thereof to be used for drinking, household, or domestic purposes, and of all water so used or intended to be used in said city.

Be it ordained by the city council of the city of Omaha:

SECTION 1. That on and after thirty days from the passage and approval of this ordinance all water furnished or offered for sale or sold in the city of Omaha for drinking, household, or domestic uses and pusposes and all water brought into the city through pipes or otherwise for such purposes and uses and so offered for sale, delivery, or distribution by any person, firm, company, or corporation shall be of the standard of purity, both chemical and bacteriological, as follows: It shall not contain more than one hundred bacteria per cubic centimeter, examined according to the standard methods adopted by the American Public Health Association, and shall exhibit fermentation in not more than thirty per cent examined when ten cubic centimeters or less of water is used in such examinations, standard lactose bouillon being the medium used.

The chemical water factors must never exceed the following proportions:

	Parts per million.
Total solids	500.00
Free ammonia	.06
Albuminoid ammonia	.08
Oxygen absorbed in ten minutes at 100° C	3.00
Nitrogen as nitrites	None.

Provided, That after fifteen months from the approval of this ordinance all water shall be of the standard of purity, both chemical and bacteriological, as follows, to wit: It shall be free from any gas-producing bacteria, colon bacilli, or other pathogenic bacteria. The number of bacilli must not exceed fifty per cubic centimeter. The chemical water factors must never exceed the following proportions:

	Parts per million.
Total solids	475.00
Free ammonia	.04
Albuminoid ammonia	.05
Oxygen absorbed in ten minutes at 100° C	2.00
Nitrogen as nitrites	None.

And water must be clear without suspended matter.

No person, firm, company, or corporation shall hereafter furnish for sale, or sell, deliver, offer to deliver, or distribute in the city of Omaha any water or waters for drinking, household, or domestic purposes that is not of the standard of purity, both chemical and bacteriological, hereinabove required.

SEC. 2. The commissioner of health of the city of Omaha shall have the right to inspect any and all reservoirs, tanks, or other receptacles from which water is furnished to the residents of the city and to inspect any and all intakes, pumping stations, water plants, and all bodies of water from which any portion of the supply of water for the city is obtained for the purpose of making such tests and examinations as he may deem necessary for the protection of the public health; and he shall require a bacteriological and chemical test of the water from any water plant or system and all reservoirs from which water is furnished, sold, or distributed to the residents of the city at least once each month, and such report in detail shall be submitted to the mayor and city council. And the commissioner of health may cause to be made whenever he may see fit an examination of any and all water from any source sold, delivered, furnished, used, existing, or kept within the city for drinking or other domestic purposes, including the water from any well within the city, to determine the standard of purity of such water.

SEC. 3. For all purposes and in any suit in court where the provisions of this ordinance or the quality of the water provided for herein may be material or involved, whether on account of violations of the provisions of this ordinance or otherwise, all tests made by the bacteriologist or chemist appointed by the commissioner of health of the city of Omaha of water furnished, sold, distributed, or offered for sale to consumers within the city by any firm, person, company, or corporation, when certified to or supported by the oath of such bacteriologist or chemist employed, appointed, or otherwise authorized by the commissioner of health shall be deemed and accepted as prima facie correct, and all instruments used by said bacteriologist or chemist in making such tests, where such tests are made, shall for all such purposes be deemed and held to be prima facie correct.

SEC. 4. If any person, firm, company, or corporation shall furnish to consumers within the city of Omaha for drinking or other domestic or household purposes water below the standard of purity required by this ordinance such person, firm, company, or corporation shall forfeit and pay to the city of Omaha for such violation of this ordinance the sum of one thousand dollars ($1,000) per week for each and every week during which such person, firm, company, or corporation shall violate the provisions of this ordinance by furnishing to the residents of the city of Omaha and selling or offering for sale and distribution for drinking or other household or domestic purposes water below the standard of purity herein required, and said amount may be recovered in a civil action in any court at law having jurisdiction thereof: *Provided*, That the city of Omaha shall, through its mayor and council, deduct from any amount due to any firm, person, company, or corporation engaged in supplying water to the residents of the city of Omaha in said city an amount equal to all sums which by the terms of this ordinance have been forfeited to the city by reason of a violation of the terms and provisions hereof; and before any appropriation shall be made by the mayor and council in favor of any such person, firm, company, or corporation for any purpose the amount so forfeited to the city under the provisions of this ordinance shall be deducted by the comptroller from the amount due any such person, firm, company, or corporation under contract or otherwise.

SEC. 5. In case any person, firm, company, or corporation shall, after receiving notice of the result of any test as provided for herein, continue to furnish, sell, distribute, or offer for sale and distribution to the residents of the city of Omaha water below the standard of purity fixed by this ordinance such person, firm, company, or corporation and the managing officers and agents thereof shall be deemed guilty of a misdemeanor and upon conviction thereof shall be punished by a fine of not more

than one hundred dollars ($100) or by imprisonment for a term not exceeding ninety days in jail, and each day that such person, firm, company, or corporation shall so continue to furnish, sell, or distribute to the residents of the city of Omaha for drinking, household, or other domestic purposes water below the standard of purity fixed by the terms of this ordinance shall be deemed a separate offense and punishable as such.

SEC. 6. This ordinance shall take effect and be in force from and after its passage.

LOUIS BURMESTER,
President City Council.
DAN B. BUTLER,
City Clerk.

Passed April 26, 1910; approved April 28, 1910.

JAMES C. DAHLMAN,
Mayor.

II. THE WATER SUPPLY OF WILLIAMSON, W. VA., AND ITS RELATION TO AN EPIDEMIC OF TYPHOID FEVER.

II. THE WATER SUPPLY OF WILLIAMSON, W. VA., AND ITS RELATION TO AN EPIDEMIC OF TYPHOID FEVER.[a]

By W. H. Frost,

Passed Assistant Surgeon, United States Public Health and Marine-Hospital Service.

INTRODUCTION.

The excessive prevalence of typhoid fever in the town of Williamson, W. Va., during the fall and winter of 1909–10 aroused the citizens of the town to a realization of the ever increasing danger from their present water supply, which is derived from the Tug Fork Branch of the Big Sandy River. Accordingly a bond issue was voted for improvement of the public water supply and other municipal improvements; and a request made through the West Virginia state board of health to the Surgeon-General of the Public Health and Marine-Hospital Service to detail an officer for the purpose of making an investigation and giving advice as to the best means of so purifying the town's water supply as to prevent typhoid fever.

In response to this request the writer was directed to proceed to Williamson, confer with the representatives of the state board of health and make such investigation as might be required.

On arrival at Williamson a conference was held with Dr. H. A. Barbee, secretary and executive officer of the West Virginia state board of health, and Dr. Tunis Nunemaker, health officer of Williamson, and a brief preliminary survey of the situation made in their company.

It was ascertained that the town had suffered during the winter of 1909–10 from a serious outbreak of typhoid fever, which was generally ascribed to the use of water from Tug River, the pollution of this stream being so gross that its danger as a source of drinking water was evident to the most casual observer. Even a brief survey of the town, however, showed conditions other than pollution of the water supply which must necessarily be favorable to the spread of typhoid fever, and it was evident that for a successful campaign against typhoid fever these factors must be appreciated, the experience of many cities, especially in the southern section of the United

[a] Manuscript submitted for publication August 19, 1910.

States, having shown that the reduction in the typhoid rate following an improvement in water supply without a coincident campaign against other factors in the spread of the disease is often, if not usually, disappointing It was determined, therefore, while the laboratory investigation of the water supply was in progress, to devote such time as could be spared from this work to a field study of typhoid fever in Williamson, to determine its prevalence and causes.

The investigation, begun May 27, 1910, and continued until June 18, included:

(1) A study of sanitary conditions in general at Williamson.

(2) A study of the water supply of the town and of Tug River, with the object of ascertaining the degree of pollution and the available means of purification.

(3) The collection of data concerning the cases of typhoid fever occurring in Williamson since November 1, 1909.

(4) An investigation of the acute diarrhea, locally prevalent.

The necessary laboratory outfit was supplied from the Hygienic Laboratory; and a temporary laboratory established in Williamson.

Upon completion of the investigation a verbal report was made to the town council and board of health, and a written report with recommendations, forwarded to the Surgeon-General, Public Health and Marine-Hospital Service, embodying in slightly briefer form the substance of this report.

It is a pleasure to acknowledge my indebtness for cooperation and assistance in this investigation, to Dr. H. A. Barbee, secretary and executive officer of the state board of health, to Dr. Tunis Nunemaker, health officer of Williamson, to Mr. Alonzo Pinson, mayor of Williamson, to the members of the town council and board of health, and to many other citizens of Williamson. I am especially indebted to Doctor Nunemaker for the devotion of much of his time to assisting in the investigation. The courtesy of the medical profession of Williamson and vicinity in supplying information and opportunities for clinical studies is gratefully acknowledged. To the Norfolk and Western Railway Company, especially Mr. W. H. Lewis, superintendent of motive power, Mr. James H. Gibbony, chief chemist, and Mr. D. G. Cunningham, superintendent of machinery and repairs at the Williamson yards, I am indebted for their cooperation and for information which would not have been available from other sources.

TOPOGRAPHY AND GENERAL SANITARY CONDITIONS.

Williamson, the county seat of Mingo County, W. Va., is situated on the east bank of the Tug Fork of the Big Sandy River, on the southwestern border of the State. It is a thriving town whose present population is locally estimated at between 4,000 and 5,000

inhabitants. Its growth has been very rapid, the population in 1900 having been only about 600.

The town is situated on the main line of the Norfolk and Western Railway, and is the terminus of a division of this road. The Norfolk and Western Railway Company has here a large yard, which furnishes employment to a considerable proportion of the population. East Williamson, the section of the town adjacent to the Norfolk and Western Railway Company's yard, is made up almost entirely of this company's employees and their families.

Although situated in a productive portion of the Tug River coal fields, Williamson is not properly a mining town; there are, however, two coal mines in operation within the town limits, both situated upon a small stream which empties into Tug River below East Williamson, and just above the main portion of the town. The population on the upper portion of this branch is largely composed of employees of the larger of these two mines above mentioned, operated by the Williamson Coal Company.

Williamson is fairly distinctly divided into three sections. The larger portion of the town, comprising the business section and the better residences, is situated below the Williamson Branch, on a tract of flat ground, rising on all sides in steep slopes, forming a kind of amphitheater. The Williamson Branch section of the town, a narrow row of houses along the banks of this small stream, is separated from the main portion of the town by a high ridge. At the head of this branch is the Williamson Coal Company's mine and a settlement of employees and their families. East Williamson, separated from this section by a second ridge, extends east, parallel to the river, from which it is separated by the Norfolk and Western Railway Company's yard. Williamson proper and East Williamson are shown on the accompanying map. The settlement at the Williamson Coal Company's mine is not shown on this map, being located above the upper margin.

The town is at present only partially provided with a closed sewer system, consisting of a 41-inch brick main, and smaller branch sewers of tiling. This system, constructed in 1907, and gradually extended since that time, connects now only with the main portion of the town, and some sections of even this portion of the town are still without sewer connections, notably some outlying blocks and many of the houses which are situated directly upon the river bank. The main sewer terminates in an open ravine about 100 yards distant from the river, and through this ravine empties into Tug River about opposite the center of the town below the intake for the water supply. There are several criticisms to be made of this sewer system. It is not sufficiently extensive. It is in many places in bad repair. It is designed as a combined system, but is of insufficient capacity to carry

off the large volume of surface water following a heavy rain, with the result that flooding is by no means infrequent. The termination of the closed sewer in an open ravine more than 100 yards from the river is highly objectionable.

The Williamson Branch section of the town has no closed sewer system. Some of the houses on the lower portion of this branch have sewers which empty directly into the stream. Their openings are often high in the bed of the stream, far above the average water level. The remainder of the houses in this section are provided with open privies of the most insanitary kind as regards construction, location, and care. The privies are practically all within 100 yards of the stream and within less than that distance of their respective houses. They are almost invariably situated above the houses to which they belong, often on sharply sloping ground. Every rain must wash a large portion of the contents of these privies through the yards of the residences and into the stream, which thus becomes an open sewer. This stream, usually of small volume, in its passage down to the river several times crosses the street, is at all times in close proximity to the houses along this street, and often passes through yards within a few feet of residences or even directly under them. In dry seasons the stream goes dry, at which times the volume of sewage being insufficient to flow through the bed of the stream must accumulate there. This stream empties into Tug River about 200 yards above the present intake for the town water supply and upon the same side of the river. The water of Williamson Branch, being extremely black from coal washings, forms a marked contrast with the water of Tug River at times when the latter is laden with clay, and at such times it can readily be observed that the water from Williamson Branch passes over the town's intake before becoming thoroughly mixed with the water of the river.

The third section of the town, East Williamson, with the exception of the large Y. M. C. A. building, has no sewers. The houses in this section of the town are provided with open privies, very rarely with cesspools. The privies are invariably situated within a few yards of the residences—often less than 20 feet from a kitchen—and in a large proportion of cases are on a hillside above the houses. They are open privies, badly cared for, and at this season swarming with flies. The Y. M. C. A. building in East Williamson, which receives the sewage from 100 to 200 persons, has a private sewer emptying into the river above the opening of the Williamson Branch. The surface washings of the whole of East Williamson are carried into the river within a mile above the intake for the city water supply.

Upon request, the board of health of Williamson authorized a sanitary survey of the town under the direction of the health officer. This survey, owing to several unfavorable circumstances, had not

been completed at the time of my departure, but from a compilation of the inspection reports from ten representative blocks of the main portion of the town, the following figures were obtained:

	Number.
Residences inspected	105
Residences having water-closets	79
Residences having open privies	33
Residences in good sanitary condition	33
Residences in fair sanitary condition	40
Residences in bad sanitary condition	32
Occupants in above residences	544
Occupants using city water for drinking	367
Occupants using well water for drinking	130
Occupants using safe (deep well, boiled, distilled) water for drinking	47

These blocks include some of the poorest as well as some of the best of the main section of the town. All of these premises are within a short distance of sewers, yet nearly one-third have open privies. Almost one-third of the residences are reported as in bad sanitary condition, while more than a third are reported as in "fair" condition. Taking into consideration the fact, omitted in these reports, that premises otherwise in the best of sanitary condition are often rendered dangerously insanitary by proximity to other premises in bad condition, it is safe to assume that fully one-half of these residences are, by reason of neglect or location, in a bad, really dangerous sanitary condition.

WATER SUPPLY.

The water supply of Williamson is obtained from Tug River. Water is pumped from the river to all sections of the town. East Williamson, however, has been thus supplied only since the summer of 1909.

The Tug Fork branch of the Big Sandy River rises in the eastern part of McDowell County, W. Va., and, flowing in a general northwest direction, joins the Louisa Fork at Louisa, below Williamson, to form the Big Sandy River. Above Williamson this river drains practically all McDowell County, W. Va., and the western portions of Wyoming and Mingo counties, W. Va., Tazewell and Buchanan counties, Va., and Pike County, Ky. This watershed is mountainous, with very steeply sloping ridges, generally wooded, for the most part sparsely inhabited, little cultivated, and but little used for grazing.

In the absence of any hydrographic data, no definite statement can be made as to the area of this water-shed and the volume and discharge of this river. It is evident, however, from the contour of the country and from local information that the volume of the river is subject to extreme fluctuations. Following heavy rains its rise is very rapid and extreme, while in dry seasons it is said to become so

small that one can cross it dry-shod at Williamson; and its tributary streams at such seasons are often completely dry. The flow of the river is very rapid. Except after heavy rainfalls its water is naturally fairly clear, but in this respect, as in volume, it shows great and sudden variations. The large amount of coal dust discharged into the stream throughout its course gives it a very dark color and adds considerably to its turbidity. A deposit of coal dust in the river bed adds to the apparent dark color of the water, giving it an extremely dirty and displeasing appearance.

The population on this watershed has increased so rapidly within the last ten years that no approximate estimate can be given of it. It is probable that the number of inhabitants per square mile is very small as compared with the watershed of many rivers whose waters are used for municipal supplies; but even if the population per square mile could be stated it would be misleading in an estimation of the pollution, owing to the concentration of population upon the banks of the river and some of its larger tributaries.

The Norfolk and Western Railroad follows Elkhorn Creek from Coaldale, W. Va., to the junction of this creek with Tug River at Welch, W. Va., whence the railroad follows the river closely to a point below Williamson. From Coaldale to Williamson (a distance of about 92 miles) the banks of the river are lined with an almost continuous chain of coal camps and mining towns, varying in population from a few score to 4,000 or 5,000. In these towns the population is almost invariably spread out along the banks of the river or one of its tributaries, probably 90 per cent of the population being within 200 yards of these streams. Welch, at the mouth of Elkhorn Creek, Matewan, at the mouth of Mate Creek (about 9 miles above Williamson) and very probably other towns along the river have closed sewers discharging into Tug River. In the smaller towns and coal camps, however, open privies are in general use. These are situated almost invariably near and often overhanging the stream, so that heavy rains must invariably wash a large part of their contents directly into the stream. Hogpens, mulepens, and stables are very commonly seen immediately upon the banks of the river. Add to this the garbage from hundreds of squalid back yards on the river banks and the refuse from scores of coal mines, all going into this small stream, and the result is a pollution peculiarly offensive to the eye and dangerous to health. Lewis, in a publication of the Geological Survey, in 1906,[a] says of this river:

> Tug Fork of the Big Sandy * * * probably carries more offensive pollution than any stream in West Virginia, which is saying a great deal.

[a] Lewis, Samuel James, Quality of water in the upper Ohio River Basin and at Erie, Pa., U. S. Geological Survey, Water Supply and Irrigation Paper, No. 161, 1906, p. 101.

Since 1906 the population on the banks of the river has increased very considerably, with a corresponding increase in the pollution of the stream.

Several factors add to the dangerous nature of the pollution of Tug River. The sudden rise and rapid run-off after rains wash into the river the filth which has accumulated upon its banks. On the other hand, the small volume of the river in dry seasons increases the concentration of such sewage as gets into it. Whether the degree of pollution is greater at high or at low stages of the river could be determined only by investigation, and would depend, of course, on various factors. The rapid flow of the river decreases the natural purification by sedimentation and diminishes the effect of unfavorable environment upon pathogenic organisms by decreasing the time during which they must be exposed to such environment. From such reports as were available, typhoid fever seems to be excessively prevalent along the river, and the insanitary condition of the residences certainly warrants the inference that careful disinfection of typhoid excreta is by no means universal.

The coal dust which finds its way into the stream and which adds so greatly to its appearance of pollution can not be considered as rendering the water more dangerous for drinking, although it certainly renders it less fit for other domestic purposes, and, by increasing the sulphates, less suitable for use in boilers.

The present intake for the water supply of Williamson, constructed in 1907, consists of a cement caisson, open at the top, 4 feet in depth, sunk below the bed of the river. This caisson is filled with coke, gravel, and sand to a depth of about 4 feet. The intake pipes, passing through this material, open at the bottom of the caisson. The suction of the pumps forces the water through the rough filter bed of the caisson into the intake pipes. When the filter becomes clogged it is cleaned by reversing the pumps. This has to be done at intervals varying from two to twelve hours. While this arrangement removes a considerable part of the coarser suspended matter from the water, it is an extremely crude and inefficient process of filtration, more properly speaking, only a "screening process."

This intake is situated about opposite the central portion of the town, above the outlet of the main sewer, but below the points at which the sewage from Williamson Branch and from East Williamson (aggregating about 1,500 people) enters the river. The water of Williamson Branch, as mentioned above, passes over the intake before being thoroughly diffused into the river, and is therefore especially dangerous.

From the pump house, situated on the bank of the river at the intake, water is distributed directly to the town. A reservoir of about 250,000 gallons capacity is situated beyond and above the

town; this reservoir, however, serves only to receive the surplus of water pumped over water used from the mains, and has no function as a distributing or storage reservoir, serving chiefly as a "safety valve" to equalize the pressure in the mains.

The water supplied is of very displeasing appearance, as is to be expected from the nature of its source and the crudity of the filtration process. It is frequently too black to be fit for bathing and laundry purposes, and often has an offensive odor. The water pipes apparently contain a considerable accumulation of sediment, as it frequently happens that there is a large amount of dirt and coal dust forced out when a tap is first turned on.

Owing to the dangerous and unpleasant character of the city water supply, a considerable proportion of the population of Williamson use other water for drinking purposes. A distilling plant in the town furnishes distilled water to the small number of people who are willing to bear the expense of such a supply. Comparatively little bottled water from distant springs is sold; a few people use water from nearby country springs, usually safe from dangerous pollution. The only deep well of importance in the vicinity furnishing water of good potable quality is one 330 feet deep at the Williamson Coal Company's mines, which supplies the employees at the mine and is piped to the houses in the immediate vicinity, where, however, it is, unfortunately, little used. The well at present in use failed to supply sufficient water during the dry weather of the fall of 1909. A new well 500 feet deep has been bored near by, but not yet put into use.

A very small proportion of people boil the river water before use. From a canvass of over 500 people it was ascertained that less than 10 per cent were at that time using water which might be considered assuredly safe, and it is certain that many of these people were not consistent in the practice.

The town has numerous shallow wells, which are almost without exception subject to gross pollution, but are nevertheless used by a considerable proportion of people on account of the clearness of the water. Probably 20 per cent of the population of the main portion of the town use water from shallow wells.

Water from shallow springs, subject to dangerous pollution, is largely used, especially by the people living on the Williamson Branch. So far as could be ascertained, the majority of the people in East Williamson have been, and still are, in the habit of using well water for drinking purposes. The wells in this section of the town are mostly pipe-lined driven wells, sufficiently deep to be probably safe from pollution.

The Norfolk and Western Railway Company, having been unable to obtain from driven wells sufficient water of suitable quality for

use in their boilers, have constructed an infiltration gallery opposite their roundhouse above Williamson. Perforated brass pipes are laid horizontally several feet below the bed of the river, covered with gravel and sand. The water obtained is fairly clear and is satisfactory for steam purposes, but by no means safe bacteriologically. The company, realizing the importance of a safe water supply, has recently installed a distilling plant to furnish drinking water to the employees in the yard, and allows the men to take home, without charge, sufficient of this water to supply their families.

EXAMINATIONS OF THE WATER SUPPLY.

Bacteriological examinations of water were made at Williamson from May 30 to June 15, 1910, as follows:

	Samples.
Tug River at Williamson intake	7
Water from taps in the town	9
Tug River above Pond Creek	10
Shallow wells	6
Springs	1
Deep wells	3
Infiltration gallery, Norfolk and Western yards	4
	40

Bacterial counts were made from standard agar plates incubated at room temperature from forty-eight to seventy-two hours. A few counts were made from plates incubated at 38° C., but results at this temperature proved less satisfactory than at room temperature.

Tests for *B. coli* were made mostly with lactose bile; gas formation in this medium within forty-eight hours was taken as presumptive evidence of the presence of *B. coli*. In seven instances these presumptive tests were confirmed by plating on lactose-litmus-agar or Endos's medium, obtaining in all these cases plate colonies characteristic of *B. coli*. In a few instances, owing to shortage in supply of lactose bile, lactose bouillon was used in the fermentation tests. In these cases gas formation within twenty-four hours was considered presumptive evidence of the presence of *B. coli*.

Additional quantitative estimations of *B. coli* were made by a plate method advocated by Marmann,[a] which, with slight modification, has been used with very satisfactory results in the Hygienic Laboratory. One cubic centimeter of water was spread upon a plate of Endos's fuchsinagar, the plate placed uncovered in the incubator until dry, then covered, incubated fourteen to twenty-four hours, and the red colonlike colonies counted. Control plates with sterile water showed no growth.

[a] Marmann Centralbl. f. Bakt. etc., I Abt., Orig., 1909, Bd. 50, pp. 267–282.

The following tables give in detail the results of turbidity readings and bacteriological examinations of water made at Williamson:

TABLE NO. 1.—*Water from Tug River above Williamson.*

Date.	Turbidity.	Bacteria per c. c. agar (48–72 hours).	B. coli (presumptive) present in—	
			0.1 c. c.	1 c. c.
1910.				
May 30	(a)	420	+	+
May 31	(a)	1,900	+	+
June 1	20	380	+	+
June 2	20	310	+	+
June 3	15	480	−	+
June 4	15	145	+	+
June 6	200	6,100	+	+
June 7	200	10,000	+	+
June 8	160	5,200	+	+
June 13	(?)	4,200	+	+

a Turbid.

TABLE NO. 2.—*Water from Tug River at Williamson intake.*

Date.	Turbidity.	Bacteria per c. c. agar (48–72 hours).	B. coli (presumptive) present in—	
			0.1 c. c.	1 c. c.
1910.				
May 30	(?)	1,450	+	+
June 2	20	1,075	+	+
June 4	20	3,600	+	+
June 6	130	9,200	+	+
June 7	200	10,000	+	+
June 10	50	3,000	+	+

TABLE NO. 3.—*Water from taps in Williamson.*

Date.	Turbidity.	Bacteria per c. c. agar (48–72 hours).	B. coli (presumptive) present in—	
			0.1 c. c.	1 c. c.
1910.				
May 30		530	+	+
June 1		600	+	+
June 2	20	480	+	+
June 3	18	1,200	+	+
June 4	10	1,170	+	+
June 6	15	690	+	+
June 7	25	1,400	+	+
June 8	35	1,600	+	+
June 10	10+	550	+	+

TABLE NO. 4.—*Water from pump (infiltration gallery).*

Date.	Turbidity.	Bacteria per c. c.	B. coli (presumptive) present in—	
			0.1 c. c.	1 c. c.
1910.				
June 1	8	150	−	+
June 3	10	100	−	+
June 6	18	1,800	+	+
June 13	(?)	710	+	+

TABLE NO. 5.—*Water from wells and springs in Williamson.*

Date.	Source.	Turbidity.	Bacteria per c. c.	B. coli (presumptive) present in—		
				0.1 c. c.	1 c. c.	10 c. c.
1910.						
May 31	230-foot driven well (W. C. C.)	Turbid..	34	.....	−	+
June 9	.....do	Clear....	40	.....	−	−
June 4	Driven well (J. K. A.)	...do.....	58	.....	+(?)	+(?)
June 3	Shallow dug well (J. R.)	...do.....	700	+	+	+
June 10	Shallow dug well, tile-lined (G. R. C.) [a]	...do.....	1,000	.....	+	+
June 14	Shallow well, tile-lined	...do.....	4	−	−	−
June 14	Surface spring	...do.....	80	−	−	+
June 15	Shallow well (W. C. C.) [b]	...do.....	(c)	.....	+	.....
June 15	Shallow well (A. B.) [d]	...do.....	(c)	.....	+	.....

[a] B. coli on Endo plate=20 per c. c.
[b] B. coli=25 per c. c.
[c] No count.
[d] B. coli=15 per c. c.

TABLE NO. 6.—*Summary of the results of the examinations of Tug River water.*

Source of samples.	Number of samples examined.	Average number of bacteria per c. c.	Percentage of samples showing B. coli in—			Number of B. coli per c. c. as estimated by plate method (Endo's medium).
			0.1 c. c.	1 c. c.	10 c. c.	
Tug River above Williamson	10	2,900	90	100	.....	.....
Tug River at Williamson intake	7	4,700	100	100	.....	[a] 50+
Taps in Williamson	9	895	100	100	.....	[a] 16
Tap at Norfolk and Western pump house	4	690	50	100	.....	.....

[a] Two samples.

From these figures it is seen—

(1) That Tug River, even above Williamson, is highly polluted, 90 per cent of 0.1 c. c. samples and 100 per cent of 1 c. c. samples showing the presence of *B. coli*, indicating an average number of at least 9 *B. coli* per c. c. for the samples examined.

2. That the water at the Williamson intake is very considerably more polluted than at a point above the town, showing a bacteria count almost twice as high, with *B. coli* constantly present in 0.1 c. c. Quantitative estimations of *B. coli* on two occasions indicated that 50 *B. coli* per c. c.—an extreme pollution—is not an excessive estimate.

3. That the present intake filter, though considerably decreasing the turbidity and removing a considerable proportion of bacteria from the water, is altogether inadequate as a means of purification.

4. That the Norfolk and Western Railway Company's well, situated below the bed of the river above Williamson, gives an effluent of considerably better quality, but that this water is still very far from being bacteriologically safe.

5. Of the six shallow wells examined, five were found to be grossly polluted; one showed no evidence of pollution. This is probably accounted for by the fact that the bottom of this well is in a very dense layer of clay, probably impervious, and that the well is tile lined to the bottom.

6. The two deep wells examined both showed doubtful evidence of slight pollution at the first examination, these wells having been stagnant for some time previous to the collection of the samples. A second sample from one of these wells proved to be of good quality according to bacteriological standards.

The one spring examined, situated on a hillside outside of the town showed *B. coli* in 10 c. c., not in 1 c. c. nor 0.1 c. c. This slight pollution was probably due to animal excreta, washed in by recent heavy rains.

The conditions at Williamson from May 30 to June 15, 1910, were unfavorable for obtaining an accurate idea of the variations in bacteriological quality of the water under various conditions. The river during this time was uniformly high and the weather moderately cool, so that no information could be obtained as to the effect of low water and high temperatures on the bacterial content of the water.

PHYSICAL AND CHEMICAL EXAMINATION OF TUG RIVER WATER.

From the limited observations which could be made on this point it is inferred that Tug River does not show the constant high turbidity characteristic of many southern and western streams. Following is a summary of the turbidity readings taken:

	Above Williamson.	At Williamson intake.	Taps in Williamson.
1910.			
June 1	20		
June 2	20	20	20
June 3	15		18
June 4	15	20	10
June 5			
June 6	200	130	15
June 7	200	200	25
June 8	160		35
Average	90	92.5	20.5
Maximum	200	200	35
Minimum	15	20	10

The occasional high turbidity, due to suspended clay, and the usual dark color, indicate the necessity of a coagulant to satisfactorily clarify the water.

Samples were collected June 1, 1910, and sent to the Hygienic Laboratory for chemical examination. Following is an abstract of the report of Passed Asst. Surg. Norman Roberts on the chemical examination of these samples:

	Sample from Tug River.	
	Above Williamson.	At Williamson intake.
Color	Very slight.	Very slight.
Odor	Decided, aromatic.	Faint, aromatic.
	Parts per million.	
Total solids (exclusive of sediment)	71,000	99,000
Loss on ignition	19,000	26,000
Nonvolatile residue	52,000	73,000
Chlorine	1,000	10,000
Nitrogen as—		
Free ammonia	.030	.018
Albuminoid ammonia	.058	.042
Nitrites	.001	.001
Nitrates	.400	.400

PREVALENCE OF TYPHOID FEVER.

There are no records from which information can be gathered as to the number of cases of typhoid fever in Williamson or deaths therefrom in previous years. I was informed that about 1900, when the population of the town was approximately 500, there was an epidemic of more than 60 cases of typhoid fever. This epidemic occurred in the summer and fall. The water supply at the time was from shallow wells and springs, and, it being prior to the construction of a sewerage system, privies were then in general use. The mortality of this epidemic is unknown.

Since 1900 there had been no epidemic of typhoid fever in Williamson until the winter of 1909–10. The disease had, however, been constantly endemic; and while no approximate estimate can be given of the average annual case rate, it must have been excessive. This is inferred from the knowledge that conditions have been favorable for its spread and measures for its prevention extremely lax. The inference is further justified by the large proportion of people in Williamson who give a history of having had typhoid fever, as well as by the general statements of local physicians and others. It is the impression of the physicians in Williamson that there is usually no marked seasonal variation in the prevalence of typhoid fever there, it being about as common at one season as at another.

Prior to January 1, 1910, no attempt had been made to have cases of typhoid fever or even deaths reported; in fact, for some two years prior to that time the town had been without a board of health or a health officer. Since January 1, 1910, physicians have been required to report to the health officer all cases of typhoid fever, but it was ascertained that less than half of the cases diagnosed by the attending physician as typhoid fever had been so reported, and that the death records were equally deficient.

The health officer had on his records reports of some 50 cases of typhoid fever occurring since January 1, 1910; but was certain that there had been many more than this, estimating the number as 125 since November, 1909, about which time typhoid fever commenced to be unusually prevalent.

A request was made of each physician in town to furnish a memorandum of all cases of typhoid fever seen by him since November 1, 1909, giving the address, sex, race, and age of patient, date of onset of symptoms, duration and termination of illness. From these reports, courteously and promptly supplied, it was ascertained that from November 1, 1909, to June 1, 1910, 152 cases had been diagnosed and treated as typhoid fever, with 11 deaths. Two other fatal cases, as to the diagnosis of which some doubt was expressed, may be considered as possibly typhoid fever.

DIAGNOSIS.

The low death rate (7.29 to 8.5 per cent according as deaths are estimated at 11 or 13) is more remarkable in view of the unfavorable circumstances under which many of the cases had to be treated. It was reported by all the local physicians that a considerable proportion of their cases were of mild type and short duration, some being confined to bed not more than two to three weeks. The low death rate and the number of mild cases suggest that a considerable proportion of the cases may have been paratyphoid infection, this suspicion being strengthened by the reported frequency of onset with gastro-intestinal disturbances. Unfortunately no bacteriological nor serological examinations of any of the cases had been possible. A few blood cultures and Widal tests were made during my stay in Williamson, and since that time Widal tests have been made with two specimens of blood sent to the Hygienic Laboratory by Doctor Nunemaker. In making blood cultures, 5 c. c. of blood, withdrawn from a vein of the forearm, were planted in ox bile, incubated twenty-four hours, at 38° C., then plated out on lactose litmus agar. The organisms isolated have been fully identified at the Hygienic Laboratory by cultural and agglutinating tests.

TABLE NO. 7.—*Summary of the results of blood cultures and agglutination tests.*

Patient.	Age.	Duration of illness when specimen was taken.	Result of blood culture.	Agglutination.	Clinical course.
J. E.	50	Second week..	B. typhosus..	Positive 1-40 B. typhosus.	Mild, convalescent within 3 weeks.
A. B.	5	First week....	None made...	.....do.....	Do.
L. S.	26	.....do.....	B. paratyphosus "A."	Negative B. typhosus, 1-40.	Mild, defervescent within 3 weeks.
L. J.	10	.....do.....	Negative.....	.....do.....	Mild, short duration.
F. J.	(a)	Fourth week..	None made...	Positive B. typhosus, 1-40.	Severe hemorrhage.
McC.	(a)	.....	.....do.....	.....do.....	Unknown.

a Adult.

Four of the above cases may be considered as satisfactorily demonstrated to be typhoid fever and one paratyphoid, A. In the sixth case (L. J.), where the tests made were negative, the diagnosis is doubtful, but the clinical diagnosis of typhoid fever is justified. It is to be regretted that more clinical and bacteriological studies could not be made, but from the above it is evident that the majority of the cases prevalent in May were true typhoid infections, while paratyphoid infection was also present.

While it is probable that in this, as in all epidemics, there was a certain percentage of error in the clinical diagnosis of typhoid fever, there is no reason to suppose that this error was excessive or more than would be counterbalanced by mild, unrecognized cases.

EXTENT AND DISTRIBUTION OF EPIDEMIC.

The 152 cases of typhoid fever occurring in a population of 5,000 are equivalent to a case rate of 3,040 per 100,000, or one case among every 33 people. An epidemic of only 152 cases can hardly be called extensive; the figures, in fact, are very small as compared, for instance, to the number of cases occurring annually in many large cities where the disease is not epidemic. Considering, however, that one out of every 33 inhabitants of the town was attacked within seven months, the epidemic may well be called intensive.

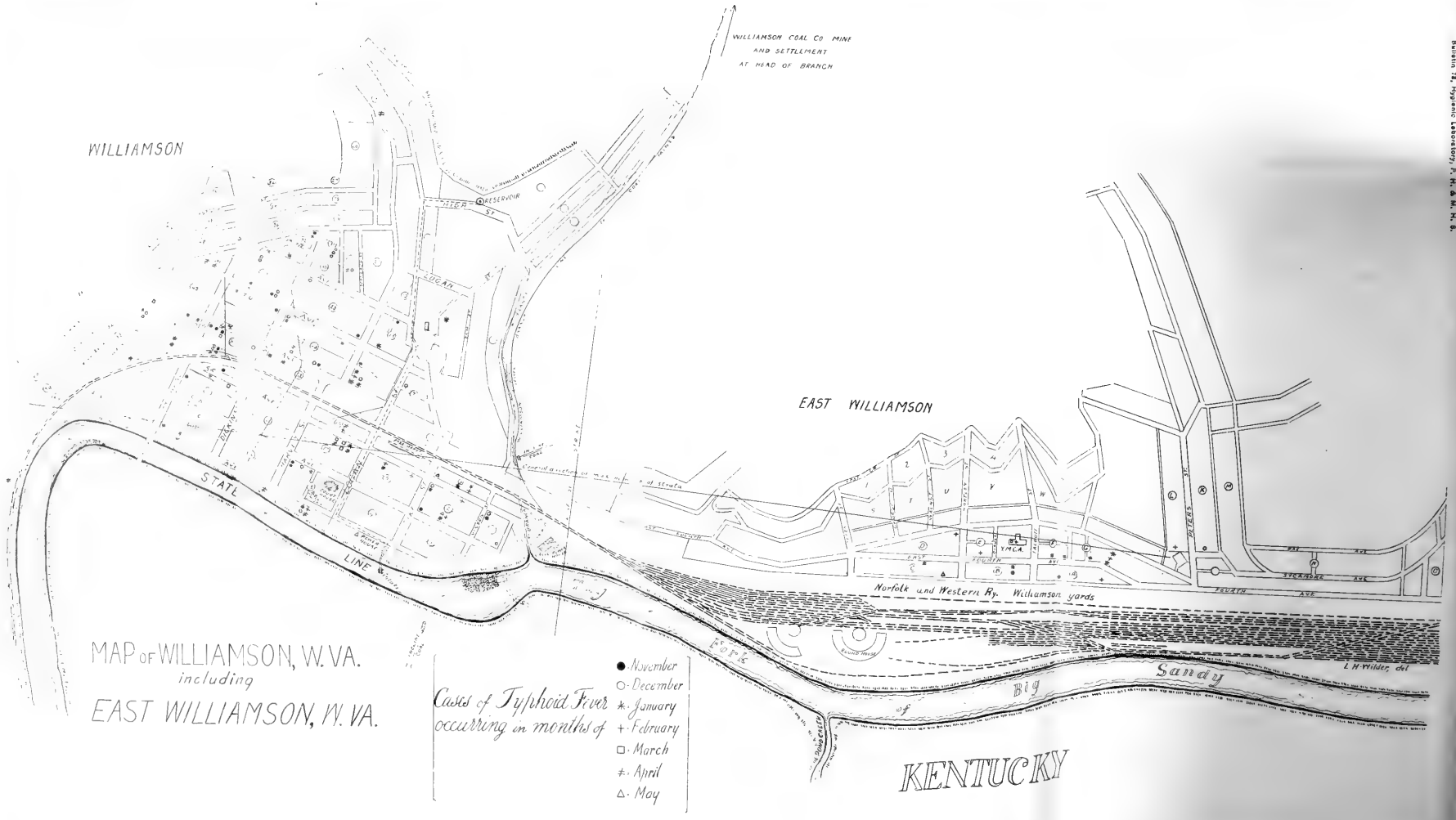
Although the mortality was low, the eleven deaths among a population of 5,000 constitute a death rate of 220 per 100,000 for a period of only seven months. This is just double the highest death rate from typhoid fever in any registration city of over 100,000 population, included in the Census Report of Mortality Statistics for 1908 (Columbus, Ohio, 110.5 per 100,000), and was equaled in 1908 in only two of the smaller towns included in this report, Mankato, Minn., 276 per 100,000, and Sharon, Pa., 244 per 100,000.[a]

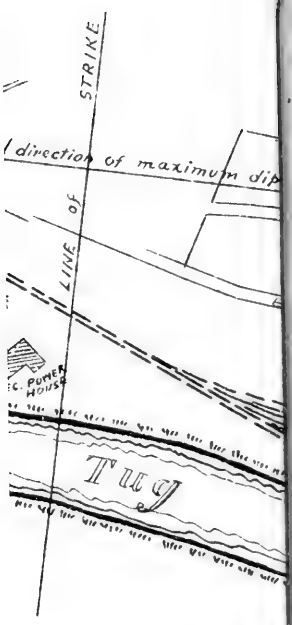
The 152 cases of typhoid fever in Williamson, had their onset by months as nearly as could be ascertained, as follows:

TABLE NO. 8.—*Onset of cases and onset of fatal cases by months, from November, 1909, to May, 1910, inclusive.*

Date.	Total cases.	Cases terminating fatally.
1909.		
November	20	
December	23	4
1910.		
January	53	2
February	21	2
March	14	1
April	12	2
May	9	
Total	152	11

[a] Ninth Annual report, Bureau of the Census, Washington, 1910, Mortality Statistics, 1908 (p. 36).





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The epidemic began quite explosively in November, reached its me in January, and from that time declined quite steadily. It n hardly be said, however, that the epidemic had altogether sub- ded at the time of investigation, for the incidence of nine cases in e month of May corresponds to an annual incidence of 108 (2,160 r 100,000), a very excessive, if not epidemic rate. The fatal cases the above summary are tabulated according to date of onset. It rather striking that none of the cases having onset in November rminated fatally, and only two (3.8 per cent) of the 53 cases occur- ng in January were fatal, while of the cases having onset in Decem- r, a high percentage (17.4 per cent) were fatal.

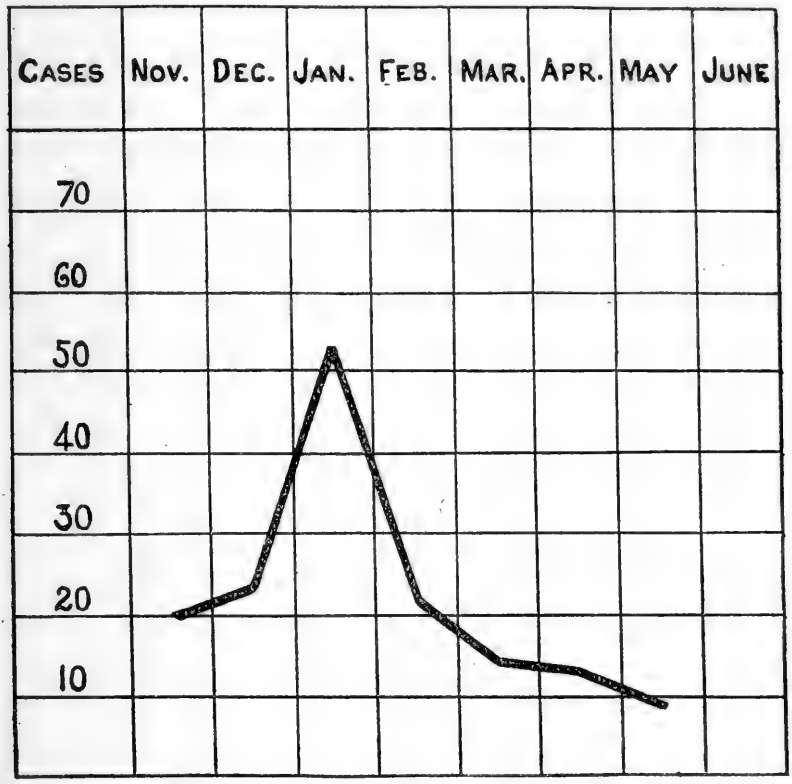


CHART No. 1.—Showing onset of cases by months.

As many cases as possible were located and platted on a map of e town. Very considerable difficulty was experienced in the cation of cases. The town has not yet reached the stage at which reet numbers are in use for locating houses, yet it is already of such ze that even those residents with the most extensive local knowledge re unable to locate all the residences. The difficulty was greatly creased by the large transient population. Of the 152 reported ses, 38 were known to have moved away from Williamson, and at ast 18 others had changed their residence in the town since their lness. Eleven cases could not be located at all, and were therefore ecessarily omitted from the map. Nine more cases, occurring on e upper portion of Williamson Branch can not be shown on the ap, as this settlement is above the limits of the map.

The 141 located cases were distributed in the three sections of the town, Williamson proper, East Williamson, and Williamson Branch as follows:

	Estimated population.	Number of cases.	Number of people to each case of typhoid.
Williamson proper	3,500	112	+31
Williamson Branch	500	9	+55
East Williamson	1,000	20	50

From these figures it appears that the incidence of typhoid fever was greater in Williamson proper than in other sections. It is probable, however, that a large proportion of the unlocated cases belonged in East Williamson or on Williamson Branch, as the population of these sections is more transient and less known locally than the population of Williamson proper. Taking into consideration also the roughness of the estimates of the relative populations of these sections, it can not be said that any marked regional prevalence of typhoid fever has been demonstrated. The appended map is somewhat misleading in this respect, as many of the blocks shown upon the map have very few or no residences upon them. In general, the distribution of cases seems to have been roughly proportionate to the distribution of population, with notable exceptions in several well-marked foci.

AGE.

The age was satisfactorily determined in 112 reported cases, from which the incidence at various ages has been computed.

TABLE NO. 9.—*Incidence of typhoid fever in persons of various ages at Williamson, W. Va.*

	Number of cases.	Percentage of total.
Under 5 years	8	7.1
5 to 9 years, inclusive	13	11.6
10 to 14 years, inclusive	14	12.5
15 to 19 years, inclusive	13	11.6
20 to 24 years, inclusive	14	12.5
25 to 29 years, inclusive	23	20.5
30 to 34 years, inclusive	13	11.6
35 to 39 years, inclusive	9	8.0
40 to 44 years, inclusive	3	2.6
45 to 49 years, inclusive	2	1.7
Over 49 years		

The above table shows that the distribution of the epidemic among the various decades was quite usual, the maximum number of cases occurring in the third decade. The proportion of cases occurring among children (31.2 per cent) is not exceptional. It is higher than in the epidemic in Omaha, investigated by Lumsden (29 per cent), and less than the average in Washington, D. C., where milk is considered an important factor in the spread of typhoid fever.

As many cases as possible were visited personally for the purpose of obtaining epidemiological data. An effort was made to visit a fair proportion of cases in each section of the town, and at the same time a fair proportion of the cases occurring in each month, but it was impossible, on account of the inaccessibility of many cases, to follow out a definite system in selecting cases for investigation. Altogether 72 cases were visited. The number and percentage of each is shown in the following summary:

TABLE NO. 10.—*Number of each month's cases investigated.*

	Total cases occurring in month.	Number of cases investigated.	Percentage of cases investigated.
November	20	11	55.0
December	23	13	56.5
January	53	22	41.5
February	21	7	33.3
March	14	6	42.0
April	12	7	58.0
May	9	6	66.6

The case card used in the investigation of these cases was identical with the one used in the investigation of the origin and prevalence of typhoid fever in the District of Columbia in 1907 and 1908,[a] and essentially the same as the one described by Lumsden elsewhere in this bulletin.

[a] Bulletin No. 35, Hygienic Laboratory, Public Health and Marine-Hospital Service, 1908, p. 13, and Bulletin No. 52, Hygienic Laboratory, Public Health and Marine-Hospital Service, p. 11.

The information so obtained is presented in summarized form in Table No. 11 following.

TABLE No. 11.—*Summary of epidemiological histories of cases of typhoid fever in Williamson.*

Date.	Number of cases investigated.	Drinking water within 30 days prior to onset.			Milk within 30 days prior to onset.		Contact within 30 days prior to onset with—	
		City.	Well.	Safe (boiled or distilled).	Used.	Suspected.	Persons in febrile stage of typhoid.	Persons or excreta of persons who had had typhoid fever within 1 month.
1909.								
November	11	11	4	.....	8	.....	.....	3
December	13	12	2	1	10	.....	4	.....
1910.								
January	22	20	4	2	16	3	9	2
February	7	6	2	1	6	.....	2	.....
March	6	5	2	.....	6	1	3	1
April	7	3	2	2	1	1	1	3
May	6	4	.....	2	3	1	2	1
Total	72	61	16	8	50	6	21	10

Date.	Sanitary condition of premises.				Sewage disposal.			Disinfection of excreta.			
	Good.	Fair.	Bad.	Unknown.	Water closet.	Privy.	Unknown.	Probably efficient.	Inefficient.	None.	Unknown.
1909.											
November	2	3	3	3	8	3	.....	.....	3	5	3
December	2	5	4	1	10	3	.....	2	4	4	3
1910.											
January	4	7	10	1	14	8	.....	2	7	8	5
February	1	1	5	.....	1	6	.....	.....	3	3	1
March	2	1	3	.....	4	1	1	3	1	1	1
April	3	.....	4	.....	6	1	.....	.....	2	2	3
May	2	.....	4	.....	4	2	.....	2	3	1	.....
Total	16	17	33	5	47	24	1	9	23	24	16

A consideration of the data obtained from the 72 cases investigated, together with other data presented, warrants some conclusion as to the probable origin of the typhoid fever in Williamson.

The sources of infection to be considered are:

(1) Foodstuffs, especially milk.

(2) Water.

(3) Contact with cases of typhoid fever, either direct personal contact or less direct contact with their excreta, through the agency of flies, dust, leaky sewers, etc.

DISCUSSION OF EPIDEMIOLOGY.

IMPORTED CASES.

Of the 72 cases investigated, four, railroad employees, had been absent from Williamson almost daily within thirty days prior to onset of illness.

Three other cases had been absent for periods of one to ten days during the thirty days prior to onset. Two cases developed between fifteen and thirty days after coming to Williamson. In none of the foregoing cases was there any known exposure to infection outside of Williamson, while in several exposure in Williamson was definitely shown. There is no strong probability of any of these cases having been contracted outside of Williamson. On the other hand information was obtained from various sources of persons who undoubtedly contracted typhoid fever in Williamson but developed it elsewhere. A few of such cases, whose residence and history in Williamson were known, have been included among the Williamson cases, but most of them have not been so included. On the whole, no deduction need be made for cases imported into Williamson, their number being in all probability more than balanced by the number of exported cases.

MILK.

The only milk supply common to more than one or two score of people in Williamson comes from Portsmouth, Ohio, and is said to be pasteurized. This milk is used at the Norfolk and Western Y. M. C. A., where meals are served to about 150 people daily. A limited number of families also purchase this milk for home use. Nothing was found in the course of the investigation to cast suspicion upon this milk supply as a source of infection.

Many of the families in Williamson keep one or two cows, for home supply, and usually sell small quantities of milk to neighbors. Peddlers bringing milk from the nearby country sell small quantities to various families. Those who buy milk, however, either from neighbors or peddlers usually do so very irregularly, often having no regular source of supply and getting their milk from perhaps half a dozen different sources in the course of a month. Under such circumstances the rôle of milk in the spread of typhoid fever is very difficult to trace. Since scarcely any source of milk supply is common to more than half a dozen families, usually living close together, milk can readily be excluded as the cause of a sudden outbreak of 20 widely scattered cases, such as occurred in November, the first month of the epidemic. In 50 of the 72 cases investigated, milk had been used either as a beverage, on cereals, or in coffee within thirty days prior to the onset of illness. In fifteen of these cases the milk was

obtained from cows owned by the patient's family. In 6 of the cases investigated the milk used within thirty days prior to onset of illness had come from premises on which a case of typhoid fever was under treatment at that time, and must therefore be reckoned as a possible source of infection. In three of these cases milk is considered the most probable source of infection, no other probable source being discovered.

No license is required for the sale of milk in Williamson, and there is no sanitary supervision over this traffic. Considering the facts that milk is often sold from premises on which a case of typhoid fever is being nursed, that it is not infrequently handled by the nurse, with little or no precautions, and that the attendant dangers are seldom realized by either vendor or purchaser, it must be admitted that raw milk is in general a dangerous foodstuff under present conditions in Williamson, and it may be suspected that it plays a considerable part in maintaining the endemic prevalence of typhoid fever there.

ICE CREAM.

The ice cream sold in Williamson is mostly obtained from Portsmouth, Ohio, said to be made of pasteurized milk. From cases of typhoid fever occurring in the fall and winter months, no reliable histories could be obtained as to their consumption of ice cream prior to illness. The majority of patients thought it improbable that they had taken ice cream within a month prior to illness and were quite certain that within that time they had not taken any which came from the drug stores—the only source of supply common to many people.

OTHER UNCOOKED FOODS.

It appears that raw shellfish are very little used in Williamson, and they can be definitely excluded as an important source of infection. No satisfactory information could be obtained as to the use of raw fruits and vegetables. During the fall and winter months, when the epidemic was at its height, very few raw vegetables were being eaten. The ultimate sources of the raw fruits and vegetables were so various that simultaneous infection of all these sources is highly improbable; such foodstuffs may therefore be excluded as important factors in the causation of the epidemic.

WATER.

The water supply of Williamson, with its known pollution, which has already been discussed, suggests itself at once as a most probable cause of the epidemic of typhoid fever.

It was found that 61 out of the 72 investigated cases (81.3 per cent) had used raw city water for drinking more or less constantly within thirty days prior to their illness.

Considering separately the investigated cases occurring in various months, it was found that of the cases occurring in—

	Per cent.
1909.	
November	100
December	92.6
1910.	
January	90.9
February	85.6
March	83.3
April	42.7
May	66.6

had used city water for drinking more or less constantly within thirty days prior to onset of symptoms.

Eight patients (11 per cent) had used only boiled or distilled water for drinking. Sixteen had used water from various wells, mostly shallow; but thirteen of these had also occasionally drunk city water, and are therefore included among the number given as having used city water.

For the cases occurring in the month of November, the drinking water is the only probable source of infection which could be shown to have been common to all cases; and the sudden outbreak of twenty cases scattered widely over the town strongly indicates the operation of some widespread, common cause.

The late summer and fall of 1909 were unusually dry and the volume of Tug River is said to have been smaller than it had been in many years. The precipitation in inches at Williamson, from August 1, to December 31, for the years 1901–1909, as given by the Weather Bureau, is shown in the following table, compiled from a publication of that bureau [a] and from additional data furnished for the period from July 1, 1909, to July 1, 1910.

TABLE No. 12.—*Total precipitation from August 1 to December 31 for the years 1901–1909.*

Year.	Precipitation.	Year.	Precipitation.
1901	22.98	1906	21.88
1902	15.52	1907	16.10
1903	8.57	1908	12.16
1904	17.06	1909	8.80
1905	16.40		

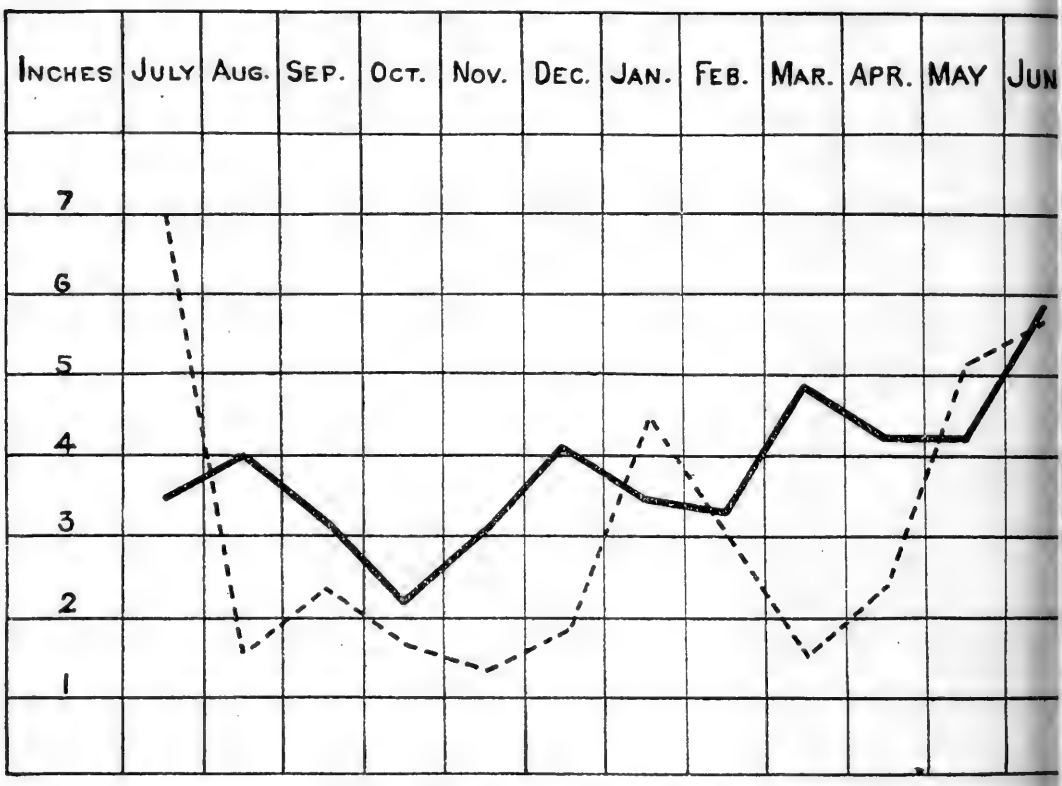
From this table it is seen that the rainfall for these months was less in 1909 than it has been in any year since 1903, and far below the average.

[a] Summary of the Climatological Data for the United States by Sections, Section No. 74, Southern West Virginia and Southwestern Virginia, U. S. Department of Agriculture, Weather Bureau

Chart No. 2, constructed from data from the same source, show the monthly precipitation from July 1, 1909, to June 1, 1910, com pared with the mean monthly precipitation from 1901 to 1908 inclusive.

Under these weather conditions it may be inferred that the amoun of sewage pollution entering Tug River was less than usual, as prob ably a large proportion of the pollution of this river comes from sur face washings, carried in by heavy rains, but on the other hand th dilution of such sewage as continued to be discharged into the rive was certainly much less than usual. Whether or not the result wa

CHART No. 2.—Showing monthly precipitation from July, 1909, to June, 1910, as compared with me monthly precipitation for previous years.



———— = Mean monthly precipitation in inches, 1901–1908, inclusive.
- - - - - - - - - - - - = Monthly precipitation, in inches, July, 1909, to June, 1910, inclusive.

a greater degree of pollution than usual can not be definitely state in the absence of any bacteriological examinations made at that time

It is certain that from the two nearest, and therefore most danger ous, sources of considerable pollution the amount of sewage discharge into the river during this period of excessively low water was no diminished in proportion to the diminution of the river's volume Matewan, a town of about 1,000 population, 9 miles above William son, has a closed sewerage system. This sewage would therefor continue to be discharged into the river independently of rainfal The other important nearby source of pollution is Williamson Branch It has already been shown that this little stream, carrying the sewag

of some 500 people, empties into Tug River only a few hundred yards above the intake for the Williamson water supply, and that the direction of flow is such that the water of this branch passes over the intake before becoming thoroughly mixed with the water of the river. During the dry season, the volume of Williamson Branch was kept up to some extent by the water used in washing coal at the Williamson Coal Company's mine, the water used for this purpose being obtained partly from a deep well and partly from the municipal supply. Tug River being greatly diminished in volume, and Williamson Branch, while also diminished, being proportionately less so than the river, it is obvious that at the intake there would be a larger proportion than usual of water from Williamson Branch, which is much more highly polluted than is Tug River.

The exact sources of typhoid infection entering Tug River at this time can not be given. It is known that during October there was at least one case of typhoid fever in the settlement at the head of Williamson Branch, and there is good reason to believe that a part of the excreta from this patient must necessarily have found its way into the stream without adequate disinfection. Typhoid fever was present also at Matewan, 9 miles above Williamson, and undoubtedly at various other points along the river

Evidence strongly suggesting that infection of Tug River water was the primary cause of this epidemic is furnished by the history of the epidemic in the settlement of mine employees at the head of Williamson Branch. This settlement was supplied with drinking water from the company's deep well until some time in December, 1909, when the well failed and city water had to be used for drinking. With the exception of one case in October, no typhoid fever is known to have occurred in this settlement during the fall and early winter of 1909. In January, within two to six weeks after the use of Williamson water was begun, seven cases developed among the 150 people in this settlement

Additional evidence is furnished by the experience of the two settlements nearest to Williamson.

Chattaroy is the town next below Williamson on Tug River. It is about 3 miles below Williamson, at the mouth of Buffalo Creek. Extending up the creek some 2 miles from Chattaroy are settlements of employees of several coal mines located there. The aggregate population of these settlements and the village of Chattaroy is about 1,000 to 1,500. All these people get their water supply from pipelined, driven wells, averaging about 100 feet in depth. Doctor Price, who has the practice of these mines, stated that there had been but one case of typhoid fever among these people since July, 1909, and that this patient had visited Williamson frequently within a month

prior to illness. At Borderland and Hatfield, mining settlements near Chattaroy, likewise supplied with deep-well water, there were no cases of typhoid fever during the winter. At Goodman, a settlement of about 150 persons on the bank of Tug River just above the mouth of Buffalo Creek, there had been four cases of typhoid fever during the winter of 1909–10. Doctor Price stated that two of these cases were imported from Williamson, and the other two had used the river water for washing and perhaps for drinking. Altogether, in the settlements of Chattaroy, Borderland, Hatfield, and Goodman, with an aggregate population of 1,500 to 2,000, there were but 5 cases of typhoid fever during the seven months in which there were 152 cases in Williamson, and 3 of these 5 cases are suspected to have been imported from Williamson. The population in these settlements is somewhat less dense than in Williamson, and sanitary conditions generally better. The difference in this respect is, however, apparently not sufficient to account for the great difference in the typhoid rate.

About 9 miles above Williamson, on Tug River, at the mouth of Mate Creek, is Matewan, a town of about 1,000 population. The public water supply here is obtained from Tug River, below the town's sewer outlet and without purification. I was verbally informed by Doctor Turner of Matewan that there had been 30 to 35 cases of typhoid fever in Matewan during the past winter—more in January than in any other month. At Redjacket, a mining settlement of possibly 900 people, a few miles above Matewan, on Mate Creek, there had been about 30 cases of typhoid fever during the winter. Deep wells supply water to this camp, but Doctor Turner was of the opinion that on account of the mineral impregnations of the deep-well water many people preferred to drink water from the near-by creek. The incidence of typhoid fever in Matewan and Redjacket, 60 to 65 cases among 2,000 population, is so near that in Williamson as to strongly indicate the operation of a common cause, most probably water.

If infection of the water of Tug River was the cause of the unusual prevalence of typhoid fever in Williamson, there must have been some unusual condition of the water operative during the fall and winter of 1909–10 to increase the infectiousness of this water, since water from the same source had been used for several years without causing such very excessive prevalence of typhoid fever.

Information as to the prevalence of typhoid fever on the Tug River watershed during the months immediately preceeding November, 1909, is wanting. There is, however, no evidence of very unusual prevalence during these months; consequently no reason to suppose that an unusual number of typhoid germs found their way into the river at that time. The only unusual condition ascertained is the extremely small volume of the river during this period, result-

ing, presumably, in unusual concentration of its sewage pollution. It is rather striking that the epidemic should have commenced to subside in February, when in all probability the amount of typhoid excreta emptied into the river immediately above Williamson was greater in January and February than in any other months of the year. The possibility of the existence of other conditions affecting the virulence or viability of typhoid bacilli in water suggests itself. What constitutes such conditions, however, is not known. Some light may perhaps be thrown upon this point by a careful comparison of the climatological conditions existing at various places during and prior to water-borne epidemics of typhoid fever and by careful studies of the biology of surface waters under varying climatological conditions.

CONTACT.

The 141 located cases of typhoid fever occurred in 99 houses. In 64 of these houses there was but 1 case in each house. In 32 houses there were two or more cases, and in 3 more houses, one case, with one or more suspected cases. Altogether, then, in approximately one-third of the houses affected there was more than one case. A study of the accompanying map will show that in the majority of instances where two or more cases occurred in a house, the interval between cases was such as to make contact infection of the later case from the earlier seem very probable.

The houses having more than one case apiece may be tabulated as follows:

Houses with—	Number.	Cases.
2 cases	23	46
1 case and 1 suspected case	2	2
3 cases	6	18
2 cases and 1 suspected case	1	2
1 case and 2 suspected cases	1	1
4 cases	1	4
4 cases and 1 suspected case	1	4
Total	35	77

In the 35 houses where there was more than one case in each house the total number of cases was 77—approximately one-half of

all reported cases. These figures are in striking contrast to the corresponding figures for the District of Columbia in 1906,[a] 1907,[b] and 1908.[c]

	Total number of cases investigated.	Number of cases occurring in houses where there was more than one case in a house.	Percentage of cases occurring in houses where there was more than one case in a house.
District of Columbia:			
1906	747	103	13.9
1907	523	73	13.9
1908	542	81	14.9
Williamson, 1909-10	141	77	54.6

These facts alone, derived simply from a study of the dates and locations of cases, would indicate that a high percentage of the cases were probably due to contact with previous cases, an assumption which is supported by the results of the investigation of individual cases.

It was found that 21 (29.4 per cent) of the 72 cases investigated gave definite histories of contact usually constant and intimate within the thirty days prior to the onset of their illness with previous cases of typhoid fever in the febrile stage.

Ten more cases gave histories of contact with persons who had recovered from typhoid fever less than one month previously, or of presumable contact with the excreta of such persons. For instance, in two cases, the patient had developed the disease within a month after moving into a house just vacated by a typhoid convalescent. Other cases included under this head are those occurring in houses less than 200 yards distant from privies into which the undisinfected excreta of typhoid-fever patients had been emptied within a month prior and when the direction of drainage or the prevalence of flies was such as to make conveyance of infection easily possible. Altogether 37 cases (51 per cent) gave a history of contact with persons who had had typhoid fever within six months prior.

The contacts determined among the 72 investigated cases, tabulated by months, are as follows:

[a] 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), Hygienic Laboratory Bulletin No. 35.

[b] 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, Hygienic Laboratory Bulletin No. 44.

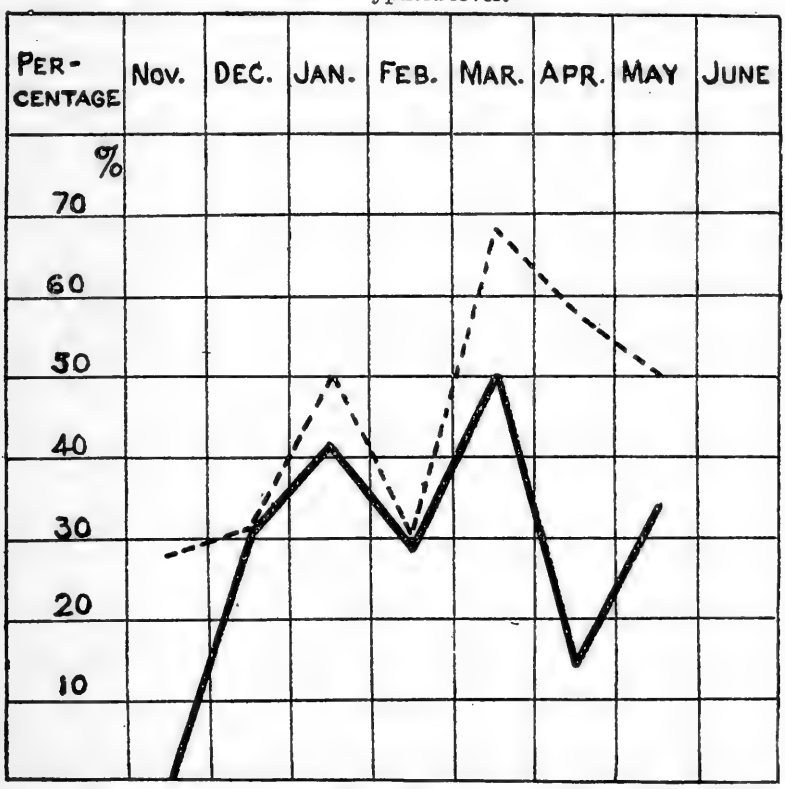
[c] Report No. 3 on the origin and prevalence of typhoid fever in the District of Columbia, by M. J. Rosenau, L. L. Lumsden, and Joseph H. Kastle, Hygienic Laboratory Bulletin No. 52.

TABLE No. 13.

Date.	Number of cases investigated.	Cases giving history of contact within thirty days prior to onset, with—			
		Persons in febrile stage of typhoid fever.		Persons who had had typhoid fever within 1 month or the excreta of such persons.	
		Number.	Per cent.	Number.	Per cent.
1909.					
November	11	.....	.....	3	27.0
December	13	4	30.7	.....	.....
1910.					
January	22	9	40.9	2	9.0
February	7	2	28.5	.....	.....
March	6	3	50.0	1	16.6
April	7	1	14.2	3	42.8
May	6	2	33.3	1	16.6
Total	72	21	30.0	10	14.0

Especially noteworthy in this table is the absence of traceable contacts to account for the cases occurring in November, contrasted with the increasing percentage of cases in subsequent months giving a history of contact. Chart No. 3 shows graphically the percentage of contacts traceable in different months.

CHART No. 3.—Showing percentage of cases in various months, giving history of contact with previous cases of typhoid fever.



———=Cases giving history of contact within thirty days prior to illness with case of typhoid in febrile stage.

- - - - - =The above, plus cases giving history of contact with persons or the excreta of persons convalescent from typhoid less than one month.

In drawing inferences from these facts it is necessary to consider: (1) That it is more difficult to trace contacts when histories are taken several months subsequent to recovery; (2) that during the months of November, December, January, and February there were progressively more cases of typhoid fever under treatment in Williamson, and consequently more chances for well persons to come into contact with such cases; (3) that nearly 3 per cent of the total population of Williamson had typhoid fever at some period between November, 1909, and June, 1910, and that considerably more than this percentage must therefore necessarily have been thrown into contact with febrile cases of typhoid fever.

There can, however, hardly have been more than 75 cases under treatment in any one month. Allowing an average of ten contacts for each case, there would be 750 out of 5,000 people (15 per cent) who would give a history of contact with cases of typhoid fever within that month. There were an average of 22 cases under treatment each month and on the above basis of calculation 220 contacts. It is probable, too, that five contacts for each case of typhoid fever in the febrile stage is a more nearly accurate estimate than ten (4.4 per cent of the total population) each month. As compared, then with this percentage of the total population who may be presumed to have been in contact with cases of typhoid fever within one month the high percentage of cases of typhoid fever (30 per cent) giving such a history clearly indicates that contact played a very considerable causative part in the prevalence of the disease.

BACILLUS CARRIERS.

In two instances where the epidemiological evidence strongly suggested a bacillus carrier as the source of infection in a household, the stools and urine of the suspected persons were examined a single time with negative results. In another instance the simultaneous infection of two members of one household seemed most probably due to a recent convalescent who aided in preparing and serving the food, but no examination of stools or urine could be made in this case. In a community where typhoid fever has been so constantly endemic as in Williamson it may be accepted as reasonably sure that there are a certain number of permanent bacillus carriers, and that the number of both permanent and temporary carriers must have been considerably augmented by the recent epidemic. The rôle of bacillus carriers in the causation of typhoid fever is, however, more difficult to trace in a community where there are numerous other probable sources of infection than in a community where typhoid fever is rare and other sources of infection more readily excluded.

FLIES.

Where open privies are so numerous as in Williamson there is every reason to suspect that flies play a considerable part in the spread of typhoid fever in summer, and in three of the investigated cases occurring in April and May flies were considered the most probable carriers of infection, all of these cases being within 100 yards of the same open privy, which, during March and April, had received the undisinfected excreta of two typhoid fever patients.

It is to be borne in mind that in the later months of the epidemic there were very numerous foci of infection, and correspondingly numerous avenues of contact, many of them unrecognizable, especially the fairly constant percentage of permanent bacillus carriers, the larger percentage of convalescent temporary carriers, and probably unrecognized ambulant cases. Especially in the warmer months, with increased prevalence of flies and other insects and increased consumption of uncooked foods, the avenues of infection must obviously have been multiplied and must at the same time have become more difficult to trace. In view of these facts, while water can not be excluded as a continued cause of the prevalence of typhoid fever in the later winter and spring months, contact alone, under existing conditions of sewage disposal and other prophylaxis in Williamson, might easily explain the continued prevalence of typhoid fever.

SANITARY CONDITION OF PREMISES ON WHICH TYPHOID FEVER OCCURRED.

The sanitary condition of the premises on which the 72 investigated cases of typhoid fever occurred was found to be good in 17 cases, fair in 17, bad in 33, these terms being used chiefly with regard to sewage disposal, general cleanliness, and such other conditions as would have a bearing upon the spread of typhoid fever.

The percentage of cases occurring under bad sanitary conditions is, so far as can be judged by the results of a sanitary survey of a part of the town, disproportionately high. The sanitary conditions of 21 premises, on each of which more than one case of typhoid fever occurred, is known. The conditions were bad on 14 of these premises (67 per cent), with an aggregate of 33 cases; fair on 3 (14 per cent), with an aggregate of 6 cases; good on 4 (19 per cent). This disproportionately high percentage of insanitary conditions in premises where typhoid fever was unusually prevalent indicates very clearly the effect of such conditions with the attendant poverty, ignorance, and neglect of prophylactic measures in maintaining a focus of typhoid fever.

PROPHYLAXIS.

In the investigation of cases the prophylactic precautions exercised in nursing the case were ascertained whenever possible. This information was satisfactorily obtained with regard to 56 cases. The

measures used in disinfection of the excreta were probably efficient in 9 cases (16 per cent), certainly inefficient in 23 (41 per cent), while in the remaining 24 (43 per cent) no disinfection had been attempted. Altogether, then, in 84 per cent of the cases investigated disinfection of excreta was, according to the statements of the nurse, either not attempted or was carried out in such a way as to be certainly inefficient. Prophylactic measures other than disinfection of excreta were found to have been even more neglected.

This is probably the most significant fact brought out in the investigation. Taken in connection with the faulty methods of sewage disposal it fully explains the succession of cases in the same house and the large number of other contact infections. And if from the practice at Williamson any inference can be drawn as to other towns farther up the river, the probable degree of typhoid infection of the water of Tug River becomes appalling.

SUMMARY.

Typhoid fever became unusually prevalent in Williamson in November, 1909, from which time until January, 1910, the number of cases increased monthly. From January to June, 1910, the number of cases decreased monthly, but the rate of prevalence remained excessive. Evidence derived from a study of the epidemic indicates that this unusual outbreak was due to infection of the municipal water supply obtained from Tug River; and it seems highly probable that unusual weather conditions, resulting in extremely low water in the river were important contributory causes. The heavy rainfall in January was followed by a sharp decline in the number of cases occurring in the next month, notwithstanding that during January the total amount of typhoid excreta washed into the river at Williamson was certainly greater than in previous months. The same may be assumed as true of Matewan, just above Williamson, and probably of many other towns on the river. The decline in the epidemic indicates a decrease in the amount or virulence of infection in the water during January. Whether this can be explained altogether by dilution, due to increased volume of the river, is not determined.

Infection by direct contact with cases of typhoid fever was undoubtedly responsible for a large proportion of the cases occurring subsequent to November, 1909. There is good reason for supposing that infection by direct contact and less direct contact, as through flies and through uncooked foods, has been the chief factor in maintaining the prevalence of typhoid fever since January. While water can not be excluded as a factor in the causation of typhoid fever since January, there is no direct evidence that it has been a factor since then; and there are enough other probable sources of infection to reasonably account for the continued prevalence of typhoid fever in Williamson.

PREVALENCE OF DIARRHEAL DISEASES.

A severe choleraic diarrhea, affecting persons of all ages, is, according to the statements of local physicians and other residents, unusually prevalent in Williamson. The infant mortality rate could not be ascertained, and it is therefore not known whether this is in excess of the average death rate for other localities. No fatal cases of diarrhea in adults were reported, but the local physicians gave accounts of many cases so severe as to be alarming. In the more severe cases of diarrhea the temperature is subnormal, there are general muscular cramps, extreme prostration, and rapid emaciation, with profuse and frequent watery stools, seldom if ever containing blood. In the more common, less severe cases the only symptoms are diarrhea, weakness, and sometimes nausea. Elevation of temperature is unusual and never great. The economic effect of such a common and disabling malady is obviously very considerable.

This diarrhea is generally believed to be due to drinking the river water. A number of observations tend to confirm this view. In the investigations of cases of typhoid fever I was told quite often that a whole family, on first coming to Williamson and drinking river water, had been affected within a week by severe diarrhea, which ceased when the use of raw river water was discontinued. During the period of increased prevalence of typhoid fever in the winter of 1909–10 severe diarrhea was also unusually prevalent. The superintendent of machinery and repairs of the Norfolk and Western Railway Company's yard stated that since the installation in the yards of a distilling plant to supply drinking water to the employees there had been a very considerable increase in efficiency of the employees, due to decrease in the number of cases of diarrhea.

A stool from an acute case of diarrhea was examined by plating out on Endo's medium. The plates showed clear colonies resembling the typhoid or paratyphoid bacilli, almost as numerous as the red colon colonies. Several of the clear colonies were identified and found to be culturally identical with *B. paratyphosus, B*, *B. enteritidis Gärtner*, and others of the so-called "Enteritidis" group of bacilli. The organism was not agglutinated by specific typhoid agglutinating serum, paratyphoid – B serum, or paratyphoid – A serum.

Unfortunately no other case of acute diarrhea could be studied. A stool from a case of chronic diarrhea, plated out on Endo's medium, gave only typical colon-bacillus colonies. From the single case examined no conclusion is justified as to the relation of the organism isolated to the etiology of the local diarrhea. A causative relation is strongly suggested, however, by the abundance of this organism in the stool examined, the rarity of such organisms in normal stools, and the known relation of many bacilli of the Enteritidis group to acute enteritis.

MEASURES RECOMMENDED FOR THE PREVENTION OF TYPHOID FEVER IN WILLIAMSON.

The measures recommended for the prevention of typhoid fever in Williamson include:

(1) Improvement of the public water supply.

(2) Improved methods of sewage disposal.

(3) More careful disinfection and other prophylactic precautions in the care of typhoid fever patients.

(4) Supervision by the board of health over the sale of milk and the management of places where food is served to the public.

WATER SUPPLY.

It is of prime importance to obtain a water supply that will be free from sewage contamination. The supply must also be abundant, suitable for laundry and other domestic purposes, of good appearance, and palatable; otherwise its use will be supplemented by the use of unsafe water from shallow wells and springs, and the full sanitary benefits of a pure water supply will not be realized.

The ideal source of water supply, from a sanitary standpoint, is a deep well, and the practicability of obtaining a sufficient and satisfactory supply from such a source should be carefully considered by competent experts. The very slight dip of the strata in this section of the country, and the frequent occurrence of strata strongly impregnated with iron, sodium chloride, and other objectionable salts render the practicability of deep wells as a satisfactory source of water supply very doubtful.

There are said to be no mountain springs available for water supply. Small streams, draining practically uninhabited areas, are quite common in the vicinity of Williamson, but these streams are all said to fail in dry seasons and could not be used as a constant source of water supply without the construction of large and expensive impounding reservoirs. It would also be necessary to protect the catchment area from future pollution, and this would possibly necessitate the purchase of valuable coal lands.

In the event that Tug River proves the only available source of water supply, purification processes of the very highest efficiency will be necessary to render its water safe according to modern bacteriological standards, and unless measures are taken to prevent further pollution of this stream, a wide margin must be left for the inevitable future increase in its pollution.

It is quite obvious that the intake should be moved to a point above the town to escape local sewage pollution. Bacteriological and physical examinations indicate that the pollution both with sewage and with coal washings is very considerably greater at the present intake than at a point above the town.

Some process of filtration will be necessary to clarify the water and to remove as many as possible of the bacteria. The present rough intake filter is altogether inadequate, as is also the infiltration gallery of the Norfolk and Western Railway Company.

All things considered, the mechanical (American) system seems best adapted for the filtration of this water. The amount and kind of coagulant necessary will have to be determined by experience. Preliminary sedimentation before filtration would certainly improve the sanitary quality of the effluent, but it is believed that this point can be left to be settled on the basis of economy.

Any filtration system which is installed should be supplemented by a sterilization process, on account of the high degree of dangerous pollution of Tug River. The examinations of Tug River water showed *B. coli* constantly present in 0.1 c. c.—that is, at the rate of 10 per c. c. or 100 per 10 c. c. Quantative estimations of *B. coli* by Marmann's plate method showed 50 *B. coli* per c. c., which is probably not an excessive average estimate. Granting for a filtration plant a constant efficiency of 99 per cent, the effluent would still contain from 1 to 5 *B. coli* per 10 c. c., thus falling far below the standard of purity for a satisfactory filter effluent. For partial sterilization of the filter-effluent either the hypochlorite of lime or ozone may be used. Hypochlorite of lime is recommended as the more practicable because of the lesser initial cost of constructing the necessary plant and its simple and cheap application.

Pending the establishment of a safe water supply it is recommended that the people of Williamson be frequently warned by the board of health of the dangers of the present water supply, and urged to boil not only all drinking water, but also all water used for washing fruits and vegetables which are eaten raw, and for washing vessels which are used for milk.

If it is decided to adopt the hypochlorite treatment subsequent to filtration of the water, it is recommended that the plant for this purpose be installed at once, in such a location that it can subsequently be used for treatment of the filtered water, and that pending the completion of the filtration plant the water be treated with hypochlorite, as a temporary expedient.

SEWAGE DISPOSAL.

The present sewerage system should be overhauled, repaired, and extended as rapidly as possible to those sections of the town not at present provided with sewers. The use of Williamson Branch as an open sewer should be discontinued and a closed sewer laid for that section of the town. Adequate provision against flooding of the sewers should be made, either by enlarging the sewers sufficiently to

carry off the surface water following heavy rains or by diverting this water from the sanitary sewers.

All houses within a reasonable distance of a town sewer should be required to have closets connecting with the sewer. All privies on such premises should be condemned, and their contents safely disposed of. Where sewer connections are for the present impossible, all open privies should be replaced by privies of an approved sanitary model. Fly-proof can privies would be a great improvement. Dry earth or lime could be used in the cans. The care of all privies and the disposal of their contents should be under municipal control.

CARE OF TYPHOID FEVER PATIENTS.

Adequate provisions should be made for:

(1) Prompt reporting of all cases of typhoid fever.

(2) Careful instruction of the families of typhoid fever patients in the necessary prophylaxis. This should be done by a representative of the board of health.

(3) Supplying of disinfectants free of charge to the poor.

SUPERVISION OF FOOD SUPPLIES.

The sale of milk should be under some supervision by the board of health, at least to the extent of prohibiting its sale by persons living on insanitary premises or premises on which a case of typhoid fever or other infectious disease is under treatment.

The board of health should also have supervision over the public restaurants of the town. Some of these were found to be in a condition which must necessarily be a menace to the health of those who eat there.

In order that the above measures may be carried out, it is obviously necessary that the town should recognize the importance of its health department and make an adequate appropriation for the maintenance of an efficient organization.

A safe water supply may be expected with reasonable certainty to greatly reduce the prevalence of typhoid fever in Williamson, but it is to be expected with equal certainty that even with a pure water supply, if other preventive measures are neglected, the disease will remain constantly and excessively prevalent and will doubtless at times become epidemic. Compared to the cost of providing a pure water supply the cost to the town of the other measures recommended is insignificant, while their importance is, perhaps, equal to the importance of obtaining pure water.

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; Micro-photography 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 bear ing 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.

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.—Digest of comments on the United States Pharmacopœia. [Eighth Decennial Revision] For the period ending December 31, 1905. By Murray Galt Motter and Martin I. Wilbert.

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

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

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

No. 56.—Milk and its relation to the public health. By various authors.

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

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

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

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

No. 61.—Quantitative pharmacological studies: Relative physiological activity of some commercial solutions of epinephrin. By W. H. Schultz.

62.—The taxonomic value of the microscopic structure of the stigmal plates in the tick genus *Dermacentor*. By Ch. Wardell Stiles.

63.—Digest of comments on the Pharmacopœia of the United States of America (eighth decennial revision) and the National Formulary (third edition) for the calendar year ending December 31, 1907. By Murray Galt Motter and Martin I. Wilbert.

64.—Studies upon anaphylaxis with special reference to the antibodies concerned. By John F. Anderson and W. H. Frost.

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71.—I. Some known and three new endoparasitic trematodes from American fresh-water fish. By Joseph Goldberger. II. On some new parasitic trematode worms of the genus *Telorchis*. By Joseph Goldberger. III. A new species of *Athesmia* from a monkey. By Joseph Goldberger and Charles G. Crane.

72.—I. Report on an outbreak of typhoid fever at Omaha, Nebr., (1909–1910). By L. L. Lumsden. II. The water supply of Williamson, W. Va., and its relation to an epidemic of typhoid fever. By W. H. Frost.

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

HYGIENIC LABORATORY.—BULLETIN No. 73
MARCH, 1911

THE EFFECTS OF A NUMBER OF DERIVATIVES OF CHOLINE AND ANALOGOUS COMPOUNDS ON THE BLOOD-PRESSURE

By

REID HUNT

and

R. de M. TAVEAU

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United States Public Health and Marine-Hospital Service.

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THE EFFECT OF A NUMBER OF DERIVATIVES OF CHOLINE AND ANALOGOUS COMPOUNDS ON THE BLOOD PRESSURE.[a]

By Reid Hunt and R. de M. Taveau.

INTRODUCTION.

Certain derivatives of choline are, as was shown in a preliminary ommunication,[b] extraordinarily active in causing changes in blood ressure; one of them (acetyl-choline) was found to be more active in his respect than any compound of any kind previously investigated. hese preliminary investigations also showed that it was possible to odify the action of choline in definite directions by the introduction f certain chemical groups. Hence it seemed not improbable that a rther study of these compounds might lead to the discovery of subances having distinct therapeutic value.[c]

Accordingly a large number of derivatives of choline and certain alogous compounds not hitherto prepared or studied were made d the degree of their toxicity determined[d] preparatory to an investgation of their effects upon the circulation.

In the present communication it is proposed to report on experients made to determine the general effects of certain of these comounds upon the circulation; it is our purpose to investigate later, in etail, the pharmacological action of certain of the more active of them.

Methods.—The present investigation was limited largely to a deterination of the effects of the compounds under investigation upon he heart rate and blood pressure as determined by the mercury

[a] Manuscript submitted for publication August 19, 1910.

[b] Hunt and Taveau, Brit. Med. Jour., 1906, ii, p. 1788.

[c] Choline and its derivatives are of considerable interest in pharmacology and hysiology from other standpoints also. Thus it is one, perhaps the chief, of the lood pressure lowering bodies in the suprarenal glands (Hunt, Amer. Jour. Physiol., 99, 3, p. xviii, 1901, 5, p. vi; Lohmann, Pflüger's Archiv, 1907, 118, p. 215) and me other organs. It is closely related to, and easily converted into, neurine and uscarine and other powerful poisons. It is found in the galenical preparations of me important drugs (ergot, for example). Since it probably occurs (in combiation) in all cells, both plant and animal, and is comparatively easily converted to other and more active compounds, it has long been recognized as a possible ource of danger in foods, etc.

[d] Hunt and Taveau, Jour. Pharmacol. and Exper. Therap., 1909, 1, p. 303.

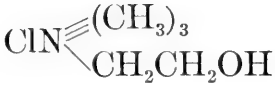
manometer in the usual manner. This method, of course, permits of but a very imperfect analysis of the action of a substance upon the circulatory organs, but it suffices to indicate which of the compounds have properties which render them worthy of further investigation.

In order to further simplify the problem the experiments were performed for the most part upon curarized animals. The latter were invariably anæsthetized, usually with urethane and chloral or ether.

The blood pressure was usually recorded from the carotid. The compounds, almost always in the form of their chlorides dissolved in normal saline solution, were injected into the saphena vein unless it is otherwise stated. Care was taken to make the injections at a uniform rate. Since the injection of small amounts of normal saline solution sometimes causes rather considerable changes in blood pressure, especially in curarized animals, control injections of this were made at frequent intervals in every experiment.

A summary of the action of each of the compounds studied will first be given, and then the influence of certain groups upon the physiological activity discussed.

I. SUMMARIES OF THE EFFECTS ON THE BLOOD PRESSURE.



CHOLINE CHLORIDE.

Historical.—Although the action of choline upon the circulation has been the subject of many investigations, it is still but imperfectly understood; this is due largely to the fact that the action is very complex.

No attempt will be made to review the literature in this place;[a] only a few of the investigations bearing more directly upon our own work will be cited.

One of the most complete investigations of the action of choline upon the circulation is that of Mott and Halliburton.[b] These investigators found that the injection of a 0.2 per cent solution of choline into dogs and cats caused a fall of blood pressure and usually a slowing of the heart; subsequent injections had less effect; they state that the heart was somewhat weakened, but that the output was increased. They found the blood vessels of the intestines to be dilated, whereas there was no effect upon those of the kidneys or limbs; the dilatation was of peripheral origin. They attributed the fall of pressure in part to the action on the heart, but mainly to the dilatation of the vessels of

[a] This has recently been done by Modrakowski, Pflüger's Archiv, 1908, 124, p. 601, and by Abderhalden and Müller, Zeitsch. f. physiol. Chemie, 1910, 65, p. 420.

[b] Mott and Halliburton, Phil. Trans., Royal Society, London, 1899, 191, B, p. 211.

the intestinal area. These writers made the interesting observation that after the administration of atropine choline no longer caused a fall of pressure, but usually caused a rise; they stated that this effect was due mainly to action on the heart.

Formánek[a] stated that choline caused first a fall of blood pressure and an acceleration of the heart; he attributed both of these effects to an action upon the heart. Later there was a rise of pressure and a slowing of the heart; he attributed the former to a stimulation of peripheral vaso-constrictors and the latter to a stimulation of the cardio-inhibitory center. Busquet and Pachon[b] stated that the effect of choline upon the blood pressure depends upon the size of the dose injected: 1 or 2 mgm. per kilo animal causing in a chloralosed or curarized dog a fall of pressure, whereas 4 or 5 mgm. caused a rise of pressure; the large doses caused marked constriction of the kidney. Larger doses sometimes had an effect on the heart which masked the vaso-motor effect. In atropinized animals these authors[c] found a summation of the effects of epinephrine and choline.

Desgrez and Chevalier[d] state that the fall of pressure is accompanied by cardiac acceleration with a diminution of the amplitude.

Modrakowski[e] believes that those investigators who report that choline causes a fall of pressure have worked with impure or deteriorated preparations; he maintains that pure choline causes only a rise of pressure. The results of Modrakowski were discussed at the meeting of the German Physiological Society.[f] Boruttau stated that he had fully confirmed Modrakowski's result that choline chloride when freshly crystallized and protected from air and especially from light causes only a rise of blood pressure. R. Müller also stated that a pure preparation of "synthetic" chlorine showed no or but a very brief and slight fall of blood pressure, whereas older preparations obtained from organs caused a marked fall of blood pressure. Von Fürth stated that pure choline obtained from the gold salt caused a fall of blood pressure. Lohmann stated that he had been unable to confirm Modrakowski's work; after passing choline through both the gold and platinum salts and recrystallizing these several times he obtained choline causing a fall of blood pressure.

The question was also investigated by Abderhalden and Müller.[g] These authors used two preparations, one from Merck (source not stated) and one prepared according to the method of Krüger and

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[a] Quoted from Biochemisches Centrallblatt, 1903, 1, p. 368.

[b] Busquet and Pachon, Comptes rend. de la Soc. de Biol., 1909, 67, p. 218.

[c] Busquet and Pachon, ibid., p. 277.

[d] Desgrez and Chevalier, Comptes rend. de la Soc. de Biol., 1909, 67, p. 251.

[e] Modrakowski, Pflüger's Archiv., 1909, 124, p. 601.

[f] Zentrbll. f. Physiol., 1909, 23, p. 291.

[g] Abderhalden and Müller, Hoppe Seyler's Zeitsch., 1910, 65, p. 420.

Bergell.[a] (Preparation of trimethylamine-bromethylium-bromide by passing dry trimethyl-amine into ethylenebromide heated to 110°–120° and converting the former into choline by heating its aqueous solution in a sealed tube for three to four hours at 150°–160°.) They reached the conclusion that the typical effect of choline is to cause a fall of blood pressure; they state that a rise is obtained only as a result of accessory factors, such as fibrillary contractions of striated muscles, etc.

Pal,[b] using a synthetic preparation from the Höchst Farbwerke and experimenting on cats, stated that choline causes a short depression followed by a rise of blood pressure; the latter he attributed to a peripheral vaso-constrictor action in the splanchnic area.

With deep curarization there was no rise of pressure. Without curare, as after section of the medulla oblongata, for example, small doses were strongly pressor. He found the action on the heart to be inconstant.

The most recent investigation on the subject is that of F. Müller[c] who states that the fall of pressure is due (*a*) to an effect upon the heart; the latter is slowed and the slowing is not prevented by section of the vagi or by atropine. The volume of the heart is increased (Stauung); (*b*) to a peripheral vasodilatation; this dilatation occurs chiefly in the extremities and not in the splanchnic area. After atropine choline no longer caused a dilatation of the peripheral vessels, but caused a contraction; atropine thus abolished the effect of choline upon the dilators and allowed its effect upon the constrictors to appear.

Experimental.—We have used choline[d] from the following sources: (1). From the suprarenal gland; the choline was obtained from extracts of the glands after the epinephrine had been removed by Doctor Abel; it was precipitated with mercuric chloride and purified by repeated crystallizations of the platinum or gold salt; as the character of the chief blood-pressure lowering body of the suprarenal was at the time of the earlier of these experiments (1899) unknown, the substance was purified with special care, and the preparations used for animal experiments were frequently passed through both the gold and platinum salts and their identity determined by determination of the platinum and gold as well as by the solubilities, melting points, etc.; (2) from egg; the lecithin was decomposed in the usual manner and the choline precipitated either by mercuric chloride or phosphotungstic acid and afterwards purified by means of the platinum salt;

[a] Krüger and Bergell, Ber., 1903, 36, p. 2901.

[b] Pal, Zentrbll. f. Physiol., 1910, 24, p. 1.

[c] F. Müller, Medzin. Klinik, 1910, No. 22.

[d] In most cases the choline was injected in the form of the chloride. The glycerophosphate, sulphate, lactate, camphorate, and nitrite were also prepared; their action was the same as that of the chloride. (Exper. 218.)

(3) synthetic from trimethylamine and ethylene chlorhydrine (Würtz's[a] method) and from trimethylamine and ethylene bromide (Bode[b]); (4) Kahlbaum's preparation; (5) Grübler's preparation; (6) choline made by what we shall call the "methyl-iodide process" (to be described later).

The effects of the last preparation differed in certain respects from that of the others; they will be discussed later. With these exceptions all of the above preparations had the same effects. These were practically those described by Mott and Halliburton; that is, they caused a fall of pressure before atropine, the effect often diminishing with successive injections, and either no effect or a rise of pressure after atropine.

Sometimes, before atropine, the fall of pressure was followed by a rise (Exper. 243[c] for example); in such cases there were frequently slight movements as described by Abderhalden and Müller. In other cases, however, curare seemed rather to increase the tendency to cause a rise,[d] especially if it had itself caused a fall of pressure. This was the case for example in Experiment 232.

Nicotine did not prevent the fall of pressure. (Exper. 278, 284.)

We did not observe that the size of the dose had a constant effect upon the result; that is, a large dose did not seem to more constantly cause a rise than did a smaller one. The condition of the blood pressure seemed to have more influence. Thus, when the blood pressure was very high, very small doses seemed especially efficacious in causing a fall. (Exper. 256.)

The heart was frequently slowed (Exper. 238); section of the vagi often, but not invariably, prevented this (Exper. 210 and 240).

Stimulation of the depressor was sometimes able to completely overcome the rise of pressure caused by choline after atropine. (Exper. 214.)

Many of the experiments were made with solutions prepared from the platinum salts immediately before their use. No difference was observed between the effects of such solutions and those prepared several days previously and kept in a cold (15°C.) and dark room (Exper. 268); a solution which had been kept for three years under these conditions gave the typical choline effects, although it was not so very active (Exper. 272).

As to the cause of the changes in blood pressure, we are inclined to attribute more importance to the changes in the heart than has been done by several of the previous writers. This is especially the case

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[a] Würtz, Liebig's Annalen, Spl. 6, p. 116.

[b] Bode, ibid., 267, p. 272.

[c] See the protocols of experiments at the end of the text.

[d] This is probably due to a partial paralysis of the endings of the inhibitory nerves in the heart.

as regards the rise of pressure which frequently occurs after atropine; here myocardiograph tracings showed a marked stimulation of the heart, i. e., the heart contracted much more completely and there was little or no increased dilatation in diastole. The tracing was very similar to that obtained with epinephrine. Before atropine the opposite occurred; the systole was less complete.

As stated above, we obtained with one preparation of choline results which differed markedly from those just described. This preparation was made as follows:

Dimethylethylalkine was prepared according to the method of Ladenburg[a] (from dimethylamine and ethylene chlorhydrine:

$$\begin{matrix} CH_3 \\ N{-}CH_3 \\ H \end{matrix} + CH_2Cl\ CH_2OH = ClHN\left\langle \begin{matrix} (CH_3)_2 \\ CH_2CH_2OH \end{matrix} \right)$$

and this treated with methyl iodide in the manner described by this author[b] in connection with diethylpropylalkine; the iodide was converted into the chloride by digestion with silver chloride. A portion was converted into the platinum double salt. This melted at 238–239° (corr.) and gave the following figures for platinum:

I. 0.1219 gm. salt gave 0.0385 gm. platinum.
II. 0.1391 gm. salt gave 0.0440 gm. platinum.

Calculated for:

$$\left(ClN\left\langle \begin{matrix} (CH_3)_3 \\ CH_2CH_2OH \end{matrix}\right)_2 PtCl_4\right.$$

	Found.	
	I.	II.
31.63 per cent platinum.	31.58 per cent.	31.62 per cent.

The choline used in the experiments was freed from the platinum salt by treatment with potassium chloride or hydrogen sulphide. As is shown by the Experiments 262, 264, 266, 267, and 268, this preparation had, before atropine, the same effect and about the same degree of activity as the others, except that in one case it was slightly more active in causing a fall, and in another case, after repeated injections, when the effects of all had become less, the effect from it diminished most rapidly; also in one case where the others had no effect, this compound caused a rise of pressure. After atropine, however, this compound was far more active in causing a rise than were the others.

It was quite active in causing a rise of pressure after this had been lowered by the injection of peptone; the rise of blood-pressure was accompanied by an acceleration of the heart.

We are unable to explain these peculiarities. The substance, from a chemical standpoint, certainly corresponded to a very pure preparation of choline. Possibly some extremely active substance, present in too small a proportion to influence the platinum content, was responsible. It seems more probable, however, that there is some

[a] Ladenburg, Ber. 1881, 14, p. 2408.
[b] Ladenburg, ibid., 1882, 15, p. 1145.

chemical difference, for we were unable to obtain an acetyl derivative[a] from this preparation, and the benzoyl derivative of it differed to some extent both chemically and physiologically from the benzoyl-choline obtained from egg choline. One suggestion would be that some of the hypothetical iso-choline $HON\begin{matrix}\equiv(CH_3)_3\\ \diagdown CHOHCH_3\end{matrix}$ had, in some way, been produced. This compound, however, is not known,[b] and, moreover, it is difficult to see how it could have been formed under the conditions.

$$BrN\begin{matrix}\equiv(CH_3)_3\\ \diagdown C_2H_4Br\end{matrix}$$

TRIMETHYL-BROMETHYL-AMMONIUM BROMIDE.

This compound was prepared by the direct addition of trimethylamine and ethylene bromide.[c]

Summary.—This compound caused a fall of blood pressure or a fall followed by a rise or simply a rise of pressure before atropine (Exper. 238); there was usually a slowing of the heart. After atropine there was a rise of pressure; this was completely overcome by stimulation of the depressor (Exper. 214). Its action did not differ very markedly from that of choline; as a rule, however, it was more active than this both in causing a fall and a rise of pressure.

$$ClN\begin{matrix}\equiv(CH_3)_3\\ \diagdown CH_2OH\end{matrix}$$

FORMOCHOLINE CHLORIDE (OXYMETHYL - TRIMETHYL - AMMONIUM CHLORIDE).

Preparation.—This compound was prepared by the method of Hofmann[d] (boiling iodomethyl-trimethyl-ammonium iodide with silver oxide).

Summary.—This compound caused a fall or a fall and a rise of blood pressure before atropine and a rise after atropine. It was distinctly more active than choline especially in causing a fall of pressure

[a] (Note during proof reading.) On repeating this experiment there was obtained an acetyl derivative the platinum salt of which contained 28.23 per cent platinum (calculated: 27.82 per cent) indicating, perhaps, incomplete acetylation. On recrystallization, a salt with 28 per cent platinum was obtained; this differed in appearance from the platinum salt of the acetyl derivative obtained from egg cholin, under precisely the same conditions, in that it was yellow whereas the latter was red.

[b] Cf. Schmidt, Liebig's Annalen, 1904, 337, p. 46.

[c] Hofmann, Jahresbericht über die Fortschritte der Chemie, 1858, p. 338.

[d] Hofmann, Jahresber. über die Fortschritte der Chemie, 1859, p. 377, cf. Litterscheid, Liebig's Annalen, 1904, 337, p. 74.

before atropine (Exper. 290). It was about nine times as toxic for mice as choline.

$$ClN\begin{matrix}(CH_3)_3\\ CH_2OCH_3\end{matrix}$$

METHYL ETHER OF FORMOCHOLINE.

Preparation.—This compound was prepared according to the method of Litterscheid and Thimme[a] (by combining chlormethyl ether and trimethylamine). It was not obtained in pure form; the platinum salt contained 32.72 per cent platinum (calculated: 31.65 per cent).

Summary.—This compound caused a fall or a fall and rise of pressure before atropine and a rise after atropine. It was about one-half as active as formocholine before atropine and about as active as this after atropine (Exper. 290). It was twice as poisonous for mice as formocholine.[b]

$$ClN\begin{matrix}(CH_3)_3\\ CH_2COOH\end{matrix}$$

BETAINE CHLORIDE.

Kalbaum's preparation was used.

Summary.—This substance had no effect upon the blood pressure when injected in doses of 1 or 2 cc. of a 1 per cent solution (Exper. 290). The results thus agree with those already reported by Mott and Halliburton.

$$ClN\begin{matrix}(CH_3)_3\\ CH_2CH_2O(CH_3CO).\end{matrix}$$

ACETYL-CHOLINE CHLORIDE.

Preparation.—This compound was prepared by the method described by Nothnagel.[c] The sirupy product, obtained by heating acetyl chloride and dry choline chloride in a sealed tube in the water bath, was poured into anhydrous ether, allowed to stand a short time, the ether decanted, and the product washed, by decantation, with dry ether. After long standing in the dessicator the product became crystalline. A small quantity was converted into the platinum double salt, the salt recrystallized from hot water and analyzed.

0.2657 gm. salt contained 0.0739 gm. platinum.

Calculated for:

$(ClN(CH_3)_3\ C_2H_4O.\ CH_3CO)_2\ PtCl_4$	Found
27.82 per cent platinum.	27.81 per cent

[a] Litterscheid and Thimme, Liebig's Annalen, v. 316, p. 166, and v. 334, pp. 5 and 63.

[b] Meyer (Liebig's Annalen, 1904, 337, p. 50) compared the toxicity of the ethyl ether of formocholine with that of the ethyl ether of choline; the former was slightly more toxic than the latter. Both were much more toxic than choline.

[c] Nothnagel, Archiv der Pharmazie, 1894, 232, p. 266.

The other acyl derivatives of choline and analogous compounds discussed in this paper were prepared in a manner similar to the above. The acetyl and similar derivatives by heating the chloride of the base with acetyl chloride in a sealed tube or by boiling it with acetic anhydride in a flask fitted with a reflux condenser; the benzoyl derivatives by heating the chloride of the base with benzoyl chloride in an evaporating dish, covered with a watch glass, on the water bath, or by heating it with benzoic anhydride in a beaker in a paraffine bath at 150° for six hours and treating the reaction product as described above).

With the exception of acetyl-choline, which underwent some hydrolysis by the treatment, all of the acyl derivatives of choline and a number of those of the other compounds were converted into the platinum or gold double salts; the chloride of the base was freed from these for the physiological experiments by the action of hydrogen sulphide.

In decomposing the gold and platinum double salts with hydrogen sulphide it was found advantageous to add the calculated amount of sodium bicarbonate to the solution or suspension of the metal salt in water to neutralize the hydrochloric acid resulting from the decomposition of the salt, since this acid tends to hydrolyze the acyl derivatives. The metal sulphide was filtered off, the solution slightly warmed, and the hydrogen sulphide displaced by a rapid stream of carbon dioxide.

Summary.—This compound is extraordinarily active in causing a fall of blood pressure before atropine; after atropine it has little, if any, effect upon the circulation. It sometimes causes a slight slowing, sometimes a slight acceleration, of the heart.

Injected subcutaneously into mice it was about three times as toxic as choline; injected into rabbits and cats intravenously it was far more toxic than choline, but the difference between the dose producing a marked effect upon the blood pressure and the fatal dose was extraordinarily great, the latter being at least several hundred times as large as the former. After atropine very large doses could be injected without causing death.

In one experiment (220) 1 cc. of a solution 1 to 5,000 caused a rapid fall of blood pressure and in about thirty seconds final stoppage of the heart. In another experiment a still smaller amount seemed to cause death. Different animals showed great differences in their response to this drug. This is shown in the protocols of the experiments (214, for example). Comparisons (on the same animal) of the activity of acetyl-choline and that of certain other drugs having a marked action on the circulation gave interesting results. In experiments 248 and 252, for example, it was found that acetyl-choline was about 100,000 times as active in causing a fall of pressure as was choline and hundreds of times as active as nitroglycerine. It was hundreds of times more active in causing a fall of pressure than was epinephrine in causing a rise of pressure.

The above experiments also show what minute amounts of acetyl choline suffice to cause a fall of pressure. Thus in some cases on two-hundred millionth of a gram (0.000,000,005 gm.) sufficed to caus a distinct effect upon the blood pressure of a large rabbit. In othe words, 1 cc. of a solution containing but one mgm. in 200 liters o saline solution (or one sixty-fourth of a grain in 50 gallons) was dis tinctly active.[a]

Experiment 252 was one of the few experiments in which a second ary rise of pressure followed the fall caused by acetyl-choline. N curare had been given, and it is possible that the rise of pressure wa the result of slight muscular movements. The usual absence of a ris after curare supports this suggestion.

Experiment 248 was somewhat unusual in that the injection o acetyl-choline, in comparatively small doses, caused a very consider able slowing of the heart after section of the vagi. In experiment 23 there was an acceleration (vagi intact).

Experiment 252 (quoted above) well illustrates the action of atropin in preventing a fall of pressure from a small dose of acetyl-choline Thus, before atropine 1 cc. of a solution 1 to 200,000,000 caused a fa of pressure; after 6 mgms. of atropine a solution of 1 to 250,000 (80 times as strong) had no effect. A solution ten times as strong as th latter, however, caused a marked fall, which was, however, completel abolished by a dose of 4 mgms. of atropine. When, however, a sti stronger solution of acetyl-choline was injected there was again a fa of pressure.[b]

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[a] We have shown elsewhere (Brit. Med. Jour., 1906, ii, p. 1791) how a test f choline, by which 0.01 and probably 0.001 mgm. of it may be detected, may be base upon this great physiological activity of acetyl-choline.

[b] The effect of atropine in abolishing the action of acetyl-choline upon the bloc pressure seems to take a somewhat different course from its effect upon the slowin of the heart rate from stimulation of the vagus. Thus in an experiment (303) upo a dog of 6.8 K. 2 cc. of a solution of acetyl-choline 1 to 1,000,000 caused a fall of pre sure of 16 mm.; 2 cc. of a solution 1 to 100,000 caused by a fall of 38 mm. After th injection of 0.002 mgr. of atropine sulphate these solutions had no effect, where there was no detectable impairment of the ability of the vagus to slow or stop th heart upon electrical stimulation of this nerve. 2 cc. of a solution of acetyl-cholin 1 to 10,000 caused the pressure to fall 51 mm.; this effect was completely abolishe by a second injection of 0.002 mgr. atropine, although this again had no detectab effect upon the electrical stimulation of the vagus. 2 cc. of a solution of acetyl-cholin 1 to 1,000 now caused a fall of pressure of 68 mm.; a third injection of 0.002 mg atropine sulphate reduced this fall to 28 mm., but still had no effect upon the ele trical stimulation of the vagus. Thus, after 0.006 mgr. of atropine sulphate, abo 2,000 times as much acetyl-choline was required to produce a given effect upon th blood pressure as before, yet there was no apparent change in the effect of stimula ing the vagus electrically. After an injection of 0.02 mgr. atropine sulphate stim lation of the vagus caused a slight acceleration of the heart; strong solutions acetyl-choline still caused a fall of pressure. Of course, it is possible that with mo

Similar results were obtained in experiments 243 and 257. Experiments 221, 235, 246, 248, and 257 illustrate how the rise of pressure caused by epinephrine may be overcome by acetyl-choline.

After repeated injections of epinephrine or homorenon, acetyl-choline seemed to become less effective in causing a fall of pressure, but the experiments were not sufficiently numerous to permit of a definite conclusion.

Nicotine, in large doses, had no effect upon the fall of pressure (Exper. 278); we found this to be true also for the fall of pressure from choline, as was also observed by Mott and Halliburton.

Several illustrations have been given in the above protocols of the effect of acetyl-choline upon the heart rate. In by far the larger number of experiments, in which doses having but a moderate effect upon the blood pressure were employed, there was no effect upon the heart rate unless the latter was unusually slow or unusually rapid. In the former case (if the vagi were intact) there was frequently a slight acceleration, in the latter a slowing.[a] Sometimes, after section of the vagi, if, for some reason, the heart was beating very slowly an injection of acetyl-choline having a very pronounced effect upon the blood pressure caused an acceleration. Thus in one experiment the heart rate had fallen to fourteen beats in ten seconds as the result of the injection of an extract of brain; acetyl-choline caused the pressure to fall from 152 to 36 mm. and the heart rate to increase to forty beats in ten seconds. In another case a small dose of acetyl-choline caused a slowly beating heart to double its rate, probably as a result of removing a heart block.

Large, toxic doses sometimes caused a slight long continued slowing of the heart after section of the vagi and also after atropine; this effect was overcome by stimulation of the accelerator nerves. (Exper. 258.) Very large doses caused great slowing (Exper. 223) and extreme irregularity and not infrequently complete final stoppage of the heart.

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carefully graduated electrical stimuli a diminution of the vagus effect could have been detected after these small doses of atropine.

Results similar to the above were obtained with hyoscyamine, eumydrine, and scopolamine; the latter was, however, more active than the others. Homatropine seemed to diminish the effect of acetyl-choline and of electrical stimulation simultaneously, but large doses were necessary. Euphthalmine seemed to abolish the effect of vagus stimulation before that of acetyl-choline; comparatively large doses were necessary (perhaps 40-80 times as much as of scopolamine).

The marked action of minute amounts of atropine and related compounds in reducing the effect of acetyl-choline upon the blood pressure might be of value in detecting these alkaloids in medico-legal and other cases. Their effect on the fall of blood pressure caused by muscarine is similar.

Caffeine, calcium chloride, and sodium oxalate, injected intravenously, had no effect upon the fall of pressure caused by acetyl-choline.

0.1 gm. of acetyl-choline injected subcutaneously into a dog weighing 6.4 K. had no distinct effects beyond causing considerable salivation.

[a] The condition is analogous to the results of the stimulation of a sensory nerve; if the heart is beating slowly, an acceleration usually results; if it is beating rapidly, a slowing. (Hunt, Amer. Jour. of Physiol., 1899, 2, p. 451.)

Acetyl-choline was fairly active when injected subcutaneously. (Exper. 264.)

We propose to investigate in detail the action of this substance upon the circulation. From the results so far obtained we are inclined to the view that the blood pressure lowering action of acetyl-choline is due largely to an effect upon the heart, probably due to a stimulation of those structures in which the "weakening" fibers of the vagus end; the fact that an amount of atropine sufficient to abolish the effect upon the heart rate of electrical stimulation of the vagus does not completely abolish (although it very greatly diminishes) the effect of the acetyl-choline, suggests that the action is on a "receptive substance" rather than upon "nerve endings;" larger amounts of atropine paralyze more and more of this substance until finally the effect of acetyl-choline is completely abolished. Support for this hypothesis was afforded by experiments with the myocardiograph: Before atropine there was a lessening of the systole, after atropine there was no effect.[a]

$$ClN\begin{matrix}(CH_3)_3\\ C_2H_4O(C_2H_5CO).\end{matrix}$$

PROPIONYL-CHOLINE CHLORIDE.

0.1827 gm. of the platinum compound contained 0.0490 gm. platinum.

Calculated for:

$(ClN(CH_3)_3\ C_2H_4O(C_2H_5CO))_2\ PtCl_4$	Found
26.76 per cent platinum.	26.82 per cent.

Summary.—This compound caused a fall of blood pressure before atropine and a rise after it. (Exper. 218, 221, 223, 243.) It was more, perhaps 100 times, active than choline in causing a fall of pressure, but of about the same activity in causing a rise of pressure. In some cases there was a considerable slowing of the heart (Exper. 223); this was abolished by section of the vagi (Exper. 222); the fall of pressure occurred independently of the slowing of the heart. The rise, after atropine, was overcome by stimulation of the depressor. (Exper. 225.)

$$ClN\begin{matrix}(CH_3)_3\\ C_2H_4O(C_3H_7CO)\end{matrix}$$

NORMAL BUTYRIL-CHOLINE CHLORIDE.

Preparation.—The platinum salt of the compound (obtained from choline chloride and the anhydride of the acid) gave the following results on analysis:

[a] Dr. Schultz found no effect upon the outflow when comparatively strong solutions of acetyl-choline were added to Ringer's solution which was being perfused through the vessels of a guinea pig.

0.1716 gm. of the salt gave 0.0443 gm. platinum.

Calculated for:

$ClN(CH_3)_3\ C_2H_4O(C_3H_7CO))_2\ PtCl_4$ — Found

25.77 per cent platinum. — 25.81 per cent.

Decomposition of the platinum salt by means of hydrogen sulphide resulted in the decomposition of the substance. The salt was therefore decomposed by adding a solution of potassium chloride to the hot solution of the platinum salt and allowing the solution to evaporate in a vacuum desiccator and extracting the residue with absolute alcohol; part of this solution was precipitated with platinum chloride and the platinum content again determined.[a]

Summary.—The most frequent effect of the injections of normal butyril-choline was a fall of pressure followed by a rise (Exper. 236, 246); sometimes there was only a rise. There was usually a marked slowing of the heart if the vagi were intact (Exper. 236); section of the vagi prevented or diminished (Exper. 254) the slowing, but it did not prevent the fall of pressure. After atropine there was a rise of pressure. The compound was more active than choline both in causing a fall and a rise of pressure. (Exper. 256.) It was more active than propionyl-choline in causing a rise. (Exper. 243.)

ISO-BUTYRIL-CHOLINE.

Preparation.—This compound was prepared in the same manner as the normal compound. The platinum salt gave the following figures on analysis:

0.1709 gm. of the salt gave 0.0443 gm. platinum.

Calculated for:

$ClN(CH_3)_3\ C_2H_4O(C_3H_7CO))_2\ PtCl_4$ — Found

25.77 per cent platinum. — 25.92 per cent.

Summary.—This compound usually caused a fall of pressure before atropine and a rise after it. Occasionally it caused a rise before atropine. It caused a slowing of the heart when the vagi were intact, but we have only a few experiments on this point.

It usually caused a somewhat greater fall of pressure than did the normal compound and sometimes caused only a fall when the latter caused a fall and a rise or had no effect. (Exper. 240, 246.) After atropine it was usually somewhat less active in causing a rise of pressure than was the normal compound. (Exper. 256.)

The normal compound was, as a rule, more active in causing a rise of pressure and the iso in causing a fall.

$$ClN\begin{matrix}(CH_3)_3 \\ C_2H_4O(C_4H_9CO)\ \text{(iso)}.\end{matrix}$$

[a] A better method of purifying this compound would doubtless be the ether method described under acetyl-choline.

ISO-VALERYL-CHOLINE CHLORIDE.

Analysis of the platinum double salt gave the following figures for platinum:

0.1706 gm. of the salt gave 0.0424 gm. platinum.

Calculated for:

$(ClN(CH_3)_3\ C_2H_4O\ (C_4H_9CO))_2\ PtCl_4$ — Found

24.85 per cent platinum. — 24.85 per cent.

Summary.—This compound had a marked action in slowing the heart; this was prevented by atropine (Exper. 225) and prevented or greatly diminished (Exper. 254) by section of the vagi; after section of the vagi (Exper. 222) and after atropine (Exper. 221) there was sometimes an acceleration of the heart. Small doses caused a fall (Exper. 255, 256) or a fall followed by a rise (Exper. 222, 225) or only a rise (Exper. 218) of blood pressure. The fall of pressure was due largely, but not entirely, to central vagus stimulation. After atropine, and usually after section of the vagi, there was a marked rise of pressure. It was far more active in causing a rise of pressure than was choline (Exper. 256). In some cases there was a marked rise of pressure notwithstanding a marked slowing of the heart (Exper. 225).

$$\left(ClN\begin{matrix}(CH_3)_3\\(C_2H_4O)\end{matrix}\right)_2 C_2H_4(CO)_2$$

SUCCINYL-CHOLINE CHLORIDE.

Analysis of the platinum salt of the compound gave the following figures for platinum:

0.1047 gm. of the salt gave 0.0290 gm. platinum.

Calculated for:

$(ClN(CH_3)_3\ C_2H_4O.\ \ COCH_2)_2\ PtCl_4$ — Found

27.91 per cent platinum. — 27.70 per cent.

Summary.—The most marked effects of succinyl-choline were slowing of the heart (from central vagus stimulation) (Exper. 222, 223) and (after section of the vagi (Exper. 221) or atropine) a marked and long continued rise of blood pressure. With intact vagi there was usually a fall of blood pressure, due apparently to the vagus effect. Occasionally, after section of the vagi, injections of the compound caused a halving of the ventricular heart rate. The compound overcame, to a very considerable extent, the fall of blood pressure from acetyl-choline (Exper. 221). Stimulation of the depressor caused a diminution of the rise from succinyl-choline (Exper. 221). Valeryl-choline, which has an effect similar to the succinyl compound, was several times (15 to 25) as active.

$$ClN\begin{matrix}(CH_3)_3\\CH_2CH_2O(C_{15}H_{31}CO)\end{matrix}$$

PALMITYL-CHOLINE CHLORIDE.

Preparation.—This compound was prepared by heating dry choline chloride with an excess of palmityl chloride for three hours at 100°. The reaction product was poured into cold alcohol, filtered, precipitated with alcoholic platinic chloride, filtered, washed with alcohol and with ether, the platinum double salt finally being boiled up with a considerable volume of water in which the double salt is difficultly soluble. Analysis of the salt, dried at 110°:

0.2281 gm. salt gave 0.0406 gm. platinum.

Calculated for:

$(C_{21}H_{44}NO_2)_2\ PtCl_6$	Found
17.84 per cent platinum.	17.80 per cent.

Summary.—This compound was very active in causing a fall of pressure before atropine (Exper. 297, 298); in one case it caused a very slight rise after atropine (Exper. 297). It seemed to be from 10 to 20 times as active as choline in causing a fall of pressure but was much less active than this in causing a rise.

$$ClN\begin{matrix}(CH_3)_3\\ CH_2CH_2O(C_5H_{10}BrCO)\end{matrix}$$

α-BROM-ISO-CAPRONYL-CHOLINE CHLORIDE.

Preparation.—This compound was prepared in the same way as the palmityl, using α-bromisocapronylchloride.

Analysis of the salt dried at 110°:

0.1287 gm. salt gave 0.0263 gm. platinum.

Calculated for:

$(C_{11}H_{23}NO_2Br)_2\ PtCl_6$	Found
20.09 per cent platinum.	20.43 per cent.

Summary.—This compound caused a rise of pressure followed by a fall before atropine (Exper. 298); it was much more active than choline in causing a rise, but about equally active in causing a fall of pressure. After atropine it caused a rise of pressure, being more active in this respect than choline (Exper. 297, 298). One cc. of a strong solution (2.7 per cent) caused the pressure to rise 82 mm. (from 95 to 177) (Exper. 298).

This compound was slightly more toxic than choline.[a]

$$ClN\begin{matrix}(CH_3)_3\\ C_2H_4O(C_6H_5CO)\end{matrix}$$

[a] See Note A at end of text.

BENZOYL-CHOLINE CHLORIDE.

Preparation.—This compound was made and described by Nothnagel.[a] Much of that used by us was made from egg choline; the preparation contained 23.63 per cent platinum: calculated for

$$\left(ClN\begin{matrix}(CH_3)_3\\ CH_2CH_2O(C_6H_5CO)\end{matrix}\right)_2 PtCl_4,\ 23.65 \text{ per cent.}$$

Later some benzoyl-choline was prepared from the choline made by the "methyl-iodide process" (which see) and benzoyl-chloride. The melting point of the platinum double salt was 239° to 242° with decomposition. The melting point given by Nothnagel for the platinum double salt of benzoyl-choline (made from choline prepared from ethylene bromide and tri-methyl-amine) was 206°.

I. 0.1294 gm. of the platinum salt gave 0.0305 gm. platinum.
II. 0.1126 gm. of the platinum salt gave 0.0267 gm. platinum.
Calculated for:

$$\left(ClN\begin{matrix}(CH_3)_3\\ CH_2CH_2O(C_6H_5CO)\end{matrix}\right)_2 PtCl_4$$

	Found	
	I.	II.
23.65 per cent platinum.	23.57 per cent.	23.71 per cent.

Summary.—Very small doses of benzoyl-choline usually caused a fall of blood pressure (Exper. 257, 258, 267) being in this respect often more active than choline (Exper. 257). Large doses caused a rise of pressure (Exper. 214, 236, 257, 258, 270, 282, 284). In either event, the vagi being intact, there was sometimes a slowing of the heart (Exper. 236), this was usually, but not always (Exper. 267), slight. Frequently, however, there was an acceleration (Exper. 214, 223, 270, 284); this was especially marked if the heart was beating slowly. After atropine there was almost always a rise of pressure from both large and small doses (Exper. 225, 236, 258, 266); this was often quite marked, much more so than after choline. The rise of pressure was not prevented by nicotine (Exper. 279, 283, 284). Stimulation of the depressor overcame the effect of benzoyl-choline (Exper. 225). Subcutaneous injections had no effect on the blood pressure (Exper. 270, 284).

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[a] Nothnagel. Archiv der Pharmacie, 1894, 232, p. 267.

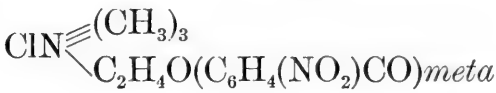
The platinum double salt of benzoyl-choline is very slightly soluble in water and is useful for identifying choline, the latter being converted into the benzoyl derivative by heating with benzoyl chloride or anhydride; 0.1 mgm. of choline may readily be detected in this manner (Hunt and Taveau, Brit. Med. Jour., 1906, ii, p. 1788).

While as a rule benzoyl-choline after repeated injections (Exper. 270, 288) or toward the end of an experiment had little effect in causing a rise of pressure (thus indicating that its action is on some mechanism easily fatigued or injured) it occasionally has a marked effect under such circumstances (Exper. 258).

In some experiments the rise of pressure from benzoyl-choline was comparable with that caused by members of the epinephrine series; in others it was almost inactive when the latter were very active (Exper. 281, 282).

The rise of pressure seemed to be due in part, perhaps largely, to a stimulation of the heart; myocardiograms showed a marked increase in the extent of systole and a much less increase in diastole.

The benzoyl-choline made from choline prepared by the "methyl-iodide process" seemed to have a more pronounced action in causing a slowing of the heart and a fall of pressure (before atropine) (Exper. 267) and a rise after atropine (Exper. 272, 279), although this difference was not constant (Exper. 272, 280).



m-NITRO-BENZOYL-CHOLINE CHLORIDE.

Analysis of the platinum salt of this compound gave the following results:

0.2548 gm. of the salt gave 0.0542 gm. platinum.

Calculated for:

$(ClN(CH_3)_3C_2H_4O\ C_6H_4(NO_2)CO)_2\ PtCl_4$	Found
21.31 per cent platinum.	21.27 per cent.

Summary.—The experiments with this compound were not very satisfactory; the compound seemed to be rather toxic. The most marked effect, after small doses, was a fall of pressure, long continued (Exper. 229, 231, 258). Usually there was a slowing of the heart (Exper. 229, 232). After section of the vagi the same effect was either not obtained (Exper. 231) or only obtained with stronger solutions (Exper. 258). After atropine still stronger solutions were necessary (Exper. 258). In one experiment (229), with a rather strong solution, the fall was followed by a rise of pressure, and this again by a fall with slow, irregular heart.

Its activity in causing a fall of pressure seemed to be about as great as that of benzoyl-choline (Exper. 258) and greater than that of choline (Exper. 232). It differed from these in not usually causing a rise of pressure after atropine.

p-NITRO-BENZOYL-CHOLINE CHLORIDE.[a]

Analysis of the platinum salt gave the following results:

0.1171 gm. of the salt gave 0.0250 gm. platinum.

Calculated for:

$(ClN(CH_3)_3C_2H_4OC_6H_4(NO_2)CO)_2\ PtCl_4$	Found
21.31 per cent platinum.	21.35 per cent.

Summary.—Weak solutions caused a fall of pressure (Exper. 231, 232) and slowing of the heart (Exper. 232) or had no effect (Exper. 258). The fall of pressure and slowing of the heart were abolished by section of the vagi (Exper. 231). After section of the vagi (Exper. 258) and still more after atropine (Exper. 231) it was fairly active in causing a rise of pressure.

The meta compound had a more pronounced tendency to lower the blood pressure, the para compound to increase it. The activity of the latter was often about the same as that of benzoyl-choline. The experiments with this compound were not, however, so very numerous.

$$ClN\begin{matrix}(CH_3)_3\\ C_2H_4O(CH_2C_6H_5CO)\end{matrix}$$

PHENYL-ACETYL-CHOLINE CHLORIDE.

Analysis of the platinum salt gave the following result:

0.1680 gm. of the salt gave 0.0385 gm. platinum.

Calculated for:

$(ClN(CH_3)_3C_2H_4O(CH_2C_6H_5CO))_2\ PtCl_4$	Found
22.87 per cent platinum.	22.91 per cent.

Summary.—One of the most marked effects of this compound, if the vagi were intact, was a slowing of the heart (Exper. 222, 223). There was sometimes a rise (Exper. 223), sometimes a fall (Exper. 257), sometimes a fall followed by a rise (Exper. 222) of blood pressure. The fall was prevented by section of the vagi (Exper. 217, 219, 222) or atropine (Exper. 223, 225); it was obviously due to stimulation of the cardio-inhibitory center. It was usually much more active in causing a rise of pressure than was benzoyl-choline (Exper. 219, 223, 225) or phenyl-propionyl-choline (Exper. 222); it was about 20 times as active as the former (Exper. 223) and twice as active as the latter (Exper. 222). Stimulation of the depressor overcame completely the rise of pressure (Exper. 225) caused by phenyl-acetyl-choline.

$$ClN\begin{matrix}(CH_3)_3\\ C_2H_4O(CH_2(C_6H_5)CH_2CO)\end{matrix}$$

[a] Efforts were made to prepare compounds of choline with benzene sulphonic acid and benzene disulphonic acid chlorides, but they were unsuccessful.

β-PHENYL-PROPIONYL-CHOLINE CHLORIDE.

Analysis of the platinum salt gave the following result:

0.1096 gm. of the salt gave 0.0243 gm. platinum.

Calculated for:

$(ClN(CH_3)_3C_2H_4O(CH_2(C_6H_5)CH_2CO)_2\ PtCl_4$ — Found

22.14 per cent platinum. — 22.17 per cent.

Summary.—With vagi intact, this compound caused a slowing of the heart (Exper. 222, 223, 226, 227) and usually a rise of pressure (Exper. 222, 223, 226, 227); occasionally there was a fall of blood pressure (Exper. 222, 227), due apparently to the slowing of the heart. After section of the vagi (Exper. 218) or the administration of atropine there was invariably a rise of pressure (Exper. 222, 227). Stimulation of the depressor overcame the rise of pressure (Exper. 221, 227). It was more active in causing a rise of pressure than was benzoyl-choline (Exper. 223), but less active than phenyl-acetyl-choline (Exper. 222, 223).

$$ClN\begin{cases}(CH_3)_3\\ C_2H_4O(C_6H_4(OCH_3)CO)\end{cases}$$

ANISYL-CHOLINE CHLORIDE.

Analysis of the platinum salt:

0.1395 gm. of the salt gave 0.0305 gm. platinum.

Calculated for:

$(ClN(CH_3)_3C_2H_4O(C_6H_4(OCH_3)CO)_2\ PtCl_4$ — Found

22.04 per cent platinum. — 21.87 per cent.

Summary.—This compound caused a slowing of the heart (Exper. 222, 223, 224) and a fall of pressure when the vagi were intact (Exper. 219, 222, 224). After section of the latter, and sometimes before it (Exper. 223), the more usual effect was a rise of pressure (Exper. 220, 221, 222). Small doses, however, sometimes caused a fall (Exper. 219, 236) after section of the vagi. After atropine there was a rise (Exper. 221, 236). There was occasionally a slight acceleration of the heart (Exper. 236). The rise of pressure was overcome by acetyl-choline (Exper. 220). It was less active than benzoyl-choline (Exper. 219, 235) in causing a rise of pressure. The fall of pressure seemed to depend largely on the cardiac slowing.

$$ClN\begin{cases}(CH_3)_3\\ C_2H_4O(C_6H_5CH{:}CHCO)\end{cases}$$

CINNAMYL-CHOLINE CHLORIDE.

Analysis of the platinum salt:

0.1363 gm. of the salt gave 0.0303 gm. platinum.

Calculated for:

$(ClN(CH_3)_3C_2H_4OC_6H_5CH:CHCO)_2\ PtCl_4$ Found

22.24 per cent platinum. 22.23 per cent.

Summary.—This compound caused a fall of pressure both before (Exper. 229, 231) and after section of the vagi (Exper. 231, 236) and after atropine (Exper. 231, 236). With intact vagi there was a slowing (Exper. 229) and sometimes an almost complete stoppage of the heart (Exper. 236, 257); in other cases there was almost no effect. There was no effect on the heart rate after section of vagi. In two cases (vagi intact) the fall of pressure was followed by a considerable rise (Exper. 236, 257). The compound seemed quite toxic.

$$\left(ClN\begin{matrix}(CH_3)_3\\ C_2H_4O\end{matrix}\right)_2 C_6H_4(CO)_2$$

PHTHALYL-CHOLINE CHLORIDE.

The analysis of the platinum salt gave the following result:

0.1710 gm. of the salt gave 0.0446 gm. platinum.

Calculated for:

$(ClN(CH_3)_3C_2H_4O)_2C_6H_4(CO)_2)\ PtCl_4$ Found

26.12 per cent platinum. 26.08 per cent.

Summary.—But four experiments were performed with this compound. Injections of 1 c.c. of 0.1 and 1 per cent solutions had no effect on blood pressure or heart rate (Exper. 221); in one case 2 c.c. of a 1 per cent solution caused a fall of 18 mm.

$$ClN\begin{matrix}(CH_3)_3\\ CH_2CH_2CH_2OH\end{matrix}$$

γ-HOMOCHOLINE.

TRI-METHYL-OXY-N-PROPYL AMMONIUM CHLORIDE.

This substance was prepared by the method of Partheil[a].

I. 0.3759 gm. of the platinum salt gave 0.1147 gm. platinum.

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[a] (Note during proof reading.) Malengreau and Lebailly (Hoppe-Seyler's Zeitschrift für physiolog. Chemie, 1910, 67, p. 35) have recently expressed doubts whether the compound prepared by Partheil had the constitution ascribed by him to it. They think the molecular structure was probably altered. Malengreau and Lebailly report a new synthesis. The experiments described in the text were completed before the appearance of Malengreau and Lebailly's paper. We have prepared γ-homocholine by the method described by these writers and have also repeated our experiments with the product obtained by the Partheil method. The latter product can be separated, by means of their platinum salts, into two fractions. One fraction yielded a platinum salt melting, with decomposition, at 225–226°. This agrees with the melting point of the γ-homocholine obtained by the method of Malengreau and Lebailly (227°). The physiological action of this fraction was the same as that of the Malengreau and Lebailly product and as that of the original product as described in the

II. 0.2317 gm. of the platinum salt gave 0.0706 gm. platinum.

Calculated for:

$(C_6H_{16}NO)_2\ PtCl_6$	Found	
	I.	II.
30.26 per cent platinum.	30.51 per cent.	30.47 per cent.

Summary.—This compound caused a fall of blood pressure before atropine (Exper. 269, 270, 271) and a rise (Exper. 269) or no effect after it. It was about as active as choline in causing a fall of pressure (Exper. 269, 270, 271), but was rather less active than this in causing a rise after atropine (Exper. 269). It did not in our experiments cause a rise before atropine as choline sometimes did. It had no effect on the heart rate unless the heart was beating slowly. Large doses of atropine were necessary to prevent the fall of pressure from strong solutions.

$$ClN\begin{matrix}\equiv(CH_3)_3\\ \diagdown CH_2CH_2CH_2O(CH_3CO)\end{matrix}$$

Analysis of the platinum salt gave the following result:

0.1661 gm. of the salt gave 0.0449 gm. platinum.

Calculated for:

$(C_8H_{18}O_2N)_2\ PtCl_6$	Found
26.77 per cent platinum.	27.03 per cent.

Summary.—This compound was extremely active in causing a fall of blood pressure, 1 c.c. of a solution 1 to 1,000,000 being very active (Exper. 275, 278); it was about 1,000 times as active as choline (Exper. 278). It had no effect on the heart rate. The fall of pressure was

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text. It had the same degree of toxicity for mice. The platinum salt of this fraction was, however, very soluble in water, thus differing from the γ-homocholine obtained by the process of Malengreau and Lebailly.

The second, more difficultly soluble, fraction of the platinum salt of the Partheil product melted, with decomposition, at 250–252°, thus agreeing with the platinum salt of β-homocholine as prepared by the method of Morley and as reported by Malengreau and Lebailly (see below). Physiologically, however, this fraction had the same effect as did the first fraction; i. e., its effects were those of a γ-homocholine and not those of β-homocholine, as will be described later. Its toxicity was also approximately that of γ-homocholine.

Both fractions of the Partheil product yielded on treatment with acetic anhydride derivatives corresponding in their gold chloride content to the acetyl derivative of a tri-methyl-oxypropyl-ammonium chloride.

Ackermann and Kutscher (Hoppe-Seyler's Zeitsch., 1908, 56, p. 220) have suggested that perhaps neosin, a trimethylamine derivative having the formula $C_6H_{17}NO_2$ and which they isolated from Liebig's meat extract and from crab extract, is a homocholine. The melting point of the gold salt of neosin (205° to 210°) does not correspond to that of either of the homocholines hitherto prepared. Light upon the structure of neosin could probably be obtained by testing the effect of its acetyl derivative upon the blood pressure. The acetyl derivatives of both of the known homocholines are extremely active physiologically.

abolished by atropine (Exper. 275), sometimes only after very large doses (Exper. 272, 276, 278).

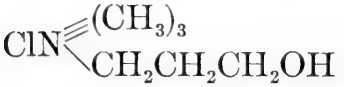
The fall of pressure was not diminished by nicotine (Exper. 278).

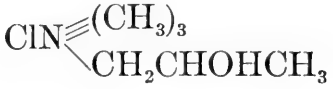
Analysis of the platinum salt gave the following result:

I. 0.2373 gm. of the salt gave 0.0543 gm. platinum.

II. 0.2059 gm. of the salt gave 0.0471 gm. platinum.

Calculated for:	Found	
$(C_{13}H_{20}O_2N)_2\ PtCl_6$	I.	II.
22.87 per cent platinum.	22.88 per cent.	22.87 per cent.

Summary.—This compound was not very active. Before atropine it caused a slight fall (Exper. 270) or a slight rise of pressure, or both (Exper. 272); after atropine it had no effect (Exper. 271, 272) or caused a slight rise. It was much less active than the compound  or benzoyl-choline or choline (Exper. 270).



β-HOMOCHOLINE.

TRIMETHYL-β-OXYPROPYL-AMMONIUM CHLORIDE.

This compound was prepared by the method described by Morley.[a]

I. 0.2054 gm. of the platinum salt contained 0.0623 gm. platinum.

II. 0.1022 gm. of salt gave 0.0311 gm. platinum.

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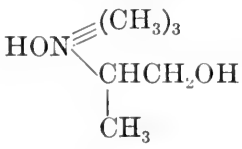
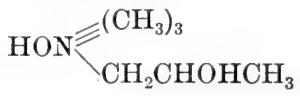
[a] Morley: Comptes rend. de l'Acad. des scien., 91 p. 333; Ber., 1880, 13, p. 1805. Two isomeric homocholines having the structural formulas

 and 

I. II.

respectively, are possible. Morley applied the formula I to the compound prepared by himself from isopropylenechlorhydrine (b. p. 128–130°) and trimethylamine. In a later paper, however (Jour. Chem. Soc., 1885, 47, p. 133), he states that propylene chlorhydrine prepared as was that used in the above synthesis "consists, at any rate for the most part," of $CH_3CH(OH)CH_2Cl$. Hence the β-homocholine prepared by Morley's method consists at least essentially of the compound corresponding to Formula II. So far as we are aware the compound corresponding to Formula I has not been prepared. Malengreau and Lebailly (Hoppe-Seyler's Zeitsch., 1910, 67, p. 35), who evidently overlooked Morley's paper on the constitution of propylene chlorhydrine, state that the compound I was prepared by this author and that compound II, for which they give a new synthesis, had not hitherto been prepared. Morley does not describe the salts of his compound in sufficient detail to enable us to determine how closely it corresponded to the compound obtained by Malengreau and Lebailly, but from the mode of its synthesis it evidently

Calculated for:	Found	
$(C_6H_{16}NO)_2PtCl_6$	I.	II.
30.26 per cent platinum.	30.33 per cent.	30.43 per cent.

consisted at least for the most part of the same. Our preparation melted (with decomposition) at 250–251°, which agrees with the figure (248°) given by these writers.

We have prepared β-homocholine by the method described by Malengreau and Lebailly; the platinum salt of the compound we obtained, however, melted at 265°, instead of at 248° as described by these authors. The product had practically the same effect upon the blood pressure as did the Morley compound except that in some experiments there seemed to be a greater tendency for the fall of blood pressure to be succeeded by a rise with the Morley product than with the Malengreau and Lebailly product. In these more recent experiments the γ-homocholine was about ten times as active as the β compound and its effects were less readily abolished by atropine.

0.15 grm. of β-homocholine injected subcutaneously into a dog weighing 6.4 K. caused no distinct symptoms; 0.1 grm. of γ-homocholine caused some salivation and lacrymation.

Dr. Menge has prepared a "β-homocholine" (or α-methyl-choline) corresponding to formula I above, starting with allyl chloride, converting this into the chlorhydrine, replacing the chlorine by an acetyl group, introducing chlorine into the β position, and then saponifying the acetyl compound, thus obtaining the chlorhydrine $CH_3CHClCH_2OH$. This, on treating with trimethyl-amine yielded a β-homocholine giving a platinum salt melting with decomposition at 253°–254° (i. e., near the point of the β-homocholine corresponding to formula II). This compound had the same effect upon the blood pressure as did the other β-homocholine; its toxicity also was the same.

This compound formed an acetyl derivative corresponding in physiological and toxicological action to similar derivatives of β-homocholine to be described later, except that it was much more active in causing a fall of blood pressure. The benzoyl derivative did not differ markedly in physiological or toxicological action from the benzoyl derivative of the other β-homocholine. 0.09 grm. of the acetyl derivative injected subcutaneously into a dog weighing 6.4 K. caused prompt emesis, defecation, salivation, marked dyspnœa and considerable prostration, followed rapidly by apparent recovery, but death occurred during the night. Death occurred in another dog (6.3 K) after 40 hours from 0.03 grm. 0.002 grm. caused in a dog of 4.75 K., defecation, some salivation, and slow respiration, but no further symptoms. 0.15 grm. of the benzoyl compound injected subcutaneously into a dog of 4.9 K. apparently had no effect.

Dr. Menge also prepared the phenyl-acetyl derivatives of γ-homocholine and of β-homocholine. Neither of these compounds had a marked effect upon the blood pressure. Thus the introduction of the phenyl group into the acetyl group had the same effect as in the case of acetyl choline; it diminished the activity in causing a fall of blood pressure. The phenyl-acetyl-γ-homocholine had a low degree of toxicity (0.5 mgr. per grm. mouse causing no distinct symptoms).

In this connection reference may be made to experiments with homoneurine chloride. Meyer (Liebig's Annalen, 1892, 268, p. 150) had already shown that the action of this compound is different from that of neurine and that it is less toxic than the latter. We found it to be decidedly more active in causing a fall of blood pressure than is β-homocholine; the effect is not easily overcome by atropine. The fall was followed by a marked rise of pressure. It was more toxic for mice than was β-homocholine; it was about as toxic as γ-homocholine. (Fatal dose about 0.2 mgr. per grm. animal.) 0.09 grm. injected subcutaneously into a dog of 6.32 K. caused vomiting and defecation.

Summary.—This compound caused a fall of blood-pressure before atropine (Exper. 269, 270, 271); after atropine it had no effect (Exper. 271) or caused a rise (Exper. 269). It seemed to be rather less active than choline in both respects (Exper. 269, 270, 271).

$$ClN\begin{matrix}(CH_3)_3\\ CH_2CHO(CH_3CO)CH_3\end{matrix}$$

Preparation.—The chloride of trimethyl-β-oxypropyl-ammonium hydroxide was heated for several hours in a sealed tube with an excess of acetyl chloride at the temperature of boiling water. The contents of the tube were filtered through hardened filter paper, the excess of acetyl chloride distilled off, the compound precipitated with an alcoholic solution of platinum chloride and recrystallized from hot water.

I. 0.2387 gm. of platinum salt contained 0.0642 gm. platinum.
II. 0.2980 gm. of platinum salt contained 0.0802 gm. platinum.

Calculated for:	Found	
$(C_8H_{18}O_2N)_2PtCl_6$	I.	II.
26.77 per cent platinum.	26.90 per cent.	26.91 per cent.

Summary.—This compound was very active in causing a fall of pressure before atropine, 1 cc. of a solution 1 to 1,000,000, sometimes causing a marked fall of pressure (Exper. 271, 272, 278); it was often about 1,000 times as active as choline in this respect (Exper. 278). The fall of pressure was much diminished, but in some cases was not completely prevented by very large doses of atropine (Exper. 271, 272, 276, 278); it was not diminished by nicotine (Exper. 278). The compound had no effect upon the heart rate.

$$ClN\begin{matrix}(CH_3)_3\\ CH_2CHO(C_6H_5CO)CH_3\end{matrix}$$

Preparation.—The chloride of the base was treated with an excess of benzoyl chloride in the same manner as in the preparation of the acetyl compound. The contents of the tube were poured slowly into cold absolute alcohol, filtered through hardened paper on a Buchner funnel and precipitated with an absolute alcohol solution of platinum chloride. The precipitate was collected on a Buchner funnel, washed thoroughly with absolute alcohol, then with ether, dried and boiled with a considerable volume of water. On cooling the product was collected on a Buchner funnel and washed successively with water, alcohol, and ether.

I. 0.1795 gm. of the platinum salt gave 0.0422 gm. platinum.
II. 0.2201 gm. of the platinum salt gave 0.0519 gm. platinum.

Calculated for:	Found	
$(C_{13}H_{20}O_2N)_2PtCl_6$	I.	II.
22.87 per cent platinum.	23.51 per cent.	23.58 per cent.

Summary.—This compound caused a fall (Exper. 271), as a rule not very marked, or a fall and a rise (Exper. 272) or simply a rise (Exper. 277) before atropine. At times the rise was marked (Exper. 272). In one case there was a slight acceleration of the heart (Exper. 271). Sometimes there was a slight fall after atropine (Exper. 271); the more usual effect was a rather marked rise of pressure (Exper. 272).

$$ClN\begin{matrix}(CH_3)_3 \\ CH_2CHOHCH_2Cl\end{matrix}$$ [a]

MONO-CHLOR-OXYPROPYL-TRIMETHYL-AMMONIUM CHLORIDE (SEPIN CHLORIDE).

Preparation.—When trimethylamine and α-dichlorhydrine in excess are heated in a closed vessel at 100° for about six hours, two products are formed, the one under discussion and oxy-propylene-hexamethyl-diammonium chloride (see later).[b]

These were separated by means of their gold salts, that of the former being much more soluble in water than that of the latter.

0.1447 gm. of the gold salt gave 0.0581 gm. gold.

Calculated for:

$$N\begin{matrix}(CH_3)_3 \\ CH_2CHOHCH_2Cl\end{matrix} AuCl_4$$

40.11 per cent gold.	Found 40.15 per cent.

Summary.—This compound caused a fall of pressure before atropine (Exper. 269, 270, 287); it was about as active as choline (Exper. 269, 270).

$$ClN\begin{matrix}(CH_3)_3 \\ CH_2CHO(CH_3CO)CH_2Cl\end{matrix}$$

Preparation.—This compound was made by boiling the chloride of the base for five hours with acetic anhydride. The platinum double salt was prepared and recrystallized once from boiling water.

0.1328 gm. of the platinum salt gave 0.0325 gm. platinum.

Calculated for:

$$\left(N\begin{matrix}(CH_3)_3 \\ CH_2CHO(CH_3CO)CH_2Cl\end{matrix}\right)_2 PtCl_6$$

24.45 per cent platinum.	Found 24.47 per cent.

Summary.—This compound caused a fall of pressure before atropine (Exper. 273, 275), the effect diminishing after repeated injections

[a] Equivalent molecular quantities of tribenzylamine and α-dichlorhydrine, α-monochlorhydrine, propylene chlorhydrine, and ethylene chloride, the last in 100 per cent excess were heated for eight hours at 100° in closed vessels without any appreciable amount of addition product resulting.

[b] Schmidt and Hartmann, Liebig's Annalen, 1904, 337, p. 107.

(Exper. 273); it seemed to be about as active as choline. The fall was easily prevented by atropine (Exper. 276).

$$ClN\equiv\begin{matrix}(CH_3)_3\\ CH_2CHO(C_6H_5CO)CH_2Cl\end{matrix}$$

Preparation.—This compound was prepared by heating the chloride of the base with an excess of benzoyl chloride for five hours at 100°. The platinum salt was recrystallized once from hot water.

0.1094 gm. of the platinum salt gave 0.0231 gm. platinum.

Calculated for:

$$\left(N\equiv\begin{matrix}(CH_3)_3\\ CH_2CHO(C_6H_5CO)CH_2Cl\end{matrix}\right)_2PtCl_6$$

	Found
21.16 per cent platinum.	21.12 per cent.

Summary.—This compound caused a fall or a slight rise of pressure before atropine (Exper. 275, 287), being about as active in this respect as choline; there was a very slight rise after atropine.

$$ClN\equiv\begin{matrix}(CH_3)_3\\ CH_2CHOHCH_2OH\end{matrix}$$

β-γ-DIOXYPROPYL-TRI-METHYL-AMMONIUM CHLORIDE[a] (HOMOISOMUSCARIN, GLYCERYL-TRIMETHY-AMMONIUM CHLORIDE).

Preparation.—A 33 per cent solution of trimethylamine in alcohol was heated with a 20 per cent excess of glycerine α-chlorhydrine for several hours in a sealed vessel in boiling water. On opening the bottle no odor of trimethylamine was noticeable. The product was transferred to a distilling flask and the alcohol distilled off in vacuo. Ten c. c. of alcohol were added and again distilled in vacuo; this was repeated three times to remove all traces of trimethylamine. A portion of the product was dissolved in alcohol and precipitated with alcoholic platinum chloride.

I. 0.2071 gm. of the platinum salt gave 0.0598 gm. of platinum.

II. 0.2134 gm. of the platinum salt gave 0.0616 gm. of platinum.

Calculated for:	Found	
$(C_6H_{16}O_2N)_2PtCl_6$	I.	II.
28.82 per cent platinum.	28.87 per cent.	28.87 per cent.

Summary.—This compound caused a fall (Exper. 281, 282, 286, 290) or a fall and rise (Exper. 277) of pressure before atropine, being about as active in this respect as choline (Exper. 281, 286, 290). After atropine it had no effect (Exper. 276, 277, 286, 290).

$$ClN\equiv\begin{matrix}(CH_3)_3\\ CH_2CHO(CH_3CO)CH_2O(CH_3CO)\end{matrix}$$

[a] This compound was prepared by Liebreich, Ber., 1869, 2, p. 167; by Meyer, ibid. p. 186; by Scholten, Inaug. Dissert., Bern., 1892; by Schmidt and Hartmann, Liebig's Annalen, 1904, 337, p. 102. Meyer (ibid., p. 48) showed that it is practically nontoxic.

Preparation.—This compound was prepared by the method of Schmidt and Hartmann, viz, by the use of acetic anhydride.[a]

0.2040 gm. of the platinum salt gave 0.0469 gm. platinum.

Calculated for:

$(C_{10}H_{20}O_4N)_2PtCl_6$ — Found

23.08 per cent platinum. — 22.99 per cent.

Summary.—This compound caused a fall of pressure (Exper. 277, 281, 282, 286) before atropine, being in this respect somewhat more active than choline (Exper. 281, 282). After atropine there was no effect (Exper. 276, 277, 286).

$$ClN\begin{matrix}\equiv(CH_3)_3\\ \diagdown CH_2CHO(C_6H_5CO)CH_2O(C_6H_5CO)\end{matrix}$$

Preparation.—This compound was made from the chloride of the base and benzoic anhydride according to the method described by Schmidt and Hartmann.[b]

0.2802 gm. of the platinum salt contained 0.0509 gm. platinum.

Calculated for:

$(C_{20}H_{24}O_4N)_2PtCl_6$ — Found

17.84 per cent platinum. — 18.17 per cent.

Summary.—This compound caused a fall of pressure both before (Exper. 277, 280, 286) and after atropine (Exper. 277, 279). It was about as active as choline in causing a fall of pressure.

$$ClN\begin{matrix}\equiv(C_2H_5)_3\\ \diagdown CH_2CH_2OH\end{matrix}$$

TRIETHYL-OXYETHYL-AMMONIUM CHLORIDE (ETHYL-CHOLINE).

This substance was prepared by the method of Würtz.[c]

0.1096 gm. of the platinum salt gave 0.0309 gm. platinum.

Calculated for:

$N\begin{matrix}\equiv(C_2H_5)_3\\ \diagdown CH_2CH_2OH\end{matrix}PtCl_6$ — Found

27.83 per cent platinum. — 28.19 per cent.

Summary.—This compound usually caused a fall of pressure before atropine (Exper. 262, 264, 267, 283); in one case it caused a rise followed by a fall (Exper. 263). It was usually slightly less active than choline (Exper. 262, 264, 267, 283). After atropine it usually had no effect (Exper. 262, 264, 276) but in one case it caused a fall (Exper.

[a] Schmidt and Hartmann found that it was impossible to completely acetylate the di-hydroxy compound by treatment with acetyl chloride; our experience was the same.

[b] Schmidt and Hartmann, Liebig's Annalen, 1904, 337, p. 104.

[c] Würtz, Liebig's Annalen, Spl. 7, p. 88. This compound was also prepared and described by Stoermer and Prall, Ber., 1897, 30, p. 1509.

267), in another a slight rise (Exper. 262), and in another a fall followed by a rise.

$$ClN\begin{matrix}(C_2H_5)_3\\ CH_2CH_2O(C_6H_5CO)\end{matrix}$$

Preparation.—This compound was prepared by heating dry triethyl-oxyethyl-ammonium chloride and benzoyl chloride for three hours in a sealed vessel in boiling water. The product was poured into absolute alcohol and precipitated by platinic chloride. The precipitate was filtered off, washed with alcohol and with ether, dried, and recrystallized from water.

0.1232 gm. of the platinum salt gave 0.0263 gm. platinum.

Calculated for:

$$\left(N\begin{matrix}(C_2H_5)_3\\ CH_2CH_2O(C_6H_5CO)\end{matrix}\right)_2 PtCl_6$$

Calculated	Found
21.46 per cent.	21.35 per cent.

Summary.—This compound had very little effect upon blood pressure; it usually caused a slight fall (Exper. 267) before atropine, and a fall (Exper. 267) or no effect after atropine (Exper. 276).

$$ClN\begin{matrix}(C_2H_5)_3\\ CH_2CHOHCH_3\end{matrix}$$

TRI-ETHYL-β-OXYPROPYL-AMMONIUM CHLORIDE.

Preparation.—This compound was prepared in a way analogous to the preparation of the trimethyl compound, triethylamine being substituted for trimethylamine. The acetyl and benzoyl derivatives were prepared in the same way as were the similar derivatives of the trimethyl compound.

0.2085 gm. of the platinum salt gave 0.0561 gm. platinum.

Calculated for:

$(C_9H_{22}ON)_2PtCl_6$

Calculated	Found
26.76 per cent platinum.	26.91 per cent.

Summary.—This compound caused a slow, prolonged fall of blood pressure (Exper. 268, 272, 276, 278, 283, 288) and a slowing of the heart (Exper. 269). It had no effect (Exper. 271, 272, 276) or caused but an insignificant rise (Exper. 269, 283) after atropine. It was sometimes about as active as choline (Exper. 269, 272, 276).

$$ClN\begin{matrix}(C_2H_5)_3\\ CH_2CHO(CH_3CO)CH_3\end{matrix}$$

Analysis of the platinum salt gave the following result:

0.2457 gm. of the salt gave 0.0591 gm. platinum.

Calculated for:

$(C_{11}H_{24}NO_2)_2,\ PtCl_6$

Calculated	Found
23.99 per cent platinum.	24.05 per cent.

Summary.—This compound caused a rather prolonged fall of pressure (Exper. 272, 276, 288) before atropine and no effect after it (Exper. 272, 276). It was not very active, being, perhaps, less active than choline in causing a fall of pressure but differing from this in that the fall was more prolonged. Two c.c. of a 0.7 per cent solution injected subcutaneously caused a fall of 20 mm. in a cat weighing 3.25 k (Exper. 272).

$$ClN\begin{matrix}(C_2H_5)_3\\ CH_2CHO(C_6H_5CO)CH_3\end{matrix}$$

Analysis of the platinum salt gave the following result:

I. 0.2308 gm. of the platinum salt gave 0.0483 gm. platinum.

II. 0.3592 gm. of the platinum salt gave 0.0754 gm. platinum.

Calculated for:

$(C_{16}H_{26}O_2N)_2\ PtCl_6$

	Found	
	I.	II.
20.81 per cent platinum.	20.93 per cent.	20.99 per cent.

Summary.—This compound caused a slow, prolonged fall of pressure (Exper. 271, 272, 276, 277, 278) before atropine; sometimes there was a prolonged slowing of the heart (Exper. 271, 278). After atropine there was no effect (Exper. 272, 276) or, especially with strong solutions (Exper. 271, 278), there was a rise, usually slight, of pressure. The fall of pressure often did not differ greatly from that caused by choline except that it occurred much more slowly and continued for a very much longer time (Exper. 271).

$$ClN\begin{matrix}(C_2H_5)_3\\ CH_2CHOHCH_2Cl\end{matrix}$$

MONO-CHLOR-OXYPROPYL-TRIETHYL-AMMONIUM CHLORIDE.

Preparation.—Triethylamine and α-dichlorhydrine, the latter in 20 per cent excess (based on equivalent molecular weights), were heated in a closed vessel at 100° for about six hours. Two compounds were formed; their separation was effected by means of their gold double salts, the gold salt of the oxy-propylene-hexaethyl-diammonium chloride (see later) being more difficultly soluble in water. The more soluble salt (the one under discussion) gave the following results on analysis:

I. 0.3369 gm. of the gold salt gave 0.1252 gm. gold.

II. 0.1038 gm. of the gold salt gave 0.0385 gm. gold.

Calculated for:

$N\begin{matrix}(C_2H_5)_3\\ CH_2CHOHCH_2Cl\end{matrix}\ AuCl_4$

	Found	
	I.	II.
36.95 per cent gold.	37.16 per cent.	37.09 per cent.

Summary.—This caused a fall of pressure before atropine (Exper. 269, 276, 278). In one case (Exper. 272) there was a fall of pressure followed by a rather marked rise. Sometimes there was a marked slowing of the heart (Exper. 269). It had no effect after atropine (Exper. 272, 276, 278), or caused a slight fall of pressure (Exper. 271, 278).

There were no distinct differences between the effects of this compound and the compound $ClN\begin{matrix}(C_2H_5)_3\\ CH_2CHOHCH_3\end{matrix}$

$$ClN\begin{matrix}(C_2H_5)_3\\ CH_2CHO\ (C_6H_5CO)\ CH_2Cl\end{matrix}$$

Preparation.—This compound was prepared by heating the monochlor-oxypropyl-triethyl-ammonium chloride with an excess of benzoyl-chloride at 100° for six hours. The reaction product was poured into cold absolute alcohol and treated with alcoholic platinum chloride; the yellow gummy precipitate slowly became granular and crystalline on being rubbed with a stirring rod. The platinum salt was recrystallized from hot water. [a]

0.1067 gm. of the platinum salt gave 0.0207 gm. platinum.

Calculated for:

$$\left(ClN\begin{matrix}(C_2H_5)_3\\ CH_2CHO\,(C_6H_5CO)\,CH_2Cl\end{matrix}\right)_2PtCl_4$$

19.38 per cent platinum. Found 19.40 per cent.

Summary.—This compound caused a prolonged fall of pressure before atropine (Exper. 276, 277, 278), being moderately active; it had no effect after atropine (Exper. 276). 1 cc. of a 0.45 per cent solution injected subcutaneously caused a fall of 20 mm. in a cat of 2.72 k. (Exper. 276); in another experiment (278) it had no effect.

At times it seemed more active than the compound $ClN\begin{matrix}(C_2H_5)_3\\ CH_2CHO(C_6H_5CO)CH_3\end{matrix}$ (Exper. 276), at others rather less active (Exper. 277).

$$ClN\begin{matrix}(C_2H_5)_3\\ CH_2CHOHCH_2OH\end{matrix}$$

β-γ-DIOXYPROPYL-TRIETHYL AMMONIUM CHLORIDE.

Preparation.—This compound was prepared by the method of Bienenthal [b] from glycerin-α-chlorhydrine and triethylamine.

I. 0.1110 gm. of the platinum salt contained 0.0284 gm. platinum.

II. 0.1894 gm. of the platinum salt contained 0.0486 gm. platinum.

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[a] The acetyl derivative was prepared in an entirely analogous manner, using, however, acetic anhydride.

[b] Bienenthal, Ber., 1901, 33, p. 3500.

Calculated for:
$(C_9H_{22}O_2N)_2\ PtCl_6$ Found

	I.	II.
25.63 per cent platinum.	25.59 per cent.	25.66 per cent.

Summary.—This caused a slight fall of pressure before atropine (Exper. 276, 277); in one case (Exper. 290) it caused a slight rise After atropine it caused a slight rise or had no effect (Exper. 276, 277). Comparatively strong solutions (0.5 to 1 per cent) had but very slight effect in either case.

$$ClN\begin{matrix}\equiv(C_2H_5)_3\\ \diagdown CH_2CHO(CH_3CO)CH_2O(CH_3CO)\end{matrix}$$ [a]

Analysis of the platinum salt gave the following result:

I. 0.2112 gm. of the salt gave 0.0448 gm. platinum.

II. 0.2122 gm. of the salt gave 0.0450 gm. platinum.

Calculated for:
$(C_{13}H_{26}O_4N)_2\ PtCl_6$ Found

	I.	II.
20.99 per cent platinum.	21.21 per cent.	21.21 per cent.

Summary.—This compound caused a fall of pressure (Exper. 277, 281, 286) before atropine. There was no effect after atropine. (Exper. 276, 277). Strong solutions (0.93 per cent) were necessary to cause any effect.

$$ClN\begin{matrix}\equiv(C_2H_5)_3\\ \diagdown CH_2CHO(C_6H_5CO)CH_2O(C_6H_5CO)\end{matrix}$$ [b]

The platinum salt of this compound is precipitated as a gummy product little soluble in hot water. It was heated with water and with alcohol; on rubbing in a mortar it became granular.

I. 0.1038 gm. of the platinum salt gave 0.0173 gm. platinum.

II. 0.1783 gm. of the platinum salt gave 0.0298 gm. platinum.

Calculated for: Found
$(C_{23}H_{30}O_4N)_2\ PtCl_6$

	I.	II.
16.56 per cent platinum.	16.67 per cent.	16.71 per cent.

Summary.—This usually caused a slow, rather marked fall of pressure before atropine (Exper. 277, 280) and sometimes a rather marked

[a] It is of interest to note that both the acetyl and benzoyl derivatives of this compound were readily obtained by treatment with the acid chlorides, whereas with the corresponding trimethyl compound it was necessary to use the anhydrides to obtain acylation.

[b] The preparation of the β-benzoyl-γ-acetyl and β-acetyl-γ-benzoyl derivatives of β-γ-dioxy-propyl-tri-ethyl ammonium chloride was attempted, starting from β-benzoyl and β-acetyl derivatives of β-oxy-γ-chlor-propyl-triethyl ammonium chloride (prepared as already described). The latter were dissolved in water, digested with

slowing of the heart (Exper. 277). There was little or no effect after atropine (Exper. 276, 277).

$$ClN\begin{matrix}(C_3H_7)_3\\ CH_2CH_2OH\end{matrix}$$

TRIPROPYL-OXYETHYL-AMMONIUM CHLORIDE.

Preparation.—This compound was prepared in a manner analogous to that of the triethyl compound.

0.1004 gm. of the platinum compound contained 0.0253 gm. platinum.

Calculated for:

$$\left(N\begin{matrix}(C_3H_7)_3\\ CH_2CH_2OH\end{matrix}\right)_2PtCl_6$$

24.85 per cent platinum.

Found 25.20 per cent.

Summary.—This compound caused a fall (not as a rule marked, but prolonged) of blood pressure before atropine (Exper. 262, 264, 268, 275). It caused a fall (Exper. 268), sometimes rather marked (Exper. 267), or a slight rise (Exper. 262) or had no effect (Exper. 275) after atropine. It was rather less active than choline (Exper. 262, 264, 268) and about equal to the compound $ClN\begin{matrix}(C_2H_5)_3\\ CH_2CH_2OH\end{matrix}$ (Exper. 264).

$$ClN\begin{matrix}(C_3H_7)_3\\ CH_2CH_2O(C_6H_5CO)\end{matrix}$$

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an excess of moist oxide of silver at ordinary temperature for twenty-four hours, the silver oxide filtered off, the filtrate acidified with hydrochloric acid and concentrated to a sirup in vacuo over sulphuric acid. In the oxide of silver treatment the odor of triethyl-amine developed.

The acetyl derivative was then heated to 100° for five hours with an excess of benzoyl chloride and the benzoyl derivative was boiled with an excess of acetic anhydride for five hours. The cooled reaction products were poured into cold alcohol and alcoholic platinic chloride added. On long standing platinum double salts were precipitated.

Analysis of the platinum salt of the product obtained from the acetic anhydride treatment after recrystallizing once from hot water:

0.1059 gm. salt gave 0.0226 gm. platinum.

Calculated for:

$$\left(N\begin{matrix}(C_2H_5)_3\\ CH_2CHO(C_6H_5CO)CH_2O(CH_3CO)\end{matrix}\right)_2PtCl_6$$ 18.52 per cent platinum.

Calculated for:

$$\left(N\begin{matrix}(C_2H_5)_3\\ CH_2CHO\ (CH_3CO)\ CH_2O\ (CH_3CO)\end{matrix}\right)_2PtCl_6$$ 20.99 per cent platinum.

Found 21.34 per cent platinum.

The benzoyl group had evidently been split out at some step in the process.

Treatment of the acetyl derivatives with benzoyl chloride also failed to yield a compound containing both the acetyl and benzoyl groups.

Preparation.—This compound was prepared by heating dry tripropyl-oxyethyl-ammonium chloride with an excess of benzoyl-chloride for three hours at a temperature of 100°.[a] Alcoholic platinic chloride was added to the solution of the reaction product in absolute alcohol; there was no precipitate. The product was freed from alcohol by distillation in vacuo at a low temperature and excess of ether was added; this caused a precipitation of the platinum double salt as a sticky gum. The precipitate was washed thoroughly with ether to remove benzoic acid. The gummy salt was then thoroughly washed with cold water and finally with absolute alcohol; it became granular. It was then recrystallized from boiling water and dried at 105°.

0.1262 gm. of the platinum salt gave 0.0250 gm. platinum.

Calculated for:

$$\left(N\equiv\begin{matrix}(C_3H_7)_3\\ CH_2CH_2O(C_6H_5CO)\end{matrix}\right)_2PtCl_6$$

Found
19.64 per cent platinum.	19.81 per cent.

Summary.—This compound had very little effect upon the blood pressure; there was usually a slight, rather prolonged fall (Exper. 265, 268) before atropine and nothing (Exper. 268) or a very slight rise (Exper. 265, 267) after it.

$$ClN\equiv\begin{matrix}(C_3H_7)_3\\ CH_2CHOHCH_3\end{matrix}$$

TRIPROPYL-β-OXYPROPYL-AMMONIUM CHLORIDE.

Preparation.—Equimolecular quantities of tripropylamine and propylene chlorhydrine were heated for several hours at the temperature of boiling water. The reaction product separated as a lower layer of liquid; this was pipetted out, dissolved in water, and the aqueous solution extracted with ether to remove any free tripropylamine. The aqueous solution was evaporated down to a thick sirup, dissolved in water, and an aqueous solution of gold chloride added. The resultant gold salt was recrystallized from hot absolute alcohol.

0.1627 gm. of the salt, dried at 110°, gave 0.0596 gm. gold.

Calculated for:

$$N\equiv\begin{matrix}(C_3H_7)_3\\ CH_2CHOHCH_3\end{matrix}AuCl_4$$

Found
36.43 per cent gold.	36.63 per cent.

Summary.—This compound had almost no effect upon blood pressure when injected in doses of 1 c. c. of the 0.5 per cent or 1 per cent solution. There was sometimes an insignificant rise both before and after atropine (Exper. 271); sometimes a very slight fall (Exper. 274).

$$ClN\equiv\begin{matrix}(C_3H_7)_3\\ CH_2CHO(CH_3CO)CH_3\end{matrix}$$

[a] Efforts to prepare the acetyl compound in a similar way were unsuccessful.

Preparation.—Some of the gold double salt of tripropyl-β-oxypropyl-ammonium chloride was dissolved in an excess of acetyl chloride and heated in a sealed vessel for an hour at the temperature of boiling water. The excess of the chloride was removed by evaporation from the water bath, the residue taken up in acetic ether and filtered; the ether was evaporated and the gold salt recrystallized from hot absolute alcohol.

0.0979 gm. of the salt gave 0.0334 gm. of gold.

Calculated for:

$$N\equiv\begin{matrix}(C_3H_7)_3\\ CH_2CHO(CH_3CO)CH_3\end{matrix} \quad AuCl_4$$

33.81 per cent gold. Found 34.12 per cent.

Summary.—This compound caused a fall, usually small, of blood-pressure before atropine (Exper. 279, 283); it had no effect after it (Exper. 278, 283).

$$ClN\equiv\begin{matrix}(C_3H_7)_3\\ CH_2CHO(C_6H_5CO)CH_3\end{matrix}$$

Preparation.—Tripropyl-β-oxypropyl-ammonium chloride, prepared by decomposing the gold double salt with hydrogen sulphide, was heated with an excess of benzoyl chloride. The compound was precipitated by pouring the benzoyl chloride solution into dry ether. The product was washed with dry ether, taken up with water and converted into the platinum double salt by means of aqueous platinic chloride.

0.1347 gm. of the salt dried at 110° gave 0.0265 gm. platinum.

Calculated for:

$$\left(N\equiv\begin{matrix}(C_3H_7)_3\\ CH_2CHO(C_6H_5CO)CH_3\end{matrix}\right)_2 PtCl_6$$

19.10 per cent platinum. Found 19.67 per cent.

Summary.—This compound caused a small, rather prolonged, fall of blood-pressure before atropine (Exper. 278, 279) and practically no effect after it (Exper. 278, 279).

$$ClN\equiv\begin{matrix}(C_3H_7)_3\\ CH_2CHOHCH_2Cl\end{matrix}$$

MONO-CHLOR-OXYPROPYL-TRIPROPYL-AMMONIUM CHLORIDE.

Preparation.—Tripropylamine and α-dichlorhydrine, the latter in 20 per cent excess, based on equimolecular quantities, were heated at 100° for several hours. The reagents are miscible, but after heating separate into two layers. The upper layer consisted largely of un-

changed tripropylamine, as was determined by an analysis of the platinum double salt. The lower layer was removed with a pipette and more α-dichlorhydrine added to the residual amine and the heating continued as before; this was repeated until practically all of the amine had been combined. The reaction product was transferred to a separating funnel and shaken with water, which dissolved it, while much of the excess of the dichlorhydrine goes to the bottom and can be drawn off. The aqueous solution was evaporated on the water bath to a thick sirup; this was then thoroughly washed with ether. The product was dissolved in water and precipitated with an aqueous solution of gold chloride. The gold double salt was precipitated as a sticky mass, which quickly became crystalline. The salt was filtered off and recrystallized from boiling alcohol. When a sufficient amount of absolute alcohol was employed, most of the salt remained in solution; that which separated out on cooling had a lemon-yellow color and a gold content corresponding to that demanded by the gold double salt of oxypropylene-hexapropyl-diammonium chloride. Thus

I. 0.1025 gm. of the salt dried at 110° gave 0.0396 gm. gold.

II. 0.1015 gm. of the salt dried at 110° gave 0.0392 gm. gold.

Calculated for:

$$\begin{array}{l} CH_2N(C_3H_7)_3AuCl_4 \\ | \\ CHOH \\ | \\ CH_2N(C_3H_7)_3AuCl_4 \end{array}$$

	Found	
	I.	II.
38.59 per cent gold.	38.64 per cent.	38.62 per cent.

Only a very small amount of this hexapropyl compound was formed.[a]

The filtrate from the hexapropyl compound was concentrated; leaf-like crystals of gold double salt separated.

0.1105 gm. of the gold salt dried at 110° gave 0.0376 gm. gold.

Calculated for:

$$N\equiv\begin{array}{l}(C_3H_7)_3 \\ CH_2CHOHCH_2Cl\end{array} AuCl_4$$

	Found
34.26 per cent gold.	34.03 per cent.

The filtrate from this precipitate yielded, on further concentration, more of the salt:

0.2099 gm. of the salt gave 0.0718 gm. gold = 34.21 per cent gold.

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[a] A gram of the gold salt of this compound was boiled in acetic anhydride solution for an hour. After precipitation with dry ether and washing with absolute alcohol and with ether and allowing to stand in the desiccator, the salt became crystalline; the gold content, which was 39.27 per cent, did not correspond to that required by an acetyl derivative (37.07 per cent).

Summary.—This compound when injected in the usual doses (1 cc. of 0.1 to 1 per cent solutions) had, in most cases, practically no effect upon the blood-pressure (Exper. 271); in one experiment (274), before atropine, a strong solution caused a rise and a weak one a fall of pressure.

$$ClN\begin{matrix}\equiv(C_3H_7)_3\\ \diagdown CH_2CHO(CH_3CO)CH_2Cl\end{matrix}$$

Preparation.—The gold salt of mono-chlor-oxypropyl-tripropyl-ammonium chloride was dissolved in acetyl chloride and heated for two hours at 100°. The excess of the acid chloride was evaporated from the water bath and the sirupy residue taken up with acetic ether and filtered. The acetic ether was evaporated and the residue crystallized from hot absolute alcohol. The salt was dried at 110°.

0.1551 gm. of the salt gave 0.0496 gm. gold.

Calculated for:

$$N\begin{matrix}\equiv(C_3H_7)_3\\ \diagdown CH_2CHO(CH_3CO)CH_2Cl\end{matrix}AuCl_4$$

31.93 per cent gold. Found 31.98 per cent.

Summary—This frequently caused a very small fall of pressure before (Exper. 275, 277) and no effect after atropine (Exper. 275, 278).

$$ClN\begin{matrix}\equiv(C_3H_7)_3\\ \diagdown CH_2CHO(C_6H_5CO)CH_2Cl\end{matrix}$$

Preparation.—This compound was prepared by heating dry mono-chlor-oxypropyl-tripropyl-ammonium chloride with an excess of benzoyl chloride for three hours at 100°. The benzoyl chloride solution was poured into dry ether which precipitated the benzoyl derivative in a gummy condition; this was thoroughly washed with dry ether. The platinum double salt was precipitated from the aqueous solution by the addition of an aqueous solution of platinum chloride.

0.1068 gm. of the salt dried at 110° gave 0.0189 gm. platinum.

Calculated for:

$$\left(N\begin{matrix}\equiv(C_3H_7)_3\\ \diagdown CH_2CHO(C_6H_5CO)CH_2Cl\end{matrix}\right)_2PtCl_6$$

17.89 per cent platinum. Found 17.70 per cent.

Summary.—This usually caused a fall of pressure (not very pronounced but sometimes prolonged) (Exper. 273) before atropine (Exper. 274, 275, 279) and no effect (Exper. 279) or a slight rise after it (Exper. 273, 274, 275).

$$ClN\begin{matrix}\equiv(C_3H_7)_3\\ \diagdown CH_2CHOHCH_2OH\end{matrix}$$

β-γ-DIOXYPROPYL-TRIPROPYL-AMMONIUM CHLORIDE.

Preparation.—This compound was prepared according to the method of Bienenthal,[a] by heating glycerine-α-chlorhydrine and tripropylamine for six hours in a sealed tube at 140°. After the heating the reaction product was found in the lower layer of viscous sirupy material, while the upper layer consisted largely of unchanged tripropylamine; the latter was treated with more α-chlorhydrine and reheated, this treatment being repeated until the bulk of the amine had been combined. The sirupy reaction product was dissolved in water, shaken out with ether, to remove any free tripropylamine, and precipitated with an aqueous solution of gold chloride. The resultant gold double salt, as soon as it had become crystalline, was filtered off and washed with water, dried on the steam bath, and finally in the desiccator. The gold double salt in this condition is impure and quite soluble in absolute alcohol. It was dissolved in an excess of acetyl chloride and the solution heated for one hour in a closed vessel in boiling water. The excess of acid chloride was evaporated, the residual salt taken up in acetic ether or acetone, filtered, and the solvent evaporated from the water bath. The resultant gold double salt of the di-acetyl derivative is difficultly soluble in cold absolute alcohol and forms a sirupy mass. By boiling the salt with a convenient amount of absolute alcohol, cooling, decanting the alcohol and washing the sirupy residue with more alcohol, the gold double salt of the diacetyl derivative is obtained pure.

0.1852 gm. salt dried at 110° gave 0.0573 gm. gold.

Calculated for:

$$N\begin{matrix}(C_3H_7)_3\\ CH_2CHO(CH_3CO)CH_2O(CH_3CO)\end{matrix} AuCl_4$$

Au = 30.75 per cent gold.	Found 30.94 per cent.

The sirupy gold double salt of the diacetyl compound was treated with hot water and hydrogen sulphide passed into the mixture kept hot by boiling water until the salt was completely broken up. The filtrate from the gold sulphide was now treated with hydrochloric acid and boiled for several hours with a reflux condenser to hydrolyse the the diacetyl compound. From this solution gold chloride throws out the crystalline gold double salt of the β-γ-dioxypropyl-tripropyl ammonium chloride. Analysis of the salt dried at 110°: 0.1609 gm. salt gave 0.0565 gm. gold.

Calculated for:

$$N\begin{matrix}(C_3H_7)_3\\ CH_2CHOHCH_2OH\end{matrix} AuCl_4$$

Au = 35.39 per cent.	Found 35.11 per cent.

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a. Bienenthal. Ber. 1901, 33, p. 3501.

Summary.—The tendency of this compound was to cause a small fall of blood pressure both before and after atropine (Exper. 284, 285, 286); frequently it had no effect.

$$ClN\begin{matrix}(C_3H_7)_3\\ CH_2CHO(CH_3CO)CH_2O(CH_3CO)\end{matrix}$$

Preparation.—The preparation of this compound was described under the preceding compound.

Summary.—Rather large doses of this compound caused a very small fall (Exper. 285, 286) of pressure or a small fall and slight rise (Exper. 284) before and the same or no effect after atropine (Exper. 284, 286). It was scarcely as active as the compound without the acetyl groups (Exper. 286).

$$ClN\begin{matrix}(C_3H_7)_3\\ CH_2CHO(C_6H_5CO)CH_2O(C_6H_5CO)\end{matrix}$$

Preparation.—This compound was prepared by evaporating a solution of dioxypropyl-tripropyl-ammonium chloride to a thick sirup and heating this on the water bath with an excess of benzoyl chloride. After two hours' heating the reaction product was poured into dry ether which precipitated the compound; this was then well washed with more ether. A sample of the material was dissolved in water and converted to its platinum double salt by addition of aqueous platinic chloride.

Analysis of the platinum salt dried at 100°:

0.2680 gm. salt gave 0.0425 gm. platinum.

Calculated for:

$$\left(N\begin{matrix}(C_3H_7)_3\\ CH_2CHO(C_6H_5CO)CH_2O(C_6H_5CO)\end{matrix}\right)_2PtCl_6$$

15.46 per cent platinum. Found 15.86 per cent.

Summary.—This compound was very active in causing a slow, prolonged fall of pressure both before and after atropine (Exper. 284, 286). The fall persisted after nicotine and large doses of atropine (Exper. 284). There was sometimes a marked slowing of the heart before nicotine and a slight acceleration after it (Exper. 284). 1 c.c. of a 1 per cent solution injected subcutaneously into a cat of 3.25 k. had no effect on the blood pressure (Exper. 286).

$$ClN\begin{matrix}(C_5H_{11})_3\\ CH_2CH_2OH^a\end{matrix}$$

[a] Efforts to make a similar compound from triisobutylamine and ethylene chlorhydrine or ethylene bromide were unsuccessful.

TRIAMYL-OXYETHYL-AMMONIUM CHLORIDE.

Preparation.—Kahlbaum's "triamylamine" and ethylene chlorhydrine were brought together in monomolecular proportions and heated for two or three hours in a sealed vessel in the boiling water bath. The reagents are miscible, but at the end of the heating two layers of liquid were present. The upper one was found to consist chiefly of unaltered triamylamine while the lower one was composed largely of triamyl-oxyethyl-ammonium chloride. The lower layer was pipetted out, more chlorhydrine added and the heating continued; this was repeated until nearly all of the amine was used up. The product was transferred to a separating funnel, diluted with a considerable volume of water, and thoroughly shaken. The chloride of the quaternary base went into solution while there remained a supernatant oily layer which was evidently unchanged triamylamine. The aqueous portion was evaporated on the water bath to a thick sirup; this was thoroughly washed with anhydrous ether. The ether deposited a small quantity of colorless crystals; it was not determined whether these were triamylamine hydrochloride or the chloride of the quaternary base. The sirupy reaction product, after thorough washing with dry ether and evaporation of all traces of the latter on the boiling water bath, became a crystalline paste on stirring. Some of this product was dissolved in water and aqueous platinic chloride solution added; a cheesy platinum double salt was thrown down which quickly crystallized on rubbing and kneading with a glass rod.

0.1088 gm. of the platinum salt gave 0.0223 gm. platinum.

Calculated for:

$$\left(N\begin{matrix}(C_5H_{11})_3\\CH_2CH_2OH\end{matrix}\right)_2PtCl_6.$$

	Found
20.46 per cent platinum.	20.50 per cent.

Summary.—This compound usually caused a very small fall of pressure before (Exper. 262, 264, 267) and a very slight rise or nothing after atropine (Exper. 262, 267). It was far less active than choline (Exper. 262).

$$ClN\begin{matrix}(C_5H_{11})_3\\CH_2CH_2O(CH_3CO)\end{matrix}$$

Preparation.—This compound was prepared by heating triamyl-oxyethyl-ammonium chloride with an excess of acetyl chloride in a sealed vessel for three hours in boiling water. The product was transferred to an evaporating dish and the excess of acid chloride evaporated on the water bath at a low temperature. The product was then thoroughly washed with dry ether, dissolved in water, and aqueous

platinic chloride solution added. A gummy red platinum double salt was precipitated. This was washed thoroughly with water and kept for some time in a vacuum desiccator. It slowly became brittle and granular; it was somewhat hygroscopic.

I. 0.1535 gm. of the salt gave 0.0300 gm. of platinum.

II. 0.1189 gm. of the salt gave 0.0233 gm. of platinum.

Calculated for:

$$\left(N\begin{matrix}(C_5H_{11})_3\\CH_2CH_2O(CH_3CO)\end{matrix}\right)_2PtCl_6.$$

	Found	
	I.	II.
19.55 per cent platinum.	19.54 per cent.	19.60 per cent.

Summary.—This usually caused a slight fall (Exper. 268, 269), or a slight rise (Exper 264) of blood pressure before and nothing (Exper. 268) or a very slight rise (Exper. 269) after atropine. Its activity was not very different from that of the compound $ClN\begin{matrix}(C_5H_{11})_3\\CH_2CH_2OH\end{matrix}$

$$ClN\begin{matrix}(C_5H_{11})_3\\CH_2CH_2(C_6H_5CO)\end{matrix}$$

Preparation.—Triamyl-oxyethyl-ammonium chloride was heated with an excess of benzoyl-chloride for three hours at 100°. The reaction product was poured into an excess of aqueous platinic chloride contained in a separatory funnel, ether was added, and the whole shaken. The platinum double salt was thrown out as a red gum adhering to the walls of the funnel. The liquid was removed and the salt shaken first with water, in which it was practically insoluble, then with alcohol, in which it is but slightly soluble, and finally with ether. The salt was then dissolved in a little chloroform, in which it dissolved readily. After evaporation of the chloroform there remained a red sticky gum which, after thoroughly drying in the vacuum desiccator and rubbing for a long time with a glass rod, finally became granular.

0.1296 gm. of the salt (dried for three hours at 115°–120°) gave 0.0217 gm. of platinum.

Calculated for·

$$\left(N\begin{matrix}(C_5H_{11})_3\\CH_2CH_2O(C_6H_5CO)\end{matrix}\right)_2PtCl_6$$

	Found
16.79 per cent platinum.	16.74 per cent.

Summary.—This compound caused a slow and prolonged fall of pressure both before and after atropine (Exper. 265, 266, 267, 268, 269); the fall seemed to be diminished by large doses of atropine

(Exper. 269, 271). The heart was sometimes slowed (Exper. 265, 267). The compound was rather toxic, 1 cc. of a 0.93 per cent solution causing death in two cases.

It was much more active than the compound $ClN\begin{matrix}(C_5H_{11})_3\\CH_2CH_2OH\end{matrix}$

The fall of pressure seemed to be due to a direct action of the drug upon the heart; the myocardiograph showed a diminution of both systole and diastole.

$$ClN\begin{matrix}(C_5H_{11})_3\\CH_2CHOHCH_3\end{matrix}$$

TRIAMYL-β-OXYPROPYL-AMMONIUM CHLORIDE.

Preparation.—This compound was prepared in the same way as the tripropyl compound. The gold double salt was precipitated in a gummy condition, but after washing and drying became crystalline.

0.1344 gm. of the salt dried at 110° gave 0.0423 gm. gold.

Calculated for:

$$\left(N\begin{matrix}(C_5H_{11})_3\\CH_2CHOHCH_3\end{matrix}\right)AuCl_4$$

	Found
31.54 per cent gold.	31.47 per cent.

Summary.—This compound caused a slow and prolonged fall of pressure both before and after atropine (Exper. 270, 271, 273); rather large doses were necessary. Occasionally, after atropine, a weak solution caused a slight rise (Exper. 271). There was sometimes a slowing of the heart (Exper. 270).

$$ClN\begin{matrix}(C_5H_{11})_3\\CH_2CHO(CH_3CO)CH_3\end{matrix}$$

Preparation.—The gold salt of tri-amyl-β-oxypropyl-ammonium chloride is soluble in acetyl chloride, but was not acetylated after heating for an hour at 100° with an excess of the reagent. The acetyl derivative was therefore prepared by heating the dry chloride of the base for two hours at 100° with an excess of acetyl chloride; the excess of acetyl chloride was evaporated and the residual sirupy mass thoroughly washed with dry ether and dissolved in water. Aqueous gold chloride precipitated a sticky gold double salt which crystallized on stirring. The salt was recrystallized from a small amount of absolute alcohol and dried at 110°.

0.0810 gm. of the salt gave 0.0240 gm. gold.

Calculated for:

$$N\begin{matrix}(C_5H_{11})_3\\CH_2CHO(CH_3CO)CH_3\end{matrix}AuCl_4$$

	Found
29.55 per cent gold.	29.63 per cent.

Summary.—This compound had very little action on the blood pressure (Exper. 280); sometimes a slight but prolonged fall (Exper. 282, 288), sometimes a slight rise (Exper. 281, 282) resulted from rather large doses before atropine; after atropine there was a slight rise (Exper. 283).

The action did not differ markedly from that of the compound without the acetyl group.

$$ClN\begin{matrix}\equiv(C_5H_{11})_3\\ \diagdown CH_2CHO(C_6H_5CO)CH_3\end{matrix}$$

Preparation.—This compound was prepared in a manner analogous to that of the acetyl derivative. The platinum double salt was obtained as a soft gummy material and in an impure condition. Owing to the small amount of material available further purification was not attempted.

0.1862 gm. of the salt, dried at 110°, gave 0.0329 gm. platinum.

Calculated for:

$$\left(N\begin{matrix}\equiv(C_5H_{11})_3\\ \diagdown CH_2CHO(C_6H_5CO)CH_3\end{matrix}\right)_2 PtCl_6$$

16.40 per cent platinum. Found 17.67 per cent.

Summary.—We had but one satisfactory experiment (Exper. 282) with this compound and that with an impure preparation; in this 1 c. c. of a solution 1 to 1,000 caused a marked, long-continued fall of pressure (no atropine had been given). It seemed to be much more active than the acetyl derivative.

$$ClN\begin{matrix}\equiv(C_5H_{11})_3\\ \diagdown CH_2CHOHCH_2Cl\end{matrix}$$

MONO-CHLOR-OXYPROPYL-TRIAMYL-AMMONIUM CHLORIDE.

Preparation.—Triamylamine and α-dichlorhydrine were brought together in equimolecular proportions and heated in a sealed bottle in boiling water for three hours. The liquid mixture, which was at first homogeneous, separated into two layers after heating for about an hour and a half. The upper layer consisted largely of triamylamine as was shown by a determination of the platinum in a double salt prepared from it. The vessel was allowed to cool and the lower layer removed; more α-dichlorhydrine was added and the heating continued as before. This was repeated until all of the triamylamine had disappeared.

The material was transferred to a large separating funnel and sufficient water added to dissolve the heavy oil at the bottom. Part of the solution was concentrated first on the water bath, then in a vacuum desiccator; crystals of triamylamine hydrochloride separated out.

The solution of the reaction product was then treated with an excess of aqueous gold chloride; the resultant plastic precipitate of the gold double salt was rubbed into a ball with a stirring rod and the residual aqueous liquid decanted. The mass of salt was washed with water several times and then dried in the vacuum desiccator. It was then dissolved in chloroform in a large flask, dry ether was added with shaking until needle crystals of a gold salt began to separate out; more ether was carefully added until after the precipitation of a considerable quantity of the needle crystals an oily gold double salt began to appear. The needle crystals were now filtered off and an excess of ether added to the filtrate when a large quantity of the oily gold salt separated out. The oily salt, together with some of the crystalline salt, was transferred to a separatory funnel and the oily salt drawn off. The needles proved to be the gold double salt of triamylamine hydrochloride. The oily salt, on being dried at 105°, formed a thick semi-crystalline mass. A specimen dried at 110° gave the following results for gold:

I. 0.2178 gm. gave 0.0645 gm. gold.
II. 0.3252 gm. gave 0.0959 gm. gold.

Calculated for:

$$N\equiv\begin{matrix}(C_5H_{11})_3\\ CH_2CHOHCH_2Cl\end{matrix}\ AuCl_4$$

	Found	
	I.	II.
29.89 per cent gold.	29.61 per cent.	29.49 per cent.

The chloride of the base was freed from the gold salt by dissolving the latter in glacial acetic acid and passing hydrogen sulphide through the solution; the gold sulphide was filtered off and thoroughly washed with hot absolute alcohol. The filtrate was subjected to distillation, under diminished pressure, from the boiling water bath to remove the acetic acid and alcohol. The chloride of the base remained in the flask as an extremely viscous sirupy residue.

No oxypropylene-hexaamyl-diammonium chloride could be found among the reaction products of triamylamine and α-dichlorhydrine.

Summary.—This compound caused a small fall of blood pressure both before and after atropine (Exper. 269, 270, 271, 273). The heart was sometimes slowed before atropine (Exper. 270). No distinct difference was observed between the action of this compound and the one without the chlorine.

$$ClN\equiv\begin{matrix}(C_5H_{11})_3\\ CH_2CHO(CH_3CO)CH_2Cl\end{matrix}$$

Preparation.—Some of the gold salt of the mono-chlor-oxypropyl-triamyl-ammonium chloride was heated in a sealed vessel in boiling water for two hours with an excess of acetyl chloride. The excess of acid chloride was evaporated on the water bath, the residual sirupy

product taken up with acetic ether, filtered and precipitated from the acetic ether solution by dry ether as a dark brown sirup that crystallized on stirring. When the precipitation with ether was conducted slowly a crystalline product was obtained at once. The crystalline material was filtered off, washed with dry ether and recrystallized from a rather small volume of boiling absolute alcohol; after washing with a little absolute alcohol and with ether and drying at 105° the gold content was determined.

I. 0.1833 gm. of the salt gave 0.0519 gm. of gold.

II. 0.2626 gm. of the salt gave 0.0753 gm. of gold.

Calculated for:

$N\equiv\begin{matrix}(C_5H_{11})_3\\ CH_2CHO(CH_3CO)CH_2Cl\end{matrix}$ $AuCl_4$

	Found	
	I.	II.
28.10 per cent gold.	28.31 per cent.	28.26 per cent.

Summary.—This compound sometimes caused a slow, prolonged fall (Exper. 278, 288), sometimes a rise (Exper. 273) of blood pressure; after atropine it caused a slight but rather prolonged rise of pressure (Exper. 272, 283). The heart was sometimes slowed somewhat (Exper. 288). Subcutaneous injections sometimes caused a slight fall (Exper. 288).

It seemed at times to be slightly more active than the compound

$ClN\equiv\begin{matrix}(C_5H_{11})_3\\ CH_3CHO(CH_3CO)CH_3\end{matrix}$ (Exper. 283).

$ClN\equiv\begin{matrix}(C_5H_{11})_3\\ CH_2CHO(C_6H_5CO)CH_2Cl\end{matrix}$

Preparation.—The mono-chlor-oxypropyl-triamyl ammonium chloride was benzoated with benzoyl chloride and the reaction product, when cold, was poured into dry ether and allowed to stand until the ether had become nearly clear. The ether was poured off, and the sirupy benzoyl product washed several times with dry ether. It was then taken up with water, filtered, and aqueous gold chloride added to the filtrate. The resultant sticky precipitate of the gold double salt was boiled with sufficient absolute alcohol to completely dissolve it; when the solution cooled, the salt fell out again as a sticky mass. The salt was finally washed with ether and dried at 110° for analysis.

0.1059 gm. of the salt gave 0.0275 gm. gold.

Calculated for:

$N\equiv\begin{matrix}(C_5H_{11})_3\\ CH_2CHO(C_6H_5CO)CH_2Cl\end{matrix}$ $AuCl_4$

	Found
25.82 per cent gold.	25.97 per cent.

Summary.—This compound usually caused a slow, long continued fall (Exper. 273, 281, 282), sometimes a rise followed by a fall (Exper.

273, 275) of pressure; atropine did not seem to influence the result (Exper. 275, 281). The heart was sometimes slowed (Exper. 273). The injection of this compound seemed to prevent a rise of pressure from adrenalin (Exper. 282).

It was more active than the compound without the benzoyl group (Exper. 273) or the compound without chlorine.

$$ClN\begin{matrix}(C_5H_{11})_3\\ CH_2CHOHCH_2OH\end{matrix}$$

β-γ-dioxypropyl-triamyl-ammonium chloride.[a]

This substance was obtained in the same manner as was the corresponding tripropyl derivative. Here as before the upper layer consisted of unchanged amine, and the lower contained the reaction product. Thus: 0.2114 gm. of platinum double salt got from the upper layer gave, on drying at 110° and igniting, 0.0472 gm. platinum = 22.33 per cent platinum. Calculated for $(N(C_5H_{11})_3HCl)_2\ PtCl_4$: Pt = 22.54 per cent. The lower layer of viscous liquid material was dissolved in water and extracted with ether for the removal of any free triamylamine. Aqueous gold chloride was now added to the solution, precipitating a mass of sticky gold double salt. The gold double salt thus obtained is impure, being contaminated with the gold double salt of triamylamine hydrochloride, this substance evidently resulting in the reaction. The mass of salt was washed thoroughly with water by decantation, dried in the vacuum desiccator or on the water bath, taken up in a convenient amount of chloroform and dry ether cautiously added. A crystalline gold double salt is first precipitated; 0.1699 gm. of this, dried at 110°, gave on ignition 0.0580 gm. gold = 34.14 per cent gold. This was evidently the gold double salt of triamylamine for which Au = 34.76 per cent. Further addition of ether caused the precipitation of an oily gold double salt; all crystalline salt was therefore filtered off and the filtrate treated with more ether for precipitation of the sirupy salt. Analysis of the sirupy salt dried at 110°:

0.1447 gm. salt gave 0.0450 gm. gold.

Calculated for:

$$N\begin{matrix}(C_5H_{11})_3\\ CH_2CHOHCH_2OH\end{matrix}\ AuCl_4$$

30.75 per cent gold. Found 31.10 per cent.

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[a] Efforts to prepare an analogous diamyl compound:

$$\left(HClN\begin{matrix}(C_5H_{11})_2\\ CH_2CHOHCH_2OH\end{matrix}\right)$$

by heating diamylamine and α-monochlorhydrine or α-dichlorhydrine resulted only in the formation of diamylamine hydrochloride.

The above product was also obtained by heating triamylamine with a large excess of the chlorhydrine, ten times the calculated quantity, in a sealed test tube in the boiling water bath for several days or until the layer of amine had practically disappeared. The impure gold double salt was washed with water and decomposed by means of hydrogen sulphide. The filtrate from the gold sulphide was freed from hydrogen sulphide by evaporation on the water bath and aqueous cadmium chloride added to the solution, precipitating a thick sirupy cadmium double salt. The salt was washed with warm water, in which it is difficultly soluble, and decomposed with hydrogen sulphide. The filtrate from the cadmium sulphide yielded on evaporation a thick straw-colored sirupy product that gave a sirup-like gold double salt, having a gold content corresponding to that required for dioxy-propyl-tri-amyl-ammonium chloride. Analysis of the salt dried at 110°:

I. 0.2597 gm. salt gave 0.0798 gm. gold.
II. 0.2286 gm. salt gave 0.0701 gm. gold.

Calculated for:

$N\begin{matrix}\equiv(C_5H_{11})_3\\ \diagdown CH_2CHOHCH_2OH\end{matrix}\ AuCl_4$

	Found	
	I.	II.
30.75 per cent gold.	30.73 per cent.	30.66 per cent.

Summary.—This compound sometimes caused a fall (Exper. 289), sometimes a rise (Exper. 289, 290) of blood pressure both before and after atropine (Exper. 290).

$ClN\begin{matrix}\equiv(C_5H_{11})_3\\ \diagdown CH_2CHO(CH_3CO)CH_2O(CH_3CO)\end{matrix}$ [a]

Preparation.—Acetylation of β-γ-dioxypropyl triamyl-ammonium chloride did not occur when this compound was heated for several hours in a sealed tube in boiling water with an excess of acetyl chloride. The acetyl product was prepared by boiling the chloride of the base for an hour with an excess of acetic anhydride. The reaction product was dissolved in water, shaken with ether, and the aqueous solution precipitated by an aqueous gold chloride solution; the double salt was precipitated in the form of a sirup which gradually became crystalline.

[a] Efforts to obtain a dibenzoyl product by heating the chloride of the base for three hours in the boiling water bath with an excess of benzoyl chloride were unsuccessful. The benzoyl product was prepared by heating the chloride of the base for six hours at 160° with an excess of benzoic anhydride, treating the product with ether and water and precipitating the aqueous solution with aqueous gold chloride solution. But little material was available and the physiological experiments were not very satisfactory; it was about four times as toxic as the chloride of the base.

0.2371 gm. of the salt dried at 110° gave 0.0649 gm. gold.

Calculated for:

$$N\begin{matrix}=(C_5H_{11})_3\\ \backslash CH_2CHO(CH_3CO)CH_2O(CH_3CO)\end{matrix}\ AuCl_4$$

	Found
27.18 per cent gold.	27.37 per cent.

Summary.—This compound caused a slow, prolonged fall of blood pressure both before (Exper. 289, 290) and after atropine (Exper. 290).

It was somewhat more active than the compound without the acetyl group.

$$ClN\begin{matrix}=(CH_3)_2\\ -C_5H_{11}\ (iso)\\ \backslash CH_2CH_2OH\end{matrix}$$

DIMETHYL-ISOAMYL-OXYETHYL-AMMONIUM CHLORIDE.

Preparation.—Dimethyl-ethyl-alkine and isoamyl-iodide, the latter in slight excess, were brought together in a small flask and boiled, using a reflux condenser. When the liquid appeared to be homogeneous the excess of iodide was evaporated on the water bath; the product crystallized to a solid cake on standing in the desiccator for some time. The product was dissolved in water and digested with an excess of silver chloride and filtered. The filtrate was evaporated to sirupy consistency and a portion dissolved in alcohol and its platinum double salt prepared by the addition of platinic chloride in alcohol. The precipitated platinum salt, after recrystallizing once from water and drying at 105°, gave the following figures for platinum:

I. 0.1723 gm. salt gave 0.0462 gm. platinum.
II. 0.1058 gm. salt gave 0.0284 gm. platinum.

Calculated for.

$$\left(N\begin{matrix}=(CH_3)_2\\ -C_5H_{11}\\ \backslash CH_2CH_2OH\end{matrix}\right)_2 PtCl_6$$

	Found	
	I.	II.
26.76 per cent platium.	26.81 per cent.	26.84 per cent.

Summary.—This compound usually caused a fall of pressure (Exper. 262, 264, 268) but sometimes a rise (Exper. 264) before atropine and no effect after atropine (Exper. 262, 264, 267, 268).

It was about as active as choline in causing a fall of pressure (Exper. 262, 268) but less active than this in causing a rise (Exper. 264, 267, 268).

It was far more active than the tri-amyl compound.

$$ClN\begin{matrix}=(CH_3)_2\\ -C_5H_{11}\\ \backslash CH_2CH_2O(CH_3CO)\end{matrix}$$

Preparation.—This compound was made by boiling the dimethyl-isoamyl-oxyethyl ammonium chloride with acetic anhydride for three hours. The platinum salt, which was difficultly soluble, was recrystallized from boiling water.

0.1144 of the salt gave 0.0275 gm. platinum.

Calculated for:

$$\left(\begin{array}{l} N\!\!\begin{array}{l}\diagup\!\!\diagup (CH_3)_2 \\ -C_5H_{11} \\ \diagdown CH_2CH_2O(CH_3CO)\end{array}\end{array}\right)_2 PtCl_6$$

23.99 per cent platinum. Found 24.04 per cent.

Summary.—Sometimes this compound caused a fall (Exper. 268), sometimes a rise or a fall followed by a rise of pressure (Exper. 263, 264) before atropine; it had very little effect after atropine (Exper. 264, 268). It sometimes caused a slowing of the heart. (Exper. 264.)

It was more active than the triamyl compound or the acetyl derivative of the triamyl compound (Exper. 264, 268) or the compound $ClN\!\!\begin{array}{l}\diagup\!\!\diagup (CH_3)_2 \\ -C_5H_{11} \\ \diagdown CH_2CH_2OH\end{array}$ (Exper. 268). It was often about as active as choline in causing a fall of pressure (Exper. 264).

$$ClN\!\!\begin{array}{l}\diagup\!\!\diagup (CH_3)_2 \\ -C_5H_{11} \\ \diagdown CH_2CH_2O(C_6H_5CO)\end{array}$$

Preparation.—This compound was prepared by heating dimethyl-isoamyl-oxyethyl ammonium chloride with benzoyl chloride for three hours at 100°. The platinum salt was dried at 105°

0.1722 gm. of the platinum salt gave 0.0357 gm. of platinum.

Calculated for:

$$\left(\begin{array}{l} N\!\!\begin{array}{l}\diagup\!\!\diagup (CH_3)_2 \\ -C_5H_{11} \\ \diagdown CH_2CH_2O(C_6H_5CO)\end{array}\end{array}\right)_2 PtCl_6$$

20.81 per cent platinum. Found 20.73 per cent.

Summary.—The effect of this compound was very variable; sometimes it caused a fall, sometimes a rise both before and after atropine (Exper. 267, 268, 269, 280). It sometimes caused a slowing of the heart before atropine (Exper. 269).

In some cases it was about as active as the benzoyl derivative of the triamyl compound in causing a fall of pressure (Exper. 267).

It partook of the activities of choline, benzoyl-choline, and the triamyl compounds.

$$HClN\!\!\begin{array}{l}\diagup\!\!\diagup (CH_3)_2 \\ \diagdown CH_2CH_2OH\end{array}$$

DIMETHYL-ETHYL-ALKINE HYDROCHLORIDE (OXYETHYL-DIMETHYLAMINE HYDROCHLORIDE).

Preparation.—This compound was prepared by the method of Ladenburg[a] from ethylene chlorhydrine and dimethylamine.

Summary.—This compound when injected in doses of 1 cc. of the 1 per cent solution had no effect upon the blood pressure either before or after atropine (Exper. 267).

$$ClN\begin{matrix}(CH_3)_2\\(CH_2CH_2OH)_2\end{matrix}$$

IOXYETHYL-DIMETHYL-AMMONIUM CHLORIDE.

Preparation.—Dimethyl-ethyl-alkine was prepared by the method of Ladenburg[a] and this compound was further treated by the method of Knorr[b] (heating with chlorhydrine) to obtain the dioxyethyl-dimethyl-ammonium chloride.

I. 0.1315 gm. of the platinum salt gave 0.0377 gm. platinum.

II. 0.1337 gm. of the platinum salt gave 0.0380 gm. platinum.

Calculated for:

$$\left(N\begin{matrix}(CH_3)_2\\(CH_2CH_2OH)_2\end{matrix}\right)_2 PtCl_6.$$

	Found	
	I.	II.
28.83 per cent platinum.	28.67 per cent.	28.42 per cent.

Summary.—This compound had but little effect upon blood pressure; before atropine it caused a small fall (Exper. 278, 281, 282) or rise (Exper. 279); after atropine it had no effect or caused a slight rise of pressure (Exper. 278, 279).

It was less active than choline in causing a fall (Exper. 278, 281), but sometimes more active than this in causing a rise of pressure (Exper. 278).

$$ClN\begin{matrix}(CH_3)_2\\(CH_2CH_2O(CH_3CO))_2\end{matrix}$$

Preparation.—This compound was prepared by boiling dioxyethyl-dimethyl-ammonium chloride for three hours with an excess of acetic anhydride; the reaction product was poured into strong alcohol and precipitated by the addition of alcoholic platinic chloride. The double salt when first precipitated was in the form of a sirup, but it slowly crystallized when rubbed with a stirring rod. The platinum salt was filtered, washed with alcohol, and dissolved in a small quantity of water, in which it is extremely soluble. The aqueous solution was then poured into a considerable volume of strong alcohol to again precipitate the salt.

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[a] Ladenburg, Ber. 1881, 14, p. 2408. [b] Knorr, Ber. 1889, 22, p. 2089.

0.1361 gm. of the salt gave 0.0316 gm. of platinum.

Calculated for:

$$\left(N\begin{matrix}(CH_3)_2\\(CH_2CH_2O(CH_3CO))_2\end{matrix}\right)_2 PtCl_6$$

Found

23.08 per cent platinum. 23.22 per cent.

Summary.—This compound was very active in causing a fall of pressure before atropine (Exper. 279, 281, 282, 286) and also after nicotine (Exper. 279, 281); it had no effect after atropine (Exper. 279, 281, 286). It caused some irregularity of the heart before atropine.

It was far more active (perhaps 1,000 times) than the compound without the acetyl groups (Exper. 279, 281, 282) and choline (Exper. 281).

$$ClN\begin{matrix}(CH_3)_2\\(CH_2CH_2O(C_6H_5CO))_2\end{matrix}$$

Preparation.—This compound was obtained by heating dioxyethyl-dimethyl-ammonium chloride with an excess of benzoyl-chloride for several hours at 100°. The platinum double salt is difficultly soluble in hot water, insoluble in cold water, and was purified by boiling with a large volume of water.

I. 0.1006 gm. of the platinum salt gave 0.0188 gm. platinum.

II. 0.1301 gm. of the platinum salt gave 0.0244 gm. platinum

Calculated for:

$$\left(N\begin{matrix}(CH_3)_2\\(CH_2CH_2O(C_6H_5CO))_2\end{matrix}\right)_2 PtCl_6$$

Found

I. II.

18.95 per cent platinum. 18.69 per cent. 18.75 per cent.

Summary.—This compound had little effect upon the blood pressure; it tended to cause a rise (Exper. 279, 280, 284), but in one case (after atropine) a strong solution caused a small fall (Exper. 284) of blood pressure.

It was much less active than benzoyl-choline (Exper. 280, 284.)

$$\begin{matrix}CH_2N(CH_3)_3Cl\\|\\CHOH\\|\\CH_2N(CH_3)_3Cl\end{matrix}$$

OXYPROPYLENE-HEXAMETHYL-DIAMMONIUM CHLORIDE (APOSEPIN CHLORIDE).

Preparation.—This compound was prepared according to the method described under mono-chlor-oxypropyl-trimethyl-ammonium chloride.

I. 0.1418 gm. of the platinum salt contained 0.0471 gm. platinum.

II. 0.1514 gm. of the platinum salt contained 0.0503 gm. platinum.

Calculated for:

$CH_2N(CH_3)_3Cl$
|
$CH(OH)$
|
$CH_2N(CH_3)_3Cl$

	Found	
	I.	II.
33.37 per cent platinum.	33.22 per cent.	33.22 per cent.

Summary.—This compound caused a slight fall of pressure before atropine (Exper. 283, 284); after atropine it had no effect (Exper. 283, 284). It was less active than choline (Exper. 283, 284).

$CH_2N(CH_3)_3Cl$
|
$CHO(CH_3CO)$
|
$CH_2N(CH_3)_3Cl$

Preparation.—This compound was prepared by boiling oxy-propylene-hexamethyl-diammonium chloride with an excess of acetic anhydride for several hours. The reaction product was poured into cold absolute alcohol, precipitated with alcoholic platinic chloride, filtered, dried, boiled with water, cooled, filtered, washed with water and with alcohol, and dried at 105°.

0.1049 gm. of the platinum salt yielded 0.0327 gm. platinum.

Calculated for:

$CH_2N(CH_3)_3Cl$
|
$CHO(CH_3CO)$. $PtCl_4$
|
$CH_2N(CH_3)_3Cl$

	Found
31.13 per cent platinum.	31.17 per cent.

Summary.—This compound was fairly active in causing a fall of pressure before atropine (Exper. 283, 284); occasionally the fall was followed by a slight rise (Exper. 282, 284). It had no effect (Exper. 284) or caused a slight fall after atropine (Exper. 283). It was perhaps 15 times as active as the compound without the acetyl group.

It was rather more active than choline in causing a fall (Exper. 283).

$CH_2N(CH_3)_3Cl$
|
$CHO(C_6H_5CO)$
|
$CH_2N(CH_3)_3Cl$

Preparation.—Benzoyl chloride had no effect on oxypropylene-hexamethyl-diammonium chloride when heated with it for six hours at 100°. Two grams of the chloride of the base were then heated for about six hours with 10 grams of benzoic acid anhydride at a temperature of 180° to 190°. When cool the reaction product was poured into alcohol and precipitated with alcoholic platinic chloride; the flocculent precipitate

was filtered off and washed with alcohol; it darkened and became gummy. Treated with water it softened to a dark, plastic mass, which became granular when transferred to alcohol. A sample was dried at 105° for an hour and the platinum determined.

0.1148 gm. of the platinum salt gave 0.0339 gm. platinum.

Calculated for:

$$\begin{array}{l} CH_2N(CH_3)_3Cl \\ | \\ CHO(C_6H_5CO) \ . \ PtCl_4 \\ | \\ CH_2N(CH_3)_3Cl \end{array}$$

Calculated	Found
29.53 per cent platinum.	29.53 per cent.

Summary.—This compound caused a rather marked rise of pressure (Exper. 282, 283, 284), but sometimes, especially in weak solutions or late in an experiment or after nicotine a slight fall (Exper. 283, 284); there seemed to be no effect after atropine (Exper. 283).

In most cases it was as active as or more active than benzoyl-choline in causing a rise of pressure (Exper. 282, 283, 284), but had, perhaps, less effect on the heart rate (Exper. 283, 284). In one experiment (283) it caused a rather marked rise before nicotine, a fall after nicotine and no effect after atropine. In another (284) it caused first a rise, later a fall followed by a rise, and, after nicotine, only a slight fall.

$$\begin{array}{l} CH_2N(C_2H_5)_3Cl \\ | \\ CHOH \\ | \\ CH_2N(C_2H_5)_3Cl \end{array}$$

OXYPROPYLENE-HEXA-ETHYL-DI-AMMONIUM CHLORIDE.

The preparation of this compound was described under that of monochlor-oxypropyl-triethyl-ammonium chloride.

I. 0.2143 gm. of the gold salt contained 0.0900 gm. gold.

II. 0.1793 gm. of the gold salt contained 0.0753 gm. gold.

Calculated for:

$$\begin{array}{l} CH_2N(C_2H_5)_3AuCl_4 \\ | \\ CHOH \\ | \\ CH_2N(C_2H_5)_3AuCl_4 \end{array}$$

Calculated	Found	
	I.	II.
42.03 per cent gold.	41.96 per cent.	42.00 per cent.

Summary.—This compound tended to cause a fall of pressure both before (Exper. 283, 284) and after atropine (Exper. 283). In one experiment (282) it caused a rise before atropine (blood pressure very low).

It was less active than choline (Exper. 283, 284) or the compound

$CH_2N(CH_3)_3Cl$

$CHOH$ (Exper. 283).

$CH_2N(CH_3)_3Cl$

$CH_2N(C_2H_5)_3Cl$
$CHO(CH_3CO)$
$CH_2N(C_2H_5)_3Cl$ [a]

This compound was obtained by boiling the chloride of the base for six hours with an excess of acetic anhydride.

0.1405 gm. of the platinum salt gave 0.0386 gm. platinum.

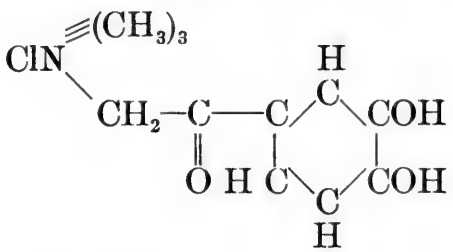
Calculated for:

$CH_2N(C_2H_5)_3$

$CHO(CH_3CO)$. $PtCl_6$

$CH_2N(C_2H_5)_3$	Found
27.44 per cent platinum.	27.47 per cent.

Summary.—This compound caused a very slight fall of pressure before atropine (Exper. 283) or had no effect (Exper. 284); it was far less active in this respect than the hexa-methyl compound (Exper. 283) and less active than the compound without the acetyl group. After atropine it caused a very slight rise of pressure (Exper. 284).



Preparation.—Chloracetopyrocatechin, prepared by the method of Dzierzgowski,[b] was dissolved in absolute alcohol and heated with a 33 per cent alcoholic solution of trimethylamine in a sealed vessel in boiling water for three hours, equimolecular quantities of the reagents being taken. On cooling, a crystalline product separated from the alcoholic solution. This was filtered off, washed with a little cold absolute alcohol, heated to the boiling point with more absolute alcohol, and again filtered when cool.

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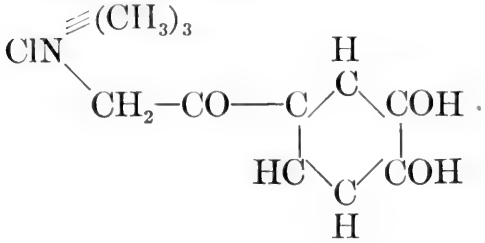
[a] Some of the oxypropylene-hexaethyl-diammonium chloride was heated for six hours at 100° with an excess of benzoyl chloride without being transformed into the benzoyl compound; fusion with benzoic anhydride at 180–190° for several hours would probably have effected the change.

[b] Dzierzgowski. Chem. Centrbll., 1893, II, p. 475; Bull. Soc. Chim., 1894 (3), 12, p. 911.

A sample of this material was dried in the air bath at 110°, then in the desiccator, and the nitrogen determined by the Kjeldahl (Gunning) method.

0.3585 gm. substance gave 0.02094 gm. nitrogen.

Calculated for:

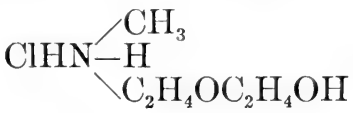


Found

N = 5.71 per cent. 5.84 per cent.

Several attempts to reduce the keto group in the above compound by means of 2 to 3 per cent sodium amalgam in the presence of an excess of aluminum sulphate proved unsatisfactory. The experiments were carried out at 0°.[a]

Summary.—This compound caused a marked, long continued rise of blood pressure (Exper. 284, 286, 287, 288, 290) both before and after atropine. It was usually more active than benzoyl-choline in causing a rise (Exper. 284), but far less active than epinephrine (Exper. 286, 288). There was frequently an acceleration of the heart (Exper. 284).



MONO-METHYL-DIOXETHYLENE-AMINE HYDROCHLORIDE.

Preparation.—The compound was prepared by the method of Morley.[b] Methylamine (33 per cent alcoholic solution) and ethylene chlorhydrine were brought together in equivalent quantities and heated in a sealed tube for twenty-four hours at about 110°. The contents of the tube, containing some crystalline material, were poured into a distilling flask connected with a condenser and distilled from the boiling water bath with the aid of the pump in order to remove the alcohol, and, as far as possible, the excess of reagents. The pump was disconnected, the end of the condenser fitted with a nitrogen bulb containing dilute hydrochloric acid, the contents of the flask diluted with water, silver oxide added in excess and the distillation carried on with a free flame until the volume of liquid in the flask was very considerably reduced and the odor of methylamine was scarcely perceptible on opening the flask. The silver oxide was filtered off, the filtrate neutralized with hydrochloric acid, evaporated to a sirup, taken up

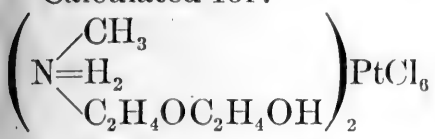
[a] Cf. reduction of dioxyaceton to glycerin (Piloty, Ber., 1898, 30, p. 3167).

[b] Morley, Ber., 1880, 13, p. 222.

with absolute alcohol, and alcoholic platinic chloride added; there resulted a precipitate of a sirupy platinum double salt that slowly became crystalline on rubbing with the stirring rod. The salt was washed with absolute alcohol and with ether and dried at 105°.

0.1312 gm. of the salt gave 0.0397 gm. platinum.

Calculated for:

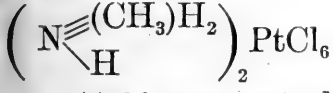


Found
30.07 per cent platinum.	30.27 per cent.

The hydrochloric acid solution in the absorption bulb was evaporated off on the bath; a crystalline white salt remained. The salt was taken up with alcohol and alcoholic platinic chloride added, the precipitate of platinum double salt resulting was filtered off and washed with alcohol, with ether, and dried at 105°.

0.1090 gm. of the salt gave 0.0450 gm. platinum.

Calculated for:



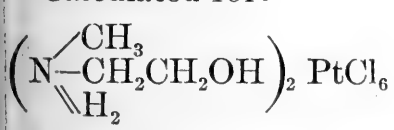
Found
41.30 per cent platinum.	41.28 per cent.

Therefore, as Morley found, only two products result from the action of methylamine on ethylene chlorhydrine under the above conditions, viz, methylamine hydrochloride and the hydrochloride of monomethyldioxethyleneamine.

A considerable quantity of the hydrochloride of the monomethyldioxethyleneamine was distilled with solid caustic potash, the distillate dried with powdered caustic potash, taken up with ether, the ether distilled off, and the residual product rectified. The distillation was interrupted when a temperature of 140° had been reached, practically all having passed over. The distillate was neutralized with hydrochloric acid, alcoholic platinic chloride added, and the solution evaporated to a sirup on the bath at a low temperature. On the addition of considerable absolute alcohol there was thrown out a sirupy platinum double salt that crystallized on stirriug with a rod. The salt was washed several times with absolute alcohol and with ether. A portion was transferred to a weighed crucible, dried at 105°, weighed, and ignited.

0.3624 gm. of the salt gave 0.1267 gm. platinum.

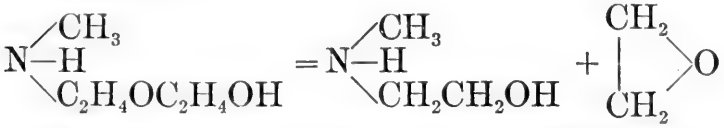
Calculated for:



Found
34.80 per cent platinum.	34.96 per cent.

The salt has the appearance of being hygroscopic and melts at a low temparature.

This is the methyloxyethylamine, described by Knorr,[a] and is evidently produced by the decomposition of Morley's monomethyldioxethyleneamine by caustic potash. Possibly the decomposition is effected thus:



The presence of monomethyloxyethylamine was further determined by means of its gold double salt;

0.1168 gm. salt gave 0.0553 gm. gold.

Calculated for:

$C_3H_{10}ON\ AuCl_4$	Found
47.51 per cent gold.	47.35 per cent.

The ethylene oxide was not identified.

Summary.—This compound had no effect upon blood pressure or heart rate when injected in doses of 1 c. c. of a 0.4 per cent solution (Exper. 296, 297).

ACETYL DERIVATIVE OF MONO-METHYL-DIOXYETHYLENE-AMINE HYDROCHLORIDE.

Preparation.—The hydrochloride of the base when boiled for two hours with acetic anhydride yielded a product that was apparently a diacetyl derivative. The reaction product was poured into cold absolute alcohol and alcoholic platinic chloride added. There resulted a well-crystallized platinum double salt. The salt was filtered off, washed with alcohol and with ether, dried at 105°, and the platinum content determined.

I. 0.1652 gm. salt gave 0.0394 gm. platinum.

II. 0.1160 gm. salt gave 0.0276 gm. platinum.

Calculated for:

	Found	
$(C_5H_{12}NO_2(CH_3CO)_2)_2\ PtCl_6$	I.	II.
23.88 per cent platinum.	23.85 per cent.	23.79 per cent.

A similar compound was obtained by treating the hydrochloride of the base with acetyl chloride for two hours at 100°. The reaction product in this case was poured into anhydrous ether contained in a small beaker, and the beaker kept in a desiccator until the acetyl body had settled to the bottom, when it was washed repeatedly with more anhydrous ether and finally dissolved in water. To the aqueous solution aqueous gold chloride was added, precipitating an oily gold

[a] Knorr, Ber., 1889, 22, p. 2087.

double salt difficultly soluble in cold water. The salt was washed with water by decantation, dried at 105° and the gold content determined.

0.3201 gm. salt gave 0.1169 gm. gold.

Calculated for:

$C_5H_{12}NO_2(CH_3CO)_2\ AuCl_4$	Found
36.30 per cent gold.	36.52 per cent.

Summary.—This compound caused a fall of pressure before atropine and no effect after it (Exper. 296, 297, 298); it was five to ten times as active as choline (Exper. 297) in causing a fall of pressure.

This compound was more toxic than choline.[a]

BENZOYL DERIVATIVE OF MONO-METHYL-DIOXYETHYLENE-AMINE HYDROCHLORIDE.

Preparation.—Exactly the same method was pursued as that employed in the acetyl chloride treatment, except that the aqueous solution of the benzoated product was extracted with ether to more completely remove any benzoic acid. The addition of an aqueous solution of platinic chloride precipitated a gummy insoluble platinum double salt which gradually became well crystallized. The salt was filtered off, washed with water, a little absolute alcohol, with ether, and a sample dried at 120° for several hours in a weighed crucible, and the percentage of platinum determined by ignition.

0.1830 gm. salt gave 0.0330 gm. platinum.

Calculated for:

$(C_5H_{12}NO_2(C_6H_5CO)_2)_2\ PtCl_6$	Found
18.31 per cent platinum.	18.03 per cent.

Here apparently a compound resulted containing two benzoyl groups. The physiological experiments were made with this compound.

In another experiment a compound was obtained the platinum salt of which corresponded to a compound with but one benzoyl group. This was prepared by heating the hydrochloride of the base with benzoyl chloride for about two hours at 100°. The platinum double salt was precipitated as a sirupy product difficultly soluble in hot water. After long rubbing in a mortar the salt became somewhat brittle and granular. The salt was dried at 105° and analyzed for platinum.

0.1586 gm. of the salt gave 0.0360 gm. platinum.

Calculated for:

$(C_5H_{12}NO_2(C_6H_5CO))_2\ PtCl_6$	Found
22.75 per cent platinum.	22.70 per cent.

Summary.—This preparation caused a fall of pressure (Exper. 297, 298) sometimes marked (Exper. 296) both before and after

[a] See Note A.

atropine. Sometimes the fall of pressure was followed by a slight rise and this again by a more prolonged fall (Exper. 297). The heart was sometimes slowed; this effect was not prevented by atropine (Exper. 296).

This compound was slightly more toxic than choline and less toxic than the acetyl derivative.[a]

II. EFFECT OF ACID RADICLES UPON THE ACTIVITY OF CHOLINE.

The above experiments show that the activity of choline is greatly modified by the introduction of acid radicles.

(*a*) *Compounds of the fatty acid series—Acetyl-choline.*—The introduction of the acetyl radicle into choline increases to an extraordinary degree (roughly 100,000 times) the power of the latter to cause a fall of blood pressure. Acetyl-choline has little, if any, activity in causing a rise of pressure either before or after atropine; this action is often quite marked with choline, especially after atropine. Neither compound has a marked or constant effect upon the heart rate. The action of choline in causing a fall of pressure was easily prevented by atropine; that of acetyl-choline was completely prevented only by large doses of atropine. Acetyl-choline is about three times as toxic as choline. Thus the introduction of the acetyl group increases greatly the activity of choline in causing a fall of blood pressure without causing a corresponding increase in its toxicity. The blood pressure lowering power of *propionyl-choline* (before atropine) is very great, although much less than that of acetyl-choline; it is perhaps 100 times as active in this respect as choline itself. After atropine this compound causes a rise of blood pressure, being about as active in this direction as choline. Propionyl-choline frequently caused a considerable slowing of the heart from central vagus stimulation; this action was more marked than with either choline or acetyl-choline.

Normal *butyril-choline* was more active than choline both in causing a fall of pressure (before atropine) and a rise after atropine; it was more active than propionyl-choline in causing a rise of pressure. It had more of a tendency to cause a rise of pressure before atropine than did choline. It had a more marked action in causing a slowing of the heart (central vagus stimulation) than did propionyl-choline. *Iso-butyril-choline* differed from the normal compound in having a greater tendency to cause a fall of pressure and a lesser tendency to cause a rise of pressure; thus there was a partial return to the choline and propionyl-choline type of action.

In *valeryl-choline* the power to cause a slowing of the heart (from central vagus stimulation), which was little marked in choline and acetyl-choline but more marked in the propionyl-choline and still more

[a] See Note A.

in the normal iso-butyril compounds, was very marked. It had less activity in causing a fall of pressure than any of the preceding compounds, but greater activity in causing a rise of pressure.

α-Bromisocapronyl-choline was about as active as choline in causing a fall of pressure, but more active than this in causing a rise of pressure; the experiments were not sufficiently numerous to determine its effect on the heart rate.

Succinyl-choline has an action very similar to that of valeryl-choline; the latter is, however, several (perhaps fifteen) times as active as the former.

Palmityl-choline was very active in causing a fall of pressure, but had little action in causing a rise; it seems to have but little action on the heart.

Thus, as we pass up the series from the acetyl to the valeryl compound we find (1) a rapid diminution in the activity of these compounds in causing a fall of blood pressure, (2) an increase in their activity in causing a rise of pressure, and (3) an increase in their activity in causing a slowing of the heart.

The fall of pressure was in all cases abolished by atropine.

(*b*) *Compounds of the aromatic series.*—The introduction into choline of acid radicles of the aromatic series had for its most marked effect an accentuation of the blood pressure raising properties of this compound; it also increased the activity of the latter in causing slowing of the heart from central vagal stimulation. *Benzoyl-choline* is essentially a blood pressure raising compound, although it frequently causes a fall of pressure, especially when injected in small doses; it is moderately active in slowing the heart (vagi intact). It is about one and one-half times less toxic than choline.

m-Nitro-benzoyl-choline had less tendency to cause a rise of pressure than did benzoyl-choline, whereas with *p-nitrobenzoyl choline* the opposite was the case. *Phenyl-acetyl-choline* was more active in causing a slowing of the heart and a rise of pressure than was benzoyl-choline; it was perhaps twenty times as active as the latter in causing a rise of pressure. It usually caused a fall of pressure (vagi intact), but this seemed to be dependent entirely upon central vagus inhibition, differing in this respect from benzoyl-choline. It is especially interesting to note that the introduction of the phenyl group into acetyl-choline had changed the latter from an extraordinarily active blood pressure lowering body into a blood pressure raising body; the characteristics of acetyl-choline were completely lost.

β-Phenyl-propionyl-choline.—The phenyl group had here an effect similar to that in the phenyl-acetyl compound; it caused a great accentuation of the blood pressure raising and cardio-inhibitory properties; it was less active (by about one-half) than the phenyl-acetyl compound in somewhat the same way as propionyl-choline is less active than

acetyl-choline. In *anisyl-choline* there was a further accentuation of the cardio-inhibitory action of benzoyl-choline. This property was still more marked in cinnamyl-choline; the latter, however, seemed to have also a greater tendency to cause a fall of pressure. *Phthalyl-choline* had almost no effect upon the blood pressure.

The fall of pressure resulting at times from the injection of some of these compounds was but incompletely prevented by atropine; this was especially the case with the cinnamyl compound.

III. EFFECTS OF VARIOUS SIDE CHAINS UPON THE ACTIVITY OF COMPOUNDS CONTAINING THE $(CH_3)_3$ GROUP.

(*a*) *Compounds containing hydroxyl* (*or certain other oxygen-containing groups*).—*Formocholine* $\left(ClN\begin{matrix}(CH_3)_3\\CH_2OH\end{matrix}\right)$.—This compound had an action on the blood pressure very similar to that of choline; it was, however, distinctly more active than the latter, at least in causing a fall of pressure. The effects of the *methyl ether of formocholine* $\left(ClN\begin{matrix}(CH_3)_3\\CH_2OCH_3\end{matrix}\right)$ were very similar to those of the formocholine; it was, however, less active than this in causing a fall of blood pressure. The toxicity of both of these compounds is much greater than that of choline itself.[a] *Betain* $\left(ClN\begin{matrix}(CH_3)_3\\CH_2COOH\end{matrix}\right)$ had practically no effect on the circulation.[b] The compound $BrN\begin{matrix}(CH_3)_3\\C_2H_4Br\end{matrix}$ had the same effect on the blood pressure as choline itself, but it seemed rather more active. The compound $ClN\begin{matrix}(CH_3)_3\\CH_2CH_2CH_2OH\end{matrix}$ had about the same effect on the circulation as did choline; the only difference, apparently, being that it did not cause a rise of pressure before atropine as choline occasionally did and the rise after atropine was rather less. It is five or six times as poisonous as choline. The compound $ClN\begin{matrix}(CH_3)_3\\CH_2CHOHCH_3\end{matrix}$ had an action similar to that of choline; it was, however less active than this. It was about one and one-half times as poisonous as choline. The compound $ClN\begin{matrix}(CH_3)_3\\CH_2CHOHCH_2Cl\end{matrix}$ seemed to have about the same activity as did choline; it is about

[a] Cf. Hunt and Taveau, Jour. Pharm. and Exper. Therap., 1909, 1, 303. The data on the toxicity of other compounds considered in this paper are taken largely from this article.

[b] Cf. Mott and Halliburton, Phil. Trans., Royal Society, London, 1899, 191, B. p. 211.

twice as toxic as the latter. The compound $ClN\begin{matrix}(CH_3)_3\\ CH_2CHOHCH_2OH\end{matrix}$ had about the same effect in causing a fall of blood pressure before atropine as did choline; it differs from the latter in not causing a rise of pressure after atropine. It was about one-half as toxic as choline.

Thus in most of the above cases changes in the side chain had increased the toxicity of the compound as compared with choline and had not increased its activity upon the blood pressure, or had not increased it in proportion to the increased toxicity. The only exception was the compound $ClN\begin{matrix}(CH_3)_3\\ CH_2CHOHCH_2OH\end{matrix}$; this retained the blood pressure lowering activity of choline, but was only about one-half as toxic as the latter.

The fall of pressure caused by these bodies was in all cases prevented by atropine.

(*b*) *Acetyl derivatives.*—The introduction of the acetyl group into choline increased the blood pressure lowering activity of this body so markedly that it was thought that the acetyl derivatives of the other members of this series might be of special interest, especially that of the di-hydroxy compound, which is, as was just stated, decidedly less poisonous than choline, but equally active in causing a fall of blood pressure. The introduction of the acetyl radicle into the compound $ClN\begin{matrix}(CH_3)_3\\ CH_2CH_2CH_2OH\end{matrix}$ increased the blood pressure lowering activity very greatly (about one thousand times). It was still far less active than acetyl-choline, from which it also differed in that its effect was not so readily abolished by atropine. The acetyl derivative of the compound $ClN\begin{matrix}(CH_3)_3\\ CH_2CHOHCH_3\end{matrix}$ was also extremely active in causing a fall of blood pressure; it differed from the preceding in that it was often impossible to completely overcome its action by atropine. The acetyl derivative of the compound $ClN\begin{matrix}(CH_3)_3\\ CH_2CHOHCH_2Cl\end{matrix}$ was scarcely more active than the original compound or than choline itself; the effect was easily abolished by atropine. The chlorine atom seemed to counteract in some way the effect of the acetyl group.

The introduction of two acetyl groups into the compound $ClN\begin{matrix}(CH_3)_3\\ CH_2CHOHCH_2OH\end{matrix}$ had very little effect; the second acetyl group seemed to counteract the effect of the first in somewhat the same way that chlorine did in the case of the preceding compound.

It may be added that the toxicity of all these compounds was increased from two to four times by the introduction of the acetyl groups.

(*c*) *Benzoyl derivatives.*—The introduction of the benzoyl group into choline increased the blood pressure raising and the cardio-inhibitory activities of choline; it also diminished the toxicity of the latter. The introduction of the benzoyl group into the compound $ClN\begin{matrix}(CH_3)_3\\ \diagdown CH_2CH_2CH_2OH\end{matrix}$ lessened somewhat the activity of this both in causing a fall and perhaps in causing a rise of pressure; the same was true in general of the compound $ClN\begin{matrix}(CH_3)_3\\ \diagdown CH_2CHOHCH_3\end{matrix}$ but this was rather more active than the former in causing a rise of pressure.

The benzoyl group seemed to have no effect in the compound $ClN\begin{matrix}(CH_3)_3\\ \diagdown CH_2CHOHCH_2Cl\end{matrix}$ except that its toxicity was doubled; the benzoyl group had no effect upon the toxicity of the two preceding compounds. The introduction of two benzoyl groups into the compound $ClN\begin{matrix}(CH_3)_3\\ \diagdown CH_2CHOHCH_2OH\end{matrix}$ had little effect except that the toxicity was increased about threefold; this compound caused a fall of pressure after atropine, however, whereas the di-hydroxy compound did not.

The fall of pressure frequently following the injection of the above bodies was not prevented, or not entirely prevented, by atropine.

IV. EFFECTS OF VARIOUS $(C_nH_{2n+1})_3$ GROUPS.

(*a*) *Compounds containing the* $-CH_2CH_2OH$ *group.*—The compound $ClN\begin{matrix}(C_2H_5)_3\\ \diagdown CH_2CH_2OH\end{matrix}$ was about thirteen times as poisonous as choline; it seemed to have the same general effect upon the blood pressure as the latter, except that it was weaker and the fall of pressure was not so easily prevented by atropine; it was less active in causing a rise of pressure after atropine. The compound containing three propyl groups was about as active as the preceding one; the fall of pressure, which was not very great, was rather prolonged. It was about six times as toxic as choline. The toxicity was still greater with the compound $ClN\begin{matrix}(C_5H_{11})_3\\ \diagdown CH_2CH_2OH\end{matrix}$ but it had little effect on the blood pressure.

The compound $ClN\begin{matrix}(CH_3)_2\\ \diagup\!\!\diagup C_5H_{11}(iso)\\ \diagdown CH_2CH_2OH\end{matrix}$ had an action more like choline; it was fairly active in causing a fall of pressure before atropine. It differed from choline in causing no rise after atropine. It was more poisonous than choline but much less poisonous than the triamyl compound.

Thus in every case the effect upon the blood pressure was lessened and the toxicity increased by the substitution of other groups

in the place of the trimethyl group of choline. The compound $HClN\begin{matrix}(CH_3)_2\\ CH_2CH_2OH\end{matrix}$ had no effect upon the blood pressure.

(*b*) *Compounds containing the* $-CH_2CH_2O(CH_3CO)$ *group.*—The triethyl and tripropyl compounds were not prepared. The triamyl compound, which was about four times as toxic as acetyl-choline, had but a very slight effect upon the blood pressure; sometimes it caused a slight fall, sometimes a slight rise. The compound $ClN\begin{matrix}(CH_3)_2\\ -C_5H_{11}(iso)\\ CH_2CH_2O(CH_3CO)\end{matrix}$, which was but slightly more toxic than acetyl-choline, was fairly active; sometimes it caused a fall, sometimes a rise of pressure. It was more active than the compound without the acetyl group or the acetyl derivative of the triamyl compound. Thus the introduction of the two methyl groups had the effect of restoring, to a limited degree, the action of this compound to that of the choline type.

(*c*) *Compounds containing the* $-CH_2CH_2O(C_6H_5CO)$ *group.*—The triethyl, the tripropyl, and the triamyl compounds were very toxic, being about eight, thirteen, and ninety times, respectively, as toxic as benzoyl-choline, but only the triamyl compound had a distinct effect upon the blood pressure; this caused a slow and prolonged fall of pressure both before and after atropine. Sometimes there was a slowing of the heart. The compound $ClN\begin{matrix}(CH_3)_2\\ -C_5H_{11}(iso)\\ CH_2CH_2O\ (C_6H_5CO)\end{matrix}$ was much less toxic than the above-mentioned ones; its effect on the blood pressure was variable, sometimes causing a fall, sometimes a rise, both before and after atropine. As stated above this compound partook of the activities of choline, benzoyl-choline, and the triamyl compounds.

(*d*) *Compounds containing the* $-CH_2CHOHCH_3$ *group.*—The trimethyl compound caused a fall of pressure before atropine; after atropine it had no effect or caused a slight rise. The triethyl compound caused a slow prolonged fall of blood pressure and a slowing of the heart; it was more than four times as poisonous as the trimethyl compound. The tripropyl compound was less active but still more poisonous. The triamyl compound caused, in large doses, a slow and prolonged fall of pressure both before and after atropine; it was the most toxic of this group.

There was thus a diminution of the activity of these compounds upon the blood pressure, beginning with the trimethyl compound up to the triamyl; the latter's effect upon the circulation seemed to depend more upon its toxic properties than upon a specific action.

(*e*) *Compounds containing the* $-CH_2CHOHCH_2Cl$ *group.*—The trimethyl compound was fairly active in causing a fall of pressure

before atropine. The triethyl compound caused a slight fall of pressure. The tripropyl compound had practically no effect upon blood pressure. The triamyl compound caused a slight fall or a slight rise of pressure. The tripropyl compound, which had the least effect upon the blood pressure, was the most toxic.

(*f*) *Compounds containing a* $-CH_2CHO(CH_3CO)CH_3$ *group.*—The trimethyl compound was extremely active in causing a fall of pressure before atropine; this action was not entirely abolished by atropine. The triethyl compound caused a rather prolonged fall of pressure before atropine and no effect after it; it was not very active. The tripropyl compound was still less active. The triamyl compound had but little activity. It is interesting to note that these compounds, all of which had some effect upon the blood pressure, were not very toxic.

(*g*) *Compounds containing a* $-CH_2CHO(C_6H_5CO)CH_3$ *group.*—The trimethyl compound had but little action on the blood pressure. The triethyl compound caused a slow prolonged fall of pressure before atropine; it had no effect after atropine. The tripropyl compound was less active. The triamyl compound seemed to be somewhat more active in causing a fall of pressure.

(*h*) *Compounds containing a* $-CH_2CHO(CH_3CO)CH_2Cl$ *group.*—The trimethyl compound caused a fall of pressure before atropine; it had no effect after atropine. The tripropyl compound was less active. The triamyl compound seemed to be slightly more active, sometimes causing a slow prolonged fall, sometimes a slight rise of pressure.

(*i*) *Compounds containing a* $-CH_2CHO(C_6H_5CO)CH_2Cl$ *group.*—The trimethyl compound was about as active as choline in causing a fall of pressure before atropine; it caused a slight rise after atropine. The triethyl compound had a similar effect, but the fall of pressure was more prolonged; the effect of the tripropyl was similar but less marked. The triamyl compound sometimes caused a slow long continued fall, sometimes a rise of pressure both before and after atropine.

(*j*) *Compounds containing a* $-CH_2CHOHCH_2OH$ *group.*—The trimethyl compound was moderately active in causing a fall of pressure before atropine; it had no effect after atropine. The effects of the triethyl and the tripropyl compounds were similar but less marked. The triamyl differed from the preceding in sometimes causing a rise of pressure.

(*k*) *Compounds containing a* $-CH_2CHO(CH_3CO)CH_2O(CH_3CO)$ *group.*—The trimethyl compound was fairly active in causing a fall of pressure before atropine; after atropine it had no effect. The triethyl compound caused a slight but slow and long-continued fall of pressure before atropine and a slowing of the heart; it had no effect after atropine. The tripropyl had a similar but less marked effect.

The triamyl compound caused a slight fall both before and after atropine.

(*l*) *Compounds containing a* $-CH_2CHO(C_6H_5CO)CH_2O(C_6H_5CO)$ *group.*—The trimethyl compound caused a slight fall of pressure both before and after atropine. The triethyl compound caused a slow, rather marked fall of pressure. The tripropyl compound was rather active in causing a slow fall of pressure both before and after atropine.

Summary.—In most cases the $(CH_3)_3$ compounds had the greatest effect upon blood pressure, whether this rose or fell. They differed from the others chiefly in that their effect was more transient and the fall of pressure more easily abolished by atropine. Thus it is possible, as a rule, to prolong the effect of these compounds on the blood pressure by introducing the higher members of the series $(C_nH_{2n+1})_3$, but larger doses are necessary. The effects produced by the higher members are less easily or completely abolished by atropine.

Hence by changing the $(C_nH_{2n+1})_3$ groups it is possible, on the one hand, to increase the effects upon the blood pressure, or it is possible to increase other effects and at the same time diminish those on the blood pressure. The chief exception to the general rule that the $(CH_3)_3$ compounds are more active is in the case of compounds containing the $-CH_2CHO(C_6H_5CO)CH_2O(C_6H_5CO)$ group. In this the ethyl and propyl groups are about as active as the trimethyl compounds.

V. EFFECT OF THE ACETYL GROUP.

(*a*) *Compounds containing the* $(CH_3)_3$ *group.*—The introduction of acetyl groups into $ClN\equiv\begin{matrix}(CH_3)_3\\CH_2CH_2OH\end{matrix}$, $ClN\equiv\begin{matrix}(CH_3)_3\\CH_2CH_2CH_2OH\end{matrix}$, and $ClN\equiv\begin{matrix}(CH_3)_3\\CH_2CHOHCH_3\end{matrix}$ increased the activity of these compounds in causing a fall of pressure very greatly; the activity of the first was increased about 100,000 times, that of the two latter about 1,000 times. Acetyl groups had no effect in the compound $ClN\equiv\begin{matrix}(CH_3)_3\\CH_2CHOHCH_2Cl\end{matrix}$ and but little effect in $ClN\equiv\begin{matrix}(CH_3)_3\\CH_2CHOHCH_2OH\end{matrix}$. The acetyl derivatives were in all cases somewhat more toxic than the original compounds.

(*b*) *Compounds containing the* $(C_2H_5)_3$ *group.*—The introduction of acetyl groups into the compounds $ClN\equiv\begin{matrix}(C_2H_5)_3\\CH_2CHOHCH_3\end{matrix}$ and $ClN\equiv\begin{matrix}(C_2H_5)_3\\CH_2CHOHCH_2OH\end{matrix}$ had no distinct effect on the activity of these upon the blood pressure; their toxicity was, however, somewhat increased.

(*c*) *Compounds containing the* $(C_3H_7)_3$ *group.*—The introduction of acetyl groups into the compounds $ClN\begin{matrix}(C_3H_7)_3\\ CH_2CHOHCH_3\end{matrix}$, $ClN\begin{matrix}(C_3H_7)_3\\ CH_2CHOHCH_2Cl\end{matrix}$ and $ClN\begin{matrix}(C_3H_7)_3\\ CH_2CHOHCH_2OH\end{matrix}$ had no distinct effects upon their activity on the blood pressure. The toxicity of the first two was lessened, that of the third increased, by the acetyl group.

(*d*) *Compounds containing the* $(C_5H_{11})_3$ *groups.*—The introduction of acetyl groups into the compounds $ClN\begin{matrix}(C_5H_{11})_3\\ CH_2CH_2OH\end{matrix}$, $ClN\begin{matrix}(C_5H_{11})_3\\ CH_2CHOHCH_3\end{matrix}$, $ClN\begin{matrix}(C_5H_{11})_3\\ CH_2CHOHCH_2Cl\end{matrix}$, and $ClN\begin{matrix}(C_5H_{11})_3\\ CH_2CHOHCH_2OH\end{matrix}$ had little if any effect upon the activity of these on the blood pressure. The toxicity was in some cases slightly increased, in others slightly diminished.

(*e*) *Compounds containing the group* $=N\begin{matrix}(CH_3)_2\\ C_5H_{11\,(iso)}\end{matrix}$.—The introduction of an acetyl group into the compound $ClN\begin{matrix}(CH_3)_2\\ C_5H_{11\,(iso)}\\ CH_2CH_2OH\end{matrix}$ increased slightly the activity of this in causing a fall of blood pressure; the toxicity was almost doubled.

(*f*) *Compounds containing the group* $(CH_3)_2$.—The introduction of two acetyl groups into the compound $ClN\begin{matrix}(CH_3)_2\\ (CH_2CH_2OH)_2\end{matrix}$ increased its activity in causing a fall of pressure very greatly (perhaps one thousand times); the toxicity[a] was diminished by about one-half.

(*g*) The introduction of an acetyl group into the compound

$$\begin{matrix}CH_2N(CH_3)_3Cl\\ |\\ CHOH\\ |\\ CH_2N(CH_3)_3Cl\end{matrix}$$

increased the activity of this in causing a fall of blood pressure very considerably (perhaps fifteen times); the toxicity was also somewhat increased.

(*h*) The introduction of an acetyl group into the compound

$$\begin{matrix}CH_2N(C_2H_5)_3Cl\\ |\\ CHOH\\ |\\ CH_2N(C_2H_5)_3Cl\end{matrix}$$

seemed to have no effect upon its activity on the circulation; the toxicity was perhaps diminished.

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[a] See Note A at end of bulletin.

(*i*) *Influences of two acetyl groups.*—The compound $ClN\begin{matrix}(CH_3)_2\\(CH_2CH_2O(CH_3CO))_2\end{matrix}$ was always more active than the compound $ClN\begin{matrix}(CH_3)_2\\CH_2CHO(CH_3CO)CH_2O)CH_3CO)\end{matrix}$; in some cases (Exper. 282 and 286) it seemed to be nearly a hundred times more active. It was about three times as toxic.

The activity of the compound $ClN\begin{matrix}CH_3\\H_2\\C_2H_4OC_2H_4OH\end{matrix}$ was much increased by the introduction of two acetyl groups.

Summary.—The acetyl group altered markedly the effects upon the blood pressure only in those compounds containing one or more methyl groups, and in these the effect was largely prevented by the presence of a second acetyl group or of a chlorine atom.

The comparatively low degree of activity of the compound $ClN\begin{matrix}(CH_3)_3\\CH_2CHO(CH_3CO)CH_2O(CH_3CO)\end{matrix}$ is another illustration of the futility of endeavoring to increase the activity of some compounds by multiplying the number of groups upon which a certain action seems dependent.

In general the fall of pressure caused by compounds containing the acetyl group was more easily prevented by atropine than was that caused by similar compounds containing benzoyl groups.

VI. EFFECT OF THE BENZOYL GROUP.

(*a*) *Compounds containing a* $(CH_3)_3$ *group.*—The introduction of a benzoyl group into choline increased very considerably the tendency of this compound to cause a rise of pressure; the toxicity was diminished. The introduction of a benzoyl group into the compound $ClN\begin{matrix}(CH_3)_3\\CH_2CH_2CH_2OH\end{matrix}$ seemed to diminish its effect upon the blood pressure; in the compound $ClN\begin{matrix}(CH_3)_3\\CH_2CHOHCH_3\end{matrix}$ it increased the blood pressure raising activity. The benzoyl group had no effect upon the toxicity of either of the above. The introduction of the benzoyl group into the compound $ClN\begin{matrix}(CH_3)_3\\CH_2CHOHCH_2Cl\end{matrix}$ had practically no effect upon the action on the blood pressure; the toxicity was about doubled. The introduction of two benzoyl groups into the compound $ClN\begin{matrix}(CH_3)_3\\CH_2CHOHCH_2OH\end{matrix}$ had no distinct effect; the toxicity was increased about threefold. Increasing the number of the benzoyl groups

had as little effect as increasing the number of acetyl groups in the analogous compound.

(*b*) *Compounds containing the* $(C_2H_5)_3$ *group.*—The introduction of a benzoyl group into the compounds $ClN\langle\begin{matrix}(C_2H_5)_3\\CH_2CH_2OH\end{matrix}$ and $ClN\langle\begin{matrix}(C_2H_5)_3\\CH_2CHOHCH_3\end{matrix}$ had no distinct effect upon the action of these upon the blood pressure; it diminished their toxicity. The introduction of a benzoyl group into the compound $ClN\langle\begin{matrix}(C_2H_5)_3\\CH_2CHOHCH_2Cl\end{matrix}$ seemed to have no effect upon the action upon the blood pressure; the toxicity was increased nearly fourfold. The introduction of two benzoyl groups into the compound $ClN\langle\begin{matrix}(C_2H_5)_3\\CH_2CHOHCH_2OH\end{matrix}$ seemed to increase the activity of this in causing a fall of blood pressure; the toxicity was increased about ninefold.

(*c*) *Compounds containing the* $(C_3H_7)_3$ *group.*—The introduction of a benzoyl group into the compounds $ClN\langle\begin{matrix}(C_3H_7)_3\\CH_2CH_2OH\end{matrix}$, $ClN\langle\begin{matrix}(C_3H_7)_3\\CH_2CHOHCH_3\end{matrix}$, and $ClN\langle\begin{matrix}(C_3H_7)_3\\CH_2CHOHCH_2Cl\end{matrix}$ seemed to have no effect upon the activity of these upon the blood pressure; it increased the toxicity in all cases. The introduction of two such groups into the compound $ClN\langle\begin{matrix}(C_3H_7)_3\\CH_2CHOHCH_2OH\end{matrix}$ increased the tendency of this to cause a fall of blood pressure; the toxicity was increased fourfold.

(*d*) *Compounds containing the* $(C_5H_{11})_3$ *group.*—The introduction of a benzoyl group into the compounds $ClN\langle\begin{matrix}(C_5H_{11})_3\\CH_2CH_2OH\end{matrix}$, $ClN\langle\begin{matrix}(C_5H_{11})_3\\CH_2CHOHCH_3\end{matrix}$, and $ClN\langle\begin{matrix}(C_5H_{11})_3\\CH_2CHOHCH_2Cl\end{matrix}$ increased somewhat the activity of these upon the blood pressure; the toxicity was increased in all cases.

(*e*) The introduction of a benzoyl group into the compound $ClN\langle\begin{matrix}(CH_3)_2\\C_5H_{11}\text{ (iso)}\\CH_2CH_2OH\end{matrix}$ increased the tendency of this to cause a rise of pressure; the toxicity was somewhat lessened.

(*f*) The introduction of two benzoyl groups into the compound $ClN\langle\begin{matrix}(CH_3)_2\\(CH_2CH_2OH)_2\end{matrix}$ had no marked effect upon the action of this upon the blood pressure; the toxicity was diminished about fivefold. (The introduction of two acetyl groups into this compound had a marked effect.)

(*g*) The introduction of a benzoyl group into the compound $\begin{matrix}CH_2N(CH_3)_3Cl\\|\\CHOH\\|\\CH_2N(CH_3)_3Cl\end{matrix}$ increased its activity in causing a rise of pressure; it was rather more active than benzoyl-choline; the toxicity was at least doubled.

Summary.—It was only in the compounds $ClN\begin{matrix}(CH_3)_3\\CH_2CH_2OH\end{matrix}$ and $\begin{matrix}CH_2N(CH_3)_3Cl\\|\\CHOH\\|\\CH_2N(CH_3)_3Cl\end{matrix}$ that the introduction of the benzoyl group had a distinct effect upon the action upon the blood pressure. In both of these compounds it increased very considerably their power to cause a rise of blood pressure. The toxicity was diminished in the former case and increased in the latter.

Atropine was less efficient in preventing a fall of pressure from compounds containing benzoyl groups than it was in the case of compounds containing acetyl groups.

VII. EFFECT OF CHLORINE IN THE SIDE CHAIN.

(*a*) *Hydroxyl compounds.*—Of the compounds $ClN\begin{matrix}(C_nH_{2n+1})_3\\CH_2CHOHCH_3\end{matrix}$ and $ClN\begin{matrix}(C_nH_{2n+1})_3\\CH_2CHOHCH_2Cl\end{matrix}$ those containing chlorine were more toxic in the case of the trimethyl and tripropyl, but less toxic in the case of the triethyl and triamyl derivatives.

The chlorine had no distinct effect in the action of these compounds upon the blood pressure.

(*b*) *Acetyl derivatives.*—Chlorine diminished the toxicity of the trimethyl and triethyl compounds of the groups $ClN\begin{matrix}(C_nH_{2n+1})_3\\CH_2CHO(CH_3CO)CH_3\end{matrix}$ and $ClN\begin{matrix}(C_nH_{2n+1})_3\\CH_2CHO(CH_3CO)CH_2Cl\end{matrix}$ and increased that of the tripropyl and triamyl compounds.

The introduction of the chlorine atom into the trimethyl compound diminished the activity of this in causing a fall of pressure most markedly (perhaps a thousand times). The effect of the chlorine compound upon the blood pressure was readily overcome by atropine; that of the hydroxy compound was not. The chlorine had no distinct or constant effect upon the action of the tripropyl and triamyl compounds.

(*c*) *Benzoyl derivatives.*—Chlorine markedly increased the toxicity of the trimethyl and triethyl, but had little effect upon that of the

tripropyl and triamyl compounds. The chlorine seemed to diminish slightly the effect of the trimethyl compound in causing a rise of pressure; this action is analogous to the effect of chlorine in the acetyl derivative. The chlorine had no marked or constant effect in the triethyl and tripropyl compounds; it seemed to somewhat increase the activity of the triamyl compound.

VIII. COMPARATIVE ACTIVITY OF NORMAL AND ISO COMPOUNDS.

We have shown[a] that of the compounds $ClN\langle\!\!\langle^{(CH_3)_3}_{CH_2CH_2CH_2OH}$ and $ClN\langle\!\!\langle^{(CH_3)_3}_{CH_2CHOHCH_3}$ and their acetyl and benzoyl derivatives the iso compounds were always the less toxic. There were also distinct differences between these two groups in their effects upon the blood pressure. Thus of the hydroxy compounds the normal was more active than the iso compound in causing a fall of pressure; it seemed from two to ten times more active in some experiments (269, 270, 271). The iso compound was more active in causing a rise of pressure.

The acetyl derivatives were extremely active in causing a fall of pressure, but the action of the normal compound was completely, although with some difficulty, abolished by atropine, whereas that of the iso compound, although much diminished, was not completely abolished even by very large doses of atropine.

Of the benzoyl derivatives the iso was more active in causing a rise of pressure (Exper. 272).

Thus in all three classes the iso compound was more active in causing a rise of pressure or less active (or less persistently active) in causing a fall of pressure. With the butyril-cholines the reverse was the case, i. e., the iso-butyril compound was more active in causing a fall of pressure; the two cases are scarcely analogous, however.

IX. EFFECT OF TWO HYDROXYL GROUPS.

It was shown in our preliminary publication that of the compounds $ClN\langle\!\!\langle^{(C_nH_{2n+1})_3}_{CH_2CHOHCH_3}$ and $ClN\langle\!\!\langle^{(C_nH_{2n+1})_3}_{CH_2CHOHCH_2OH}$ those with the two hydroxyl groups were always the least toxic.[b] In their effects upon

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[a] Jour. Pharm. and Exper. Therap., 1909, I, 303.

[b] The slight toxicity of the trimethyl-dihydroxy compound (homoisomuscarine) was attributed by Meyer to the length of the side chain (Liebig's Annalen, 1904, 337, p. 48), isomuscarine ($HON\langle\!\!\langle^{(CH_3)_3}_{CHOHCH_2OH}$ being much more toxic. An increase in the length of the side chain sometimes increases the toxicity (cf. choline, the homocholines and mono-chlor-oxypropyl-trimethyl ammonium chloride).

he blood pressure the compound $ClN\begin{matrix}(CH_3)_3\\ CH_2CHOHCH_2OH\end{matrix}$ was some-

vhat more active than $ClN\begin{matrix}(CH_3)_3\\ CH_2CHOHCH_3\end{matrix}$ in causing a fall of pres-

ure before atropine, but the latter seemed slightly more active in ausing a rise of pressure. No distinct or constant differences were bservable in the case of the triethyl, tripropyl, and triamyl compounds.

X. GENERAL CONCLUSION.

The most important general conclusion which can be drawn from he above experiments is that the greatest effects upon the blood ressure are produced by those compounds which depart least from he choline type—that is, by those compounds containing the nucleus $HON\begin{matrix}(CH_3)_3\\ CH_2CH_2O—\end{matrix}$; all changes in the group $(C_nH_{2n+1})_3$ or in the ide chain (except the substitution of the terminal hydrogen atom) ended to diminish the effect upon the blood pressure but increased, s a rule, the toxicity.[a]

When this configuration is maintained it is possible to vary the ntensity and character of action upon the circulation within extraor- inarily wide limits by substituting groups in the place of the hydrogen tom.[b]

The rôle of this group is probably to carry the compounds to efinite cell structures or, to use a comparison of Ehrlich's, to make hem fit in a certain mosaic; then the groups which have substituted he hydrogen atom enable the entire compound to exert a definite ction (groups of the lower fatty series to depress the blood pressure, hose of the higher ones to cause a slowing of the heart, those of the romatic series to cause a rise of blood pressure). The groups which n certain combinations contribute so greatly toward a definite action ave, in other combinations, either no effect or even the opposite ffect; introduced into choline the acetyl group led to a remarkable ncrease in the blood-pressure lowering activity of this compound; ntroduced into certain other compounds it had no effect. The ben- oyl group introduced into choline increased the blood-pressure raising ctivity; introduced into certain triamyl compounds it had the oppo-

[a] It is also of interest to note that the greater the variation of the compounds from ie choline type, the less effect did atropine have in preventing a fall of pressure. his was the case whether the variation occurred in the $(C_nH_{2n+1})_3$ group or in the de chain containing the hydroxyl; the resistance to the action of atropine was pecially marked when both were different from choline. The presence of benzoyl oups in the side chain was especially potent in preventing the action of atropine.

[b] We are now engaged in the study of further compounds of this character.

site effect. The amyl group, which in some compounds contributes so greatly to their blood-pressure lowering activity, increased the tendency of others to cause a rise of pressure.

The reasons for the efficiency of the group $HON\begin{matrix}=(CH_3)_3\\ \diagdown CH_2CH_2O-\end{matrix}$ as a carrier and the low toxicity of its derivatives are perhaps to be found in the fact that choline is a constituent of probably all plant and animal cells; these have, to use the rather crude comparison of a mosaic again, places into which the compounds can fit,[a] and the cells themselves containing such groups are not injured by them as they would be by new and unusual groups.[b]

Note A.—EXPERIMENTS ON TOXICITY.

The toxicity of a number of the compounds discussed in the above was determined upon mice. The results, which have not been previously reported, were as follows. The compounds were injected in aqueous solution subcutaneously.

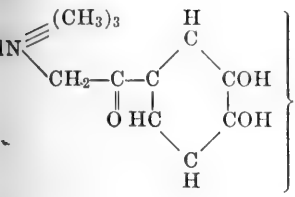
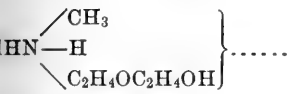
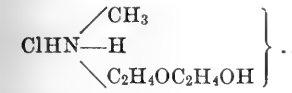
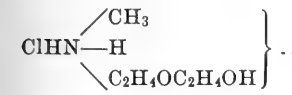
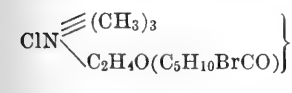
Compounds.	Dose in mgm. per gm. mouse.		Average dose in mgm. per gm.	Average fatal dose compared with choline =1.	Molecular weight.	Average fatal dose of molecule compared with choline =1.
	Survived.	Died.				
$ClN\begin{matrix}=(CH_3)_3\\ \diagdown CH_2CH_2OH\end{matrix}$	0.72	0.75	0.735	1.00	139.59	1.00
$ClN\begin{matrix}=(CH_3)_2\\ \diagdown (CH_2CH_2OH)_2\end{matrix}$	0.11	0.13	0.12	0.163	169.61	0.134
$ClN\begin{matrix}=(CH_3)_2\\ \diagdown (CH_2CH_2O(CH_3CO))_2\end{matrix}$	0.28	0.31	0.295	0.401	253.64	0.221
$ClN\begin{matrix}=(CH_3)_2\\ \diagdown (CH_2CH_2O(C_6H_5CO))_2\end{matrix}$	0.504	0.525	0.514	0.699	377.67	0.258
$\begin{matrix}CH_2N(CH_3)_3Cl\\ CHOH\\ CH_2N(CH_3)_3Cl\end{matrix}$	0.600	.....	[c] 0.60	[c] 0.816	247.16	[c] 0.461
$\begin{matrix}CH_2N(CH_3)_3Cl\\ CHO(CH_3CO)\\ CH_2N(CH_3)_3Cl\end{matrix}$	0.52	0.65	0.585	0.796	289.18	0.384
$\begin{matrix}CH_2N(CH_3)_3Cl\\ CHO(C_6H_5CO)\\ CH_2N(CH_3)_3Cl\end{matrix}$	0.248	0.347	0.297	0.404	351.20	0.160
$\begin{matrix}CH_2N(C_2H_5)_3Cl\\ CHOH\\ CH_2N(C_2H_5)_3Cl\end{matrix}$	[d] 0.42	0.40	0.41(?)	0.557	331.26	0.234

[a] Cf. Hunt, Jour Amer. Med. Assoc., 1907, 49, p. 1329.

[b] Joseph and Meltzer (Jour. Pharm. and Exp. Ther., 1909, 1, p. 1) found that the toxicity of magnesium, calcium, potassium, and sodium ions varies inversely to the proportion in which they occur in the serum of the animal.

[c] Greater than.

[d] Results irregular.

Compounds.	Dose in mgm. per gm. mouse.		Average dose in mgm. per gm.	Average fatal dose compared with choline = 1.	Molecular weight.	Average fatal dose of molecule compared with choline = 1.
	Survived.	Died.				
$H_2N(C_2H_5)_3Cl$ $HO(CH_3CO)$ $H_2N(C_2H_5)_3Cl$	0.16	.....	a 0.46	a 0.625	373.28	a 0.234
	0.33	0.35	0.34	0.462	245.61	0.263
	0.27	.....	a 0.27	a 0.367	a 155.60	0.329
iacetyl derivative of—						
	0.65	0.8	0.73	0.993	239.63	0.598
ibenzoyl derivative of—						
	1.5	1.7	1.6	2.176	363.66	0.835
	1.1	1.4	1.25	1.700	316.62	0.749

a Greater than.

ote B.—EFFECT OF FEEDING THYROID UPON THE TOXICITY OF SOME OF THE ABOVE COMPOUNDS.

It has been shown in a number of publications from this laboratory [a] hat the feeding of thyroid to mice markedly increases their resistance o a methyl compound, viz, methyl cyanide or acetonitrile. It was hought that it would be of interest to determine if a similar reaction ould be obtained with some of the methyl and other compounds escribed in these experiments. The results were entirely negative.

Experiments were made with the following compounds:

omocholine chloride and the benzoyl derivative.
cetyl and benzoyl derivatives of isooxypropyl-trimethyl-ammonium chloride.
enzoyl derivative of mono-chlor-isooxypropyl-trimethyl-ammonium chloride.
ioxyethyl-dimethyl-ammonium chloride and the diacetyl derivative.
enzoyl derivative of hexamethyl-oxypropyl-diammonium chloride.
thyl-choline.
ooxypropyl-triethyl-ammonium chloride and the acetyl derivative.
onochlor-isooxypropyl-triethyl-ammonium chloride.

[a] Cf. Hunt and Seidell, Bull. No. 47, Hyg. Lab., 1909.

Isooxypropyl-tripropyl-ammonium chloride.
Mono-chlor-isooxypropyl tripropyl-ammonium chloride and the benzoyl derivative.
Oxyethyl-triamyl-ammonium chloride and the benzoyl derivative.
Isooxypropyl-triamyl-ammonium chloride and the benzoyl derivative.
Mono-chlor-isooxypropyl-triamyl-ammonium chloride and the benzoyl derivative.

Note C.—ON THE EFFECT OF SOME OF THE ABOVE COMPOUNDS ON TASTE.

The above compounds, in approximately 1 per cent solutions, were tasted and the following notes made:

(*a*) *Compounds containing a* $(CH_3)_3$ *group.*—All of the compounds containing the trimethyl group were practically tasteless except those containing one or more benzoyl groups which were all bitter.

The most bitter of these were the compounds

$ClN\begin{matrix}(CH_3)_3\\ \diagdown\\ CH_2CHO(C_6H_5CO)CH_2O(C_6H_5CO)\end{matrix}$ and $ClN\begin{matrix}(CH_3)_3\\ \diagdown\\ CH_2CH_2CH_2O(C_6H_5CO)\end{matrix}$;

the latter was far more bitter than the compounds

$ClN\begin{matrix}(CH_3)_3\\ \diagdown\\ CH_2CHO(C_6H_5CO)CH_3\end{matrix}$ and $ClN\begin{matrix}(CH_3)_3\\ \diagdown\\ CH_2CHO(C_6H_5CO)CH_2Cl\end{matrix}$

The compounds $ClN\begin{matrix}(CH_3)_2\\ \diagdown\\ (CH_2CH_2O(C_6H_5CO))_2\end{matrix}$ and $\begin{matrix}CH_2N(CH_3)_3Cl\\ |\\ CHO(C_6H_5CO)\\ |\\ CH_2N(CH_3)_3Cl\end{matrix}$

were slightly bitter.

(*b*) *Compounds containing the* $(C_2H_5)_3$ *group.*—Those with a side chain containing one or more hydroxyl groups were slightly bitter; those containing an acetyl group were practically tasteless except that the one containing two acetyl groups was somewhat bitter. All of the compounds containing a benzoyl group were very bitter.

The compound $\begin{matrix}CH_2N(C_2H_5)_3Cl\\ |\\ CHOH\\ |\\ CH_2N(C_2H_5)_3Cl\end{matrix}$ and its acetyl derivative were tasteless.

(*c*) *Compounds containing the* $(C_3H_7)_3$ *group.*—Those compounds containing a hydroxyl group in the side chain were slightly bitter. The compound containing the $-CH_2CHO(CH_3CO)CH_2Cl$ group was very slightly bitter; the one without the chlorine atom was very bitter. All of the compounds containing a benzoyl group were extremely bitter.

(*d*) *Compounds containing the* $(C_5H_{11})_3$ *group.*—All of these compounds were extremely bitter; the one containing the group $-CH_2CHO(CH_3CO)CH_3$ was less bitter than the one containing a chlorine atom. The acetyl derivative of the oxyethyl compound was but slightly bitter.

(*e*) *Compounds containing the* $=N\begin{matrix}(CH_3)_2\\ \diagdown\\ C_5H_{11}\end{matrix}$ *group.*—All of these were bitter.

PROTOCOLS.

EXPERIMENT 210.—RABBIT, 1.89 K. URETHANE-ETHER.

Time.	Compound.	Blood pressure.
M. m. 34	Choline chloride ..... 1 to 1,000, 2 c. c.	Fell 19 mm. (68 to 49); rose 6 mm. (68 to 74). Heart slowed from 47 to 42 beats in 10 sec.
39	Vagi cut	
46	Choline ..... 1 to 1,000, 2 c. c.	Fell 17 mm. (69 to 52). Heart slowed from 52 to 49 beats in 10 sec.
48	Atropine, 8 mg	
53	Choline ..... 1 to 1,000, 2 c. c.	Rose 19 mm. (89 to 108).

EXPERIMENT 214.—HARE, 2.86 K. URETHANE AND CHLORAL; CURARE; VAGI CUT.

57	Choline ..... 1 to 100, 2 c. c.	Fell 35 mm. (from 118 to 83).
59	Acetyl-choline ..... 1 to 5,000, 0.3 c. c.	Fell 91 mm. (from 124 to 33).
9	Benzoyl-choline ..... 0.9 per cent, 0.4 c. c.	Rose 77 mm. (from 112 to 189).
14	$BrN\begin{matrix}\equiv(CH_3)_3\\ \diagdown C_2H_4Br\end{matrix}$ ..... 1 to 100, 1 c. c.	Fell 62 mm. (from 132 to 70). (Remained low for some time).
19	Benzoyl-choline ..... 0.9 per cent, 1 c. c.	Rose 100 mm. (from 84 to 184). Heart rate increased from 38+ to 43½ in 10 sec.
40	Acetyl-choline ..... 1 to 500,000, 1 c. c.	Fell 22 mm. (80 to 58).
41	Atropine sulphate ..... 10 mg.	
53	Acetyl-choline ..... 1 to 5,000, 0.4 c. c.	No change.
54	Benzoyl-choline ..... 0.9 per cent, 0.4 c. c.	Rose 68 mm. (74 to 142).
57	$BrN\begin{matrix}\equiv(CH_3)_3\\ \diagdown C_2H_4Br\end{matrix}$ ..... 1 to 100, 1 c. c.	Rose 56 mm. (79 to 135).
19	Choline ..... 1 to 100, 2 c. c.	Rose 48 mm. (87 to 135).
21	Depressor ..... (Stimulated for 10 sec.)	Fell 34 mm. (90 to 56).
24	$BrN\begin{matrix}\equiv(CH_3)_3\\ \diagdown C_2H_4Br\end{matrix}$ ..... 1 to 100, 1 c. c.	Rose 61 mm. (85 to 146).
	Depressor	Fell 54 mm. (146 to 92).
32	Depressor ..... (Stimulated for 20 sec.)	Fell.
33	Choline ..... 1 to 100, 2 c. c.	Rose 61 mm. (61 to 122).
33½	Depressor	Fell 64 mm. (122 to 58).

EXPERIMENT 218.—RABBIT, 2.15 K. URETHANE AND CHLORAL; CURARE; VAGI CUT.

Time.	Compound.	Blood pressure.
H. *m.*		
1 28	Propionyl-choline ..... 1 to 10,000, 1 c. c.	Fell 26 mm. (78 to 52).
35	Iso-valeryl-choline..... 1 to 10,000, 1 c. c.	Rose 61 mm. (75 to 136). Heart slowed for a few seconds.
49	Phenyl-propionyl-choline ..... 1 to 1,430, 1 c. c.	Rose 37 mm. (83 to 120).
2 2	Choline lactate ..... 1 to 100, 1 c. c.	Fell 20 mm. (76 to 56).
07	Choline camphorate ..... 1 to 100, 1 c. c.	Fell 16 mm. (74 to 58).
52	Propionyl-choline ..... 1 to 10,000, 2 c. c.	Fell 28 mm. (84 to 56).
55	Atropine sulphate..... 10 mgm.	
59	Propionyl-choline ..... 1 to 10,000, 1 c. c.	0.
3 4	Propionyl-choline..... 1 to 1,000, 1 c. c.	Rose 49 mm. (88 to 137). (Quickly returned.)
8	Phenyl-propionyl-choline ..... 1 to 7,000, 1 c. c.	Rose 64 mm. (93 to 157).
16	Iso-valeryl-choline..... 1 to 1,000, 1 c. c.	Rose 65 mm. (91 to 156).
21	Choline lactate ..... 1 to 100, 1 c. c.	Rose 24 mm. (85 to 109).
25	Choline camphorate ..... 1 to 1,000, 1 c. c.	Rose 24 mm. (84 to 108).

EXPERIMENT 220.—RABBIT, 1.55 K. URETHANE AND CHLORAL; CURARE; VAGI CUT.

2 53	Anisyl-choline ..... 1 to 100, 1 c. c.	Rose 40 mm. (83 to 123).
55	Acetyl-choline ..... 1 to 50,000, 1 c. c.	Fell 29 (77 to 48).
3 6	Anisyl-choline ..... 1 to 100, 1 c. c. Acetyl-choline ..... 1 to 50,000, 1 c. c. (mixed).	Fell slightly.
14	Acetyl-choline ..... 1 to 5,000, 1 c. c.	Fell (from 94) to 0. Animal died.

EXPERIMENT 221.—RABBIT, 2.75 K. URETHANE AND CHLORAL; CURARE; VAGI CUT.

2 8	Phthalyl-choline ..... 1 per cent, 1 c. c.	0.
13	Anisyl-choline..... 1 per cent, 1 c. c.	Rose 53 mm. (92 to 145). Fell slowly to 54.
25	Succinyl-choline..... 1.47 per cent, 1 c. c.	Rose 76 mm. (58 to 134).
39	Acetyl-choline..... 1 to 10,000 (?), 1 c. c.	Fell 45 mm. (89 to 44).
42	Succinyl-choline..... 1.47 per cent, 1 c. c.	Fell 13 mm. (79 to 66).
	Acetyl-choline..... 1 to 10,000 (?), 1 c. c.	Rose 48 mm. (79 to 127). Heart slow and irregular.

EXPERIMENT 221.—RABBIT, 2.75 K. URETHANE AND CHLORAL; CURARE; VAGI CUT.—Con.

Time.	Compound.	Blood pressure.
H. m. 56	Succinyl-choline 1.47 per cent, 1 c. c.	Rose 61 mm. (83 to 144). Heart slowed from 31 to 15 beats in 10 sec.
3 2	Suprarenalin 1 to 50,000, 1 c. c.	Fell 15 mm. (103 to 88).
	Acetyl-choline 1 to 10,000 (?), 1 c. c.	Rose 35 mm. (103 to 138).
9	Suprarenalin 1 to 50,00, 1 c. c.	Rose 68 mm. (95 to 163.
13	Atropine sulphate 9 mgm.	
17	Succinyl-choline 1.47 per cent, 1 c. c.	Rose 35 mm. (92 to 127). Returned slowly.
21	Phthalyl-choline 1 per cent, 1 c. c.	0.
27	Anisyl-choline 1 to 200, 1 c. c.	Rose 70 mm. (66 to 136).
45	Succinyl-choline 1.47 per cent, 1 c. c.	Rose 63 mm. (39 to 102).
49	Succinyl-choline 1.47 per cent, 1 c. c.	Rose 43 mm. (84 to 127).
	Depressor stimulated	
4 6	Valeryl-choline 1 to 1,000, 1 c. c.	Rose 36 mm. (100 to 136). Heart rate increased from 35 to 41+ beats in 10 sec.
9	Depressor stimulated	Fell 40 mm. (102 to 62).
14	Phenyl-propionyl-choline 0.07 per cent, 1 c. c.	Rose 14 mm. (98 to 112).
	Depressor	Fell 8 mm. (98 to 90).
17	Phenyl-propionyl-choline 0.07 per cent, 1 c. c.	Rose 33 mm. (95 to 128).
22	Propionyl-choline 0.9 per cent, 1 c. c.	Rose 25 mm. (100 to 125).

EXPERIMENT 222.—HARE, 2.77 K. URETHANE AND CHLORAL; CURARE.

1 3	Iso-valeryl-choline 1 to 1,000, 1 c. c.	Fell 48 mm. (92 to 44); rose 63 mm. (92 to 155). Heart rate slowed from 47 to 11 in 10 sec.
11	Anisyl-choline 1 to 200, 1 c. c.	Fell 30 mm. (88 to 58). Heart slowed from 41 to $23\frac{1}{2}$ beats in 10 sec.
20	Succinyl-choline 1.47 per cent, 1 c. c.	Fell 50 mm. (99 to 49); rose 27 mm. (99 to 126). Heart rate slowed from 36 to $20\frac{1}{2}$ in 10 sec.
24	Iso-valeryl-choline 1 to 1,000, 1 c. c.	Fell 44 mm. (100 to 56); rose 44 mm. (100 to 144). Heart slowed from 36 to 20 in 10 sec.
27	Succinyl-choline 1.47 per cent, 0.4 c. c.	Fell 41 mm. (100 to 59). Heart slowed from 36 to 31 in 10 sec.
28	Acetyl-choline 1 to 10,000, 0.3 c. c.	Fell 52 mm. (98 to 46). No change in heart rate.
51	Phenyl-propionyl-choline 1 to 1,430, 1 c. c.	Fell 28 mm. (118 to 90); rose 20 mm. (118 to 138). Heart slowed from $37\frac{1}{2}$ to 14 beats in 10 sec.
58	Propionyl-choline 1 to 1,000, 1 c. c.	Fell 41 mm. (113 to 72); rose 13 mm. (113 to 126). Heart slowed from 42 to $30\frac{1}{2}$ beats in 10 sec.

EXPERIMENT 222.—HARE, 2.77 K. URETHANE AND CHLORAL; CURARE—Continued.

Time.	Compound.	Blood pressure.
H. m.		
2 9	Phenyl-acetyl-choline 1 to 2,000, 1 c. c.	Fell 33 mm. (106 to 73); rose 43 mm. (106 to 149). Heart slowed from 42 to 14½ beats in 10 sec.
14	Vagi cut	
19	Phenyl-acetyl-choline 1 to 2,000, 1 c. c.	Rose 71 mm. (113 to 184). No change in heart rate.
29	Phthalyl-choline 1 per cent, 2 c. c.	Fell 18 mm. (107 to 89).
35	Iso-valeryl-choline 1 to 1,000, 1 c. c.	Rose 66 mm. (106 to 172). Heart rate increased from 40 to 46½ in 10 sec.
45	Anisyl-choline 1 to 200, 1 c. c.	Rose 47 mm. (100 to 147).
55	Succinyl-choline 1.47 per cent, 0.4 c. c.	Rose 57 mm. (99 to 156).
3 20	Phenyl-propionyl-choline 1 to 1,430, 1 c. c.	Rose 44 mm. (94 to 138). Heart slowed from 40 to 36 beats in 10 sec.
35	Propionyl-choline 1 to 1,000, 1 c. c.	Fell 22 mm. (74 to 52). No change in heart rate.

EXPERIMENT 223.—RABBIT, 2.56 K. URETHANE AND CHLORAL; CURARE.

11	47	Benzoyl-choline 0.9 per cent, 1 c. c.	Rose 41 mm. (44 to 85). Heart rate increased from 29 to 34 beats in 10 sec.
	53	Phenyl-acetyl-choline 1 to 2,000, 1 c. c.	Rose 52 mm. (58 to 110). Heart rate slowed from 31 to 20 beats in 10 sec.
12	6	Propionyl-choline 0.9 per cent, 1 c. c.	Fell 28 mm. (63 to 35). Heart slowed from 29 to 20⅓ beats in 10 sec.
	13	Phenyl-propionyl-choline 1 to 140, 1 c. c.	Rose 40 mm. (60 to 100). Heart slowed from 30½ to 14 beats in 10 sec.
	17	Acetyl-choline 1 to 10,000, 0.4 c. c.	Fell. Heart slowed from 33 to 15 in 10 sec.; became irregular.
	30	Succinyl-choline 1.47 per cent, 0.4 c. c.	Fell 16 mm. (61 to 45); rose 13 mm. (61 to 74). Heart slowed from 30 to 26 beats in 10 sec.
	48	Iso-valeryl-choline 1 to 2,000, 1 c. c.	Rose 54 mm. (63 to 117). Heart slowed from 36 to 15 beats in 10 sec.
	56	Anisyl-choline 1 to 100, 1 c. c.	Rose 15 mm. (44 to 59). Heart slowed from 35 to 21 beats in 10 sec.
	59	Atropine sulphate 8 mgm.	
1	17	Benzoyl-choline 0.9 per cent, 1 c. c.	Rose 52 mm. (78 to 130).
	29	Phenyl-acetyl-choline 1 to 2,000, 1 c. c.	Rose 74 mm. (70 to 144).
	37	Propionyl-choline 0.9 per cent, 1 c. c.	Rose 40 mm. (58 to 98).
	47	Phenyl-propionyl-choline 1 to 1,430, 1 c. c.	Rose 66 mm. (49 to 115).

EXPERIMENT 225.—RABBIT, 2.52 K. URETHANE AND CHLORAL; CURARE.

Time.	Compound.	Blood pressure.
H. m. 26	Iso-valeryl-choline 1 to 2,000, 1 c. c.	Fell 20 mm. (112 to 92); rose 78 mm. (112 to 190). Heart slowed from 39½ to 16 beats in 10 sec
35	Atropine sulphate, 6 mgm	
41	Iso-valeryl-choline 1 to 2,000, 1 c. c.	Rose 90 mm. (107 to 197). No change in heart rate.
55	Phenyl-acetyl-choline 1 to 4,000, 1 c. c.	Rose 55 mm. (103 to 158).
11	R. depressor stimulated	Fell 35 mm. (75 to 40).
16	Phenyl-acetyl-choline 1 to 8,000, 1 c. c.	Rose 59 mm. (86 to 145).
18	Phenyl-acetyl-choline 1 to 8,000, 1 c. c. R. depressor	Fell 35 mm. (77 to 42); returned to 60 mm.
30	Phenyl-acetyl-choline 1 to 8,000, 1 c. c.	Rose 60 mm. (76 to 136).
23	Phenyl-acetyl-choline 1 to 8,000, 1 c. c. R. depressor (stimulated for 13 sec.)	Rose 62 mm. (68 to 130); fell 12 mm. (68 to 56).
25	Depressor (stimulated for 40 sec.) Phenyl-acetyl-choline 1 to 8,000, 1 c. c.	Fell 36 mm. (66 to 30); rose to 52 mm.
27	Depressor (stimulated for 40 sec.)	Fell 46 mm. (78 to 32).
30	Vagi cut	Rose.
31	R. depressor	Fell 56 mm. (90 to 34).
42	R. depressor	Fell 30 mm. (63 to 33); rose to 44 mm.
	Benzoyl-choline 1 to 1,000 1 c. c.	Heart slowed from 40 to 34½ beats in 10 sec.
44	Benzoyl-choline 1 to 1,000, 1 c. c.	Rose 28 mm. (68 to 96).
58	Propionyl-choline 1 to 1,000, 1 c. c.	Rose 22 mm. (47 to 69).
00	Propionyl-choline 1 to 1,000, 1 c. c. R. depressor	Rose 19 mm. (43 to 62); fell to 43.

EXPERIMENT 227.—RABBIT, 2.79 K. URETHANE AND CHLORAL; CURARE.

22	Phenyl-propionyl-choline 1 to 2,900, 0.5 c. c.	Rose 39 mm. (116 to 155). Heart rate slowed from 39 to 22 in 10 sec.
27	Phenyl-propionyl-choline 1 to 2,900, 1 c. c.	Fell 11 mm. (116 to 105); rose 35 mm. (116 to 151). Heart slowed from 35½ to 18 in 10 sec.
36	Propionyl-choline 1 to 1,000, 1 c. c.	Fell 41 mm. (114 to 73).
37	Choline 1 to 100, 1 c. c.	Fell 25 mm. (114 to 89).
40	Vagi cut and atropine, 8 mgm	
33	Phenyl-propionyl-choline 1 to 2,900, 1 c. c.	Rose 32 mm. (93 to 125).
39	Depressor stimulated	Fell 27 mm. (80 to 53).

EXPERIMENT 227.—RABBIT, 2.79 K. URETHANE AND CHLORAL; CURARE—Continued.

Time.	Compound.	Blood pressure.
H. m.		
46	Phenyl-propionyl-choline 1 to 2,900, 1 c. c. Depressor	Rose 28 mm. (76 to 104); returned to 87.
54	Phenyl-propionyl-choline 1 to 2,900, 1 c. c.	Rose 22 mm. (72 to 94).
58	Depressor Phenyl-propionyl-choline 1 to 2,900, 1 c. c.	Fell 21 mm. (71 to 50); rose 3 mm. (71 to 74).

EXPERIMENT 229.—RABBIT, 2.35 K. URETHANE AND CHLORAL; CURARE.

11	41	m-Nitro-benzoyl-choline 1 to 1,000, 1 c. c.	Fell 48 mm. (124 to 76); rose 45 mm. (124 to 169). Heart slowed from 38 to 20 in 10 sec.; irregular. (Slight movements.)
	55	m-Nitro-benzoyl-choline 1 to 2,000, 1 c. c.	Fell slightly; rose slightly; fell 74 mm. (104 to 30).
12	10	m-Nitro-benzoyl-choline 1 to 2,000, 1 c. c.	Fell 25 mm. (71 to 46). Heart slowed from 56 to about 50 in 10 sec.
	16	Cinnamyl-choline 1 to 1,000, 1 c. c.	Fell 13 mm. (43 to 30). Heart slowed from 52 to 45 in 10 sec.
	38	Vagi cut	
	43	m-Nitro-benzoyl-choline 1 to 2,000, 1 c. c.	0.

EXPERIMENT 231.—RABBIT, 1.9 K. URETHANE AND CHLORAL; CURARE.

2	43	m-Nitro-benzoyl-choline 1 to 4,000, 1 c. c.	Fell 25 mm. (98 to 73).
	50	p-Nitro-benzoyl-choline 1 to 4,000, 1 c. c.	Fell 21 mm. (99 to 78).
	53½	Benzoyl-choline 1 to 1,000, 1 c. c.	Fell 8 mm. (100 to 92); rose 25 mm. (100 to 125).
	58	Cinnamyl-choline 1 to 4,000, 1 c. c.	Fell 26 mm. (100 to 74).
3	9	Vagi cut	
	15	m-Nitro-benzoyl-choline 1 to 4,000, 2 c. c.	0.
	18	p-Nitro-benzoyl-choline 1 to 4,000, 2 c. c.	0.
	22	Benzoyl-choline 1 to 1,000, 1 c. c.	Fell 18 mm. (134 to 116); rose 31 mm. (134 to 165).
	24	Cinnamyl-choline 1 to 4,000, 1 c. c.	Fell 26 mm. (137 to 111). No change in heart rate.
	31	Atropine sulphate, 8 mgm.	
	43	m-Nitro-benzoyl-choline 1 to 1,000, 1 c. c.	0.
	48	p-Nitro-benzoyl-choline 1 to 1,000, 1 c. c.	Rose 24 mm. (117 to 141); returned at once.
	53	Benzoyl-choline 1 to 1,000, 1 c. c.	Fell 19 mm. (114 to 95); rose 25 mm. (114 to 139).
	55	Cinnamyl-choline 1 to 4,000, 1 c. c.	Fell 26 mm. (118 to 92).

EXPERIMENT 232.—HARE, 3.01 K. URETHANE AND CHLORAL; CURARE.

Time.	Compound.	Blood pressure.
H. m.		
1 15	m-Nitro-benzoyl-choline. 1 to 4,000, 1 c. c.	Fell 58 mm. (102 to 44). Heart slowed from 27 to 21½ beats in 10 sec.
22	p-Nitro-benzoyl-choline 1 to 4,000, 1 c. c.	Fell 69 mm. (117 to 48). Heart slowed from 26 to 20½ beats in 10 sec.
28	Choline 1 per cent, 1 c. c.	Fell 33 mm. (107 to 74); rose 11 mm. (107 to 118).
36	Curare	
40	Choline 1 per cent, 1 c. c.	Rose 41 mm. (65 to 106).

EXPERIMENT 235.—RABBIT, 1.75 K. URETHANE AND CHLORAL; CURARE.

2	27	Acetyl-choline 1 to 200,000, 0.4 c. c.	Fell 25 mm. (88 to 63). Heart increased from 32½ to 39 beats in 10 sec.
	28	Choline 1 to 200, 1 c. c.	Fell 9 mm. (86 to 77). Heart slowed from 32 to 29 beats in 10 sec.
3	10	Vagi cut	
	46	Acetyl-choline 1 to 100,000, 0.4 c. c.	Fell 24 mm. (77 to 53). No effect on heart rate.
4	28	Suprarenalin 1 to 50,000, 1 c. c.	Rose 73 mm. (27 to 100).
	32	Suprarenalin 1 to 50,000, 1 c. c. Mixed with acetyl-choline 1 to 100,000, 1 c. c.	Rose 41 mm. (25 to 66).

EXPERIMENT 236.—RABBIT, 2.29 K. URETHANE AND CHLORAL; CURARE.

12	30	Anisyl-choline 1 to 200, 1 c. c.	Fell 33 mm. (96 to 63). (Remained low for some time.) Heart slowed from 40 to 27 beats in 10 sec.
	32	Benzoyl-choline 1 to 1,000, 1 c. c.	Rose 26 mm. (66 to 92). Heart slowed from 36 to 28½ beats in 10 sec.
	38	Benzoyl-choline 1 to 1,000, 0.3 c. c.	Fell 51 mm. (102 to 51). Heart slowed from 38 to 27½ beats in 10 sec.
	40	Cinnamyl-choline 1 to 4,000, 0.4 c. c.	Fell 71 mm. (111 to 40); rose 39 mm. (111 to 150). Heart stopped for a few seconds, began to beat slowly, then increased to 37½, then slowed to 18½ in 10 sec., then increased to 33.
	58	n-Butyril-choline 1 to 10,000, 1 c. c.	Fell 54 mm. (112 to 58); rose 46 mm. (112 to 158). Heart almost stopped; then beat at 15 per 10 sec., then increased to 29.
1	11	Acetyl-choline 1 to 50,000, 1 c. c.	Fell 42 mm. (102 to 60). Heart increased from 28 to 33½ in 10 sec.
	14	Vagi cut	
	34	Anisyl-choline 1 to 100, 1 c. c.	Rose 36 mm. (126 to 162). Heart increased from 35 to 40 in 10 sec.

EXPERIMENT 236.—RABBIT, 2.29 K. URETHANE AND CHLORAL; CURARE—Continued.

Time.	Compound.	Blood pressure.
H. m.		
40	Cinnamyl-choline ..... 1 to 4,000, 1 c. c.	Fell 19 mm. (97 to 78).
43	Anisyl-choline ..... 1 to 100, 0.4 c. c.	Fell 36 mm. (108 to 72).
51	Cinnamyl-choline ..... 1 to 1,000, 1 c. c.	Fell 46 mm. (122 to 76).
53	n-Butyril-choline ..... 1 to 10,000, 1 c. c.	Fell 20 mm. (119 to 99); rose 37 mm. (119 to 156). Heart rate not changed.
2 16	Atropine sulphate, 8 mg	
24	Cinnamyl-choline ..... 1 to 1,000, 1 c. c.	Fell 36 mm. (96 to 60). No effect on heart rate.
28	Vagi stimulated	0.
35	Acetyl-choline ..... 1 to 50,000, 1 c. c.	Fell 28 mm. (96 to 68).
48	n-Butyril-choline ..... 1 to 10,000, 1 c c.	Fell 16 mm. (104 to 88); rose 62 mm. (104 to 166). Heart slowed from 35½ to 23 beats in 10 sec.
55	Benzoyl-choline ..... 1 to 1,000, 1 c. c.	Rose 38 mm. (86 to 124).
3 00	Anisyl-choline ..... 1 to 200, 1 c. c.	Rose 39 mm. (85 to 124).

EXPERIMENT 238.—RABBIT, 2.27 K. URETHANE AND CHLORAL; CURARE; VAGI INTACT; NO ATROPINE.

1 14	Choline ..... 1 to 100, 1 c. c.	Fell 12 mm. (66 to 54); rose 34 mm. (66 to 100). Heart slowed from 38 to 27 beats in 10 sec.
20	$BrN\begin{matrix}\equiv(CH_3)_3\\ \diagdown C_2H_4Br\end{matrix}$ ..... 1 to 200, 0.5 c. c.	Fell 16 mm. (54 to 38); rose 45 mm. (54 to 99). Heart slowed from 38 to 26½ beats in 10 sec.
23	Choline ..... 1 to 100, 1 c. c.	Rose 25 mm. (40 to 65). Heart slowed from 32½ to 28½ beats in 10 sec.
24	$BrN\begin{matrix}\equiv(CH_3)_3\\ \diagdown C_2H_4Br\end{matrix}$ ..... 1 to 200, 1 c. c.	Rose 35 mm. (33 to 68). Heart slowed from 32½ to 25 beats in 10 sec.

EXPERIMENT 240.—RABBIT, 2.14 K. URETHANE AND CHLORAL; CURARE.

11 45	Choline chloride ..... 1 to 100, 1 c. c.	Fell 28 mm. (104 to 76). Heart slowed from 45 to 35½ beats in 10 sec.
47	Isobutyril-choline ..... 1 to 10,000, 1 c. c.	Fell 33 mm. (102 to 69). Heart slowed from 32 to 28 beats in 10 sec.
12 02	n-Butyril-choline ..... 1 to 50,000, 1 c. c.	0.
18	Vagi cut	
22	Choline ..... 1 to 100, 1 c. c.	Fell 17 mm. (73 to 56). Heart slowed from 46½ to 39 beats in 10 sec
24	Butyril-choline ..... 1 to 10,000, 1 c. c.	0.
43	Atropine sulphate, 8 mg	

EXPERIMENT 240.—RABBIT, 2.14 K. URETHANE AND CHLORAL; CURARE—Continued.

Time.	Compound.	Blood pressure.
H. m.		
47	Choline chloride ..... 1 to 100, 1 c. c.	0.
51	n-Butyril-choline ..... 1 to 1,000, 1 c. c.	Rose 16 mm. (29 to 45).
55	Isobutyril-choline ..... 1 to 1,000, 1 c. c.	0.

EXPERIMENT 243.—RABBIT, 1.87 K. URETHANE AND CHLORAL; CURARE; VAGI CUT.

22	Propionyl-choline ..... 1 to 10,000, 1 c. c.	Fell 28 mm. (97 to 69); rose 10 mm. (97 to 107).
27	Choline ..... 1 to 200, 0.4 c. c.	Fell 22 mm. (100 to 78); rose 22 mm. (100 to 122) (slight movements).
43	Acetyl-choline ..... 1 to 2,000,000, 1 c. c.	Fell 33 mm. (126 to 93).
54	Acetyl-choline ..... 1 to 5,000,000, 1 c. c.	Fell 29 mm. (133 to 104).
29	Acetyl-choline ..... 1 to 20,000,000, 1 c. c.	Fell 12 mm. (122 to 110).
33	Choline ..... 1 to 200, 1 c. c.	Fell 18 mm. (120 to 102); rose 29 mm. (120 to 149).
39	Acetyl-choline ..... 1 to 1,000,000, 1 c. c.	Fell 32 mm. (112 to 80).
41	Atropine sulphate, 6 mg	
42	Acetyl-choline ..... 1 to 1,000,000, 1 c. c.	0.
45	Choline ..... 1 to 200, 1 c. c.	Rose 40 mm. (81 to 121).
08	Propionyl-choline ..... 1 to 1,000, 1 c. c.	Rose 15 mm. (65 to 80).
15	Acetyl-choline ..... 1 to 50,000, 1 c. c.	Fell 4 mm. (55 to 51).
23	Acetyl-choline ..... 1 to 12,500, 1 c. c.	Fell 12 mm.
29	Choline ..... 1 to 200, 1 c. c.	Rose 52 mm. (65 to 117).
32	Propionyl-choline ..... 1 to 200, 1 c. c.	Rose 40 mm. (68 to 108).
38	n-Butyril-choline ..... 1 to 10,000, 1 c. c.	Rose 28 mm. (56 to 84).
47	n-Butyril-choline ..... 1 to 1,000, 1 c. c.	Rose 37 mm. (49 to 86).
49	iso-Butyril-choline ..... 1 to 1,000, 1 c. c.	Rose 38 mm. (46 to 84).
09	Propionyl-choline ..... 1 to 200, 1 c. c.	Rose 15 mm. (37 to 52).
11	Choline ..... 1 to 200, 1 c. c.	Rose 12 mm. (36 to 48).
34	Acetyl-choline ..... 1 to 2,500, 1 c. c.	Fell 32 mm.
	Vagus stimulated	0.

EXPERIMENT 246.—RABBIT, 2.6 K. URETHANE AND CHLORAL; CURARE; VAGI CUT; NO ATROPINE.

Time.	Compound.	Blood pressure.
H. m.		
11 23	Normal saline ..... 1 c. c.	Fell 2 mm. (114 to 112).
26	Acetyl-choline ..... 1 to 250,000,000, 1 c. c.	Fell 5 mm. (115 to 110).
35	Acetyl-choline ..... 1 to 25,000,000, 1 c. c.	Fell 21 mm. (117 to 96).
41	Acetyl-choline ..... 1 to 125,000,000, 1 c. c.	Fell 7 mm. (114 to 107).
52	n-Butyril-choline ..... 1 to 100,000, 1 c. c.	Fell 24 mm. (120 to 96).
56	Acetyl-choline ..... 1 to 100,000,000, 1 c. c.	Fell 26 mm. (125 to 99).
12 22	iso-Butyril-choline ..... 1 to 100,000, 1 c. c.	Fell 18 mm. (122 to 104).
23	n-Butyril-choline ..... 1 to 100,000, 1 c. c.	Fell 20 mm. (120 to 100); rose 10 mm. (120 to 130) (slight movements).
2 12	Acetyl-choline ..... 1 to 100,000,000, 1 c. c.	Fell 16 mm. (86 to 70).
13	Adrenalin ..... 1 to 200,000, 1 c. c.	Rose 40 mm. (78 to 118).
15	Adrenalin ..... 1 to 200,000, 1 c. c. Acetyl-choline ..... 1 to 100,000,000, 1 c. c.	Rose 10 mm. (76 to 86).
42	Choline ..... 1 to 1,000, 1 c. c.	Fell 4 mm. (72 to 68); rose 6 mm. (72 to 78).
45	n-Butyril-choline ..... 1 to 50,000, 1 c. c.	Fell 7 mm. (67 to 60); rose 34 mm. (67 to 101).
50	iso-Butyril-choline ..... 1 to 50,000, 1 c. c.	Fell 12 mm. (68 to 56).
54	n-Butyril-choline ..... 1 to 50,000, 1 c. c.	Fell 8 mm. (66 to 58); rose 26 mm. (66 to 92).

EXPERIMENT 248.—RABBIT, 2.2 K. URETHANE AND CHLORAL; CURARE; VAGI CUT.

10 47	Acetyl-choline ..... 1 to 50,000,000, 1 c. c.	Fell 21 mm. (114 to 93).
50	Saline ..... 0.8 per cent, 1 c.c.	Fell 5 mm. (114 to 109).
52	Acetyl-choline ..... 1 to 100,000,000.	Fell 16 mm. (112 to 96).
58	Saline ..... 1 c. c.	Rose 4 mm. (119 to 123); fell 2 mm. (119 to 117).
11 15	Acetyl-choline ..... 1 to 100,000,000, 1 c. c.	Fell 15 mm. (130 to 115).
17+	Saline ..... 1 c. c.	0.
33	Saline ..... 1 c. c.	0.
37	Adrenalin ..... 1 to 800,000, 1 c. c.	Rose 9 mm. (130 to 139).
41	Saline ..... 1 c. c.	0.

XPERIMENT 248.—RABBIT, 2.2 K. URETHANE AND CHLORAL; CURARE; VAGI CUT—Con.

ime.	Compound.	Blood pressure.
m.		
43	Acetyl-choline 1 to 200,000,000, 1 c. c.	Fell 5 mm. (142 to 137).
35	Acetyl-choline 1 to 2,500,000, 0.8 c. c.	Fell 15 mm. (72 to 57). Heart rate slowly decreased from 40 to 33 in 10 sec.
39	Adrenalin 1 to 200,000, 1 c. c.	Rose 39 mm. (49 to 88). Heart rate slowly increased from 33 to 40½ in 10 sec.
43	Acetyl-choline 1 to 2,500,000, 1 c. c. Adrenalin 1 to 200,000, 1 c. c.	Fell 20 mm. (62 to 42). Heart rate slowly decreased from 40 to 34½ in 10 sec.
48	Adrenalin 1 to 200,000, 1 c. c.	Rose 30 mm. (74 to 104).
52	Acetyl-choline 1 to 2,500,000, 1 c. c.	Fell 42 mm. (57 to 15). Heart rate slowly decreased from 38 to 24.

XPERIMENT 252 (JULY 31, 1906).—RABBIT, 1.85 K. URETHANE AND CHLORAL; ETHER; NO CURARE.

59	Nitroglycerin 1 to 100,000, 1 c. c.	Fell 18 mm. (106 to 88).
1	Acetyl-choline 1 to 25,000,000, 1 c. c.	Fell 18 mm. (106 to 88).
9	Saline 1 c. c,	0.
13	Acetyl-choline 1 to 100,000,000, 1 c. c.	Fell 16 mm. (108 to 92).
15	Nitroglycerin 1 to 200,000, 1 c. c.	Fell 10 mm. (110 to 100).
19	Saline 1 c. c.	0.
23	Nitroglycerin 1 to 500,000, 1 c. c.	0.
24	Acetyl-choline 1 to 200,000,000. 1 c. c.	Fell 8 mm. (114 to 106).
28	Saline 1 c. c.	0.
34	Acetyl-choline 1 to 100,000,000, 1 c. c.	Fell 21 mm. (103 to 82).
38	Choline chloride 1 to 2,000, 1 c. c.	Fell 8 mm. (112 to 104).
42	Choline chloride 1 to 1,000, 1 c. c.	Fell 30 mm. (108 to 78).
50	Saline 1 c. c.	0.
51	Suprarenalin 1 to 400,000, 0.5 c. c.	Rose 39 mm. (106 to 145).
53	Choline 1 to 1,000, 1 c. c. Suprarenalin 1 to 400,000, 1 c. c.	Rose 26 mm. (105 to 131); fell 10 mm. (105 to 95).
54	Suprarenalin 1 to 400, 000, 1 c. c.	Rose 49 mm. (98 to 147).
56	Choline 1 to 1,000, 1 c. c.	Fell 25 mm. (97 to 72).

EXPERIMENT 252 (JULY 31, 1906).—RABBIT, 1.85 K. URETHANE AND CHLORAL; ETHER; NO CURARE—Continued.

Time.	Compound.	Blood pressure.
H. m.		
3 5½	Suprarenalin ..... 1 to 800,000, 1 c. c.	Rose 15 mm. (104 to 119).
9½	Choline (egg) ..... 1 to 1,000, 1 c. c.	Fell 16 mm. (104 to 88).
14	Saline ..... 1 c. c.	0.
17	Acetyl-choline ..... 1 to 200,000,000, 1 c. c.	Fell 10 mm. (88 to 78).
19½	Saline	0.
58+	Acetyl-choline ..... 1 to 12,500,000, 1 c. c.	Fell 23 mm. (84 to 61); rose 14 mm. (84 to 98).
4 1	Choline (egg) ..... 1 to 500, 1 c. c.	Fell 15 mm. (83 to 68); rose 11 mm. (83 to 94).
5½	Choline (egg) ..... 1 to 100, 1 c. c.	Fell 26 mm. (80 to 54); rose 24 mm. (80 to 104).
8½	Acetyl-choline ..... 1 to 12,500,000, 1 c. c.	Fell 13 mm. (73 to 60).
10	Atropine sulphate, 6 mgr	
12	Acetyl-choline ..... 1 to 12,500,000, 1 c. c.	0.
23½	Acetyl-choline ..... 1 to 250,000, 1 c. c.	0.
28+	Acetyl-choline ..... 1 to 25,000, 1 c. c.	Fell 35 mm. (93 to 58).
30	Choline ..... 1 to 500, 1 c. c.	Rose 20 mm. (87 to 107).
34	Atropine sulphate, 4 mgr	
36	Acetyl-choline ..... 1 to 25,000, 1 c. c.	0.
41	Acetyl-choline ..... 1 to 2,500, 1 c. c.	Fell 15 mm. (47 to 32).

EXPERIMENT 254.—RABBIT, 2.11 K. URETHANE AND CHLORAL.

12	26	iso-Valeryl-choline ..... 1 to 1,000, 1 c. c.	0. Heart slowed from 47 to 41 beats in 10 sec.
	31	n-Butyril-choline ..... 1 to 1,000, 1 c. c.	Fell 14 mm. (45 to 31). Heart slowed from 44 to 34 beats in 10 sec.
	32	Vagi cut	
	33	n-Butyril-choline ..... 1 to 1,000, 1 c. c.	Fell 11 mm. (42 to 31). Heart slowed from 42 to 36½ beats in 10 sec.
	35	iso-Valeryl-choline ..... 1 to 1,000, 1 c. c.	0. No effect on heart.
	38	iso-Valeryl-choline ..... 1 to 100, 0.5 c. c.	0. Heart slowed from 40 to 35½ beats in 10 sec.

EXPERIMENT 256.—CAT, 3.4 K. ETHER; NO CURARE.

12	13	n-Butyril-choline ..... 1 to 100,000, 1 c. c.	Fell 14 mm. (132 to 118).
	18	iso-Butyril-choline ..... 1 to 50,000, 1 c. c.	Fell 10 mm. (136 to 126).

EXPERIMENT 256.—CAT, 3.4 K. ETHER; NO CURARE—Continued.

me.	Compound.	Blood pressure.
m.		
20	n-Butyril-choline 1 to 500,000, 1 c. c.	Fell 7 mm. (136 to 129).
22	Normal saline 1 c. c.	0.
23	Choline 1 to 1,000, 1 c. c.	Fell 31 mm. (135 to 104).
38	Choline 1 to 2,000, 1 c. c.	Fell 16 mm. (138 to 122).
48	iso-Valeryl-choline 1 to 2,000, 1 c. c.	Fell 9 mm. (139 to 130).
49	Vagi cut	
32	Choline 1 to 10,000, 1 c. c.	Fell 21 mm. (127 to 106).
35	n-Butyril-choline 1 to 100,000, 1 c. c.	Fell 22 mm. (112 to 90).
40	iso-Butyril-choline 1 to 100,000, 1 c. c.	Fell 21 mm. (117 to 96).
45	iso-Valeryl-choline 1 to 10,000, 1 c. c.	Fell 14 mm. (126 to 112).
1	Atropine sulphate, 4 mg	
35	iso-Valeryl-choline 1 to 400, 1 c. c.	Rose 45 mm. (124 to 169).
40	n-Butyril-choline 1 to 2,000, 1 c. c.	Rose 39 mm. (128 to 167).
48	iso-Butyril-choline 1 to 2,000, 1 c. c.	Rose 18 mm. (142 to 160).
51	n-Butyril-choline 1 to 4,000, 1 c. c.	Rose 12 mm. (140 to 152).
56	Choline 1 to 200, 1 c. c.	Rose 13 mm. (144 to 157).

EXPERIMENT 257.—CAT, 2.4 K. URETHANE AND CHLORAL; CURARE.

46	Benzoyl-choline 1 to 1,000, 1 c. c.	Fell 11 mm. (83 to 72).
53	Phenyl-propionyl-choline 1 to 1,000, 1 c. c.	Fell 12 mm. (80 to 68).
4	Benzoyl-choline 1 to 50,000, 1 c. c.	Fell 22 mm. (81 to 59).
10	Benzoyl-choline 1 to 100,000, 1 c. c.	Fell 14 mm. (74 to 60).
14	Normal saline 1 c. c.	0.
10	Choline 1 to 1,000, 1 c. c.	Fell 12 mm. (73 to 61).
13	Benzoyl-choline 1 to 100,000, 1 c. c.	Fell 11 mm. (73 to 62).
35	Phenyl-propionyl-choline 1 to 10,000, 1 c. c.	Fell 10 mm. (72 to 62).
49	Phenyl-acetyl-choline 1 to 1,000, 1 c. c.	Fell 14 mm. (75 to 61).
55	Benzoyl-choline 1 to 1,000, 1 c. c.	Rose 11 mm. (80 to 91); fell 11 mm. (80 to 69).

EXPERIMENT 257.—CAT, 2.4 K. URETHANE AND CHLORAL; CURARE—Continued.

Time.	Compound.	Blood pressure.
H. m.		
4 1	Cinnamyl-choline ..... 0.84 per cent, 0.4 c. c.	Rose 26 mm. (75 to 101); fell 11 mm. (75 to 64). Heart seemed to stop completely for a few sec., then beat at rate of 23½ in 10 sec. (had been 27).
6	Benzoyl-choline ..... 0.75 per cent, 1 c. c.	Rose 45 mm. (72 to 117).
10	Benzoyl-choline ..... 1 to 100,000, 1 c. c.	Fell 7 mm. (78 to 71).
12	Normal saline ..... 1 c. c.	Rose 5 mm. (78 to 83).
16	Cinnamyl-choline ..... 0.84 per cent, 1 c. c.	Rose 25 mm. (82 to 107). Heart slowed slightly.
22	Phenyl-propionyl-choline ..... 0.7 per cent, 1 c. c.	Rose 27 mm. (81 to 108).
35	Vagi cut; atropine, 4 mg	
42	Phenyl-acetyl-choline ..... 1 per cent, 1 c. c.	Fell 4 mm. (79 to 75); rose 9 mm. (79 to 88).
52	Phenyl-propionyl-choline ..... 0.7 per cent, 1 c. c.	Fell 7 mm. (64 to 57); rose 10 mm. (64 to 74).
54	Cinnamyl-choline ..... 0.84 per cent, 1 c. c.	Rose 6 mm. (76 to 82); fell 7 mm. (76 to 69).
59	Benzoyl-choline ..... 0.75 per cent, 1 c. c.	Rose 28 mm. (82 to 110).
5 28	Phenyl-acetyl-choline ..... 1 per cent, 1 c. c.	Rose 23 mm. (85 to 108).
6 15	Acetyl-choline ..... 1 to 2,500, 1 c. c.	Fell 23 mm. (72 to 49).
21	Suprarenalin ..... 1 to 80,000, 1 c. c. Acetyl-choline ..... 1 to 2,500, 2 c. c.	Rose 25 mm. (58 to 83); fell 7 mm. (58 to 51).
24	Suprarenalin ..... 1 to 80,000, 1 c. c. Acetyl-choline ..... 1 to 2,500, 3 c. c.	Rose 8 mm. (54 to 62); fell 3 mm. (54 to 51); rose 8 mm. (54 to 62). Heart slowed from 24½ to 20 beats in 10 sec.
29	Suprarenalin ..... 1 to 80,000, 1 c. c.	Rose 24 mm. (56 to 80).
33	Acetyl-choline ..... 1 to 2,500, 1 c. c.	Fell 13 mm. (54 to 41).
36	Acetyl-choline ..... 1 to 500, 1 c. c.	Fell 24 mm. (67 to 43).
39	Atropine, 6 mg	
41	Acetyl-choline ..... 1 to 500, 1 c. c.	0.

EXPERIMENT 258.—CAT, 3.22 K. URETHANE AND CHLORAL; CURARE.

1	28	m-Nitro-benzoyl-choline ..... 1 to 50,000, 1 c. c.	Fell 16 mm. (91 to 75).
	26	p-Nitro-benzoyl-choline ..... 1 to 50,000, 1 c. c.	0.
2	28	Benzoyl-choline ..... 1 to 100,000, 1 c. c.	Fell 20 mm. (92 to 72).

EXPERIMENT 258.—CAT, 3.22 K. URETHANE AND CHLORAL; CURARE—Continued.

Time.	Compound.	Blood pressure.
H. m.		
36	m-Nitro-benzoyl-choline 1 to 50,000, 1 c. c.	Fell 22 mm. (119 to 97).
37	p-Nitro-benzoyl-choline 1 to 50,000, 1 c. c.	0.
44	p-Nitro-benzoyl-choline 1 to 10,000, 1 c. c.	Rose 4 mm. (114 to 118).
2 56	Benzoyl-choline 1 to 10,000, 1 c. c.	Fell 28 mm. (120 to 92).
58	m-Nitro-benzoyl-choline 1 to 10,000, 1 c. c.	Fell 20 mm. (126 to 106).
3 1	Benzoyl-choline 1 to 10,000, 1 c. c.	Fell 24 mm. (118 to 94).
3	p-Nitro-benzoyl-choline 1 to 10,000, 1 c. c.	0.
5	p-Nitro-benzoyl-choline 1 to 1,000, 1 c. c.	0.
7	m-Nitro-benzoyl-choline 1 to 10,000, 1 c. c.	Fell 18 mm. (112 to 94).
10	Vagi cut	
16	m-Nitro-benzoyl-choline 1 to 10,000, 1 c. c.	0.
3 28	m-Nitro-benzoyl-choline 1 to 1,000, 1 c. c.	Fell 16 mm. (128 to 112).
45	p-Nitro-benzoyl-choline 0.74 per cent, 1 c. c.	Rose 26 mm. (128 to 154).
46	m-Nitro-benzoyl-choline 1 to 1,000, 1 c. c.	Fell 24 mm. (130 to 106).
4 00	Benzoyl-choline 0.75 per cent, 1 c. c.	Rose 20 mm. (132 to 152).
15	m-Nitro-benzoyl-choline 1 to 1,000, 1 c. c.	Fell 24 mm. (150 to 126).
21	Benzoyl-choline 1 to 10,000, 1 c. c.	Fell 24 mm. (146 to 122).
30	Atropine sulphate, 6 mg	
39	Benzoyl-choline 1 to 10,000, 1 c. c.	0.
41	m-Nitro-benzoyl-choline 1 to 10,000, 1 c. c.	0.
48	Benzoyl-choline. 1 to 1,000, 1 c. c.	Rose 18 mm. (100 to 118); fell 18 mm. (100 to 82).
52	Benzoyl-choline 0.75 per cent, 1 c. c.	Rose 46 mm. (100 to 146).
55	m-Nitro-benzoyl-choline 0.3 per cent, 1 c. c.	Fell 14 mm. (100 to 86).
58	p-Nitro-benzoyl-choline 0.74 per cent, 1 c. c.	Rose 34 mm. (102 to 136).
5 51	Accelerator nerves cut	
59	Acetyl-choline 1 to 2,500, 1 c. c.	Heart slowed from $27\frac{1}{5}$ to $21\frac{1}{5}$ in 10 sec.
$59\frac{1}{2}$	Accelerators stimulated	Heart rate increased to 27.
6 17	Benzoyl-choline 0.75 per cent, 2 c. c.	Rose 103 mm. (30 to 133).

EXPERIMENT 258.—CAT, 3.22 K. URETHANE AND CHLORAL; CURARE—Continued.

Time.	Compound.	Blood pressure.
H. m.		
24	Suprarenalin 1 to 50,000, 2 c. c.	Rose 52 mm. (42 to 94).
38	p-Nitro-benzoyl-choline 0.74 per cent, 2 c. c.	Rose 10 mm. (38 to 48).

EXPERIMENT 262.—CAT, 2.34 K. ETHER; CURARE.

Time.	Compound.	Blood pressure.
10 40	Choline (methyl iodide process) 1 to 1,000, 1 c. c.	Fell 45 mm. (from 153 to 108).
43	Choline (Kahlbaum) 1 to 1,000, 1 c. c.	Fell 38 mm. (151 to 113).
52	Choline (Grübler) 1 to 1,000, 1 c. c.	Fell 35 mm. (140 to 105).
54	Choline (egg) 1 to 1,000, 1 c. c.	Fell 30 mm. (141 to 111).
11 8	$ClN\begin{cases}(C_3H_7)_3\\CH_2CH_2OH\end{cases}$ 1 to 1,000, 1 c. c.	Fell 17 mm. (138 to 121).
15	$ClN\begin{cases}(C_5H_{11})_3\\CH_2CH_2OH\end{cases}$ 1 to 1,000, 1 c. c.	0.
24	$ClN\begin{cases}(CH_3)_2\\C_5H_{11}\text{ (iso)}\\CH_2CH_2OH\end{cases}$ 1 to 1,000, 1 c. c.	Rose 8 mm. (136 to 144); fell 39 mm. (136 to 97).
34	$ClN\begin{cases}(C_5H_{11})_3\\CH_2CH_2OH\end{cases}$ 1 to 100, 1 c. c.	Fell 7 mm. (130 to 123).
36	$ClN\begin{cases}(C_3H_7)_3\\CH_2CH_2OH\end{cases}$ 0.79 per cent, 1 c. c.	Fell 36 mm. (124 to 88). (Fell slowly and remained low for sometime.)
57	Choline (egg) 1 to 1,000, 1 c. c.	Fell 33 mm. (150 to 117).
12 00	$ClN\begin{cases}(C_2H_5)_3\\CH_2CH_2OH\end{cases}$ 1 to 1,000, 1 c. c.	Fell 17 mm. (150 to 133).
3	$ClN\begin{cases}(C_5H_{11})_3\\CH_2CH_2OH\end{cases}$ 1 to 1,000, 1 c. c.	0.
7	$ClN\begin{cases}(CH_3)_2\\C_5H_{11}\text{ (iso)}\\CH_2CH_2OH\end{cases}$ 1 to 1,000, 1 c. c.	Fell 13 mm. (137 to 124).
9	Atropine 5 mg.	(?)
25	Choline (methyl iodide process) 1 per cent, 1 c. c.	Rose 67 mm. (109 to 176).
28	Choline (egg) 1 per cent, 1 c. c.	0.
30	Choline (methyl iodide process) 1 per cent, 1 c. c.	Rose 48 mm. (122 to 170).
35	Choline (Grübler) 1 per cent, 1 c. c.	0.

EXPERIMENT 262.—CAT, 2.34 K. ETHER; CURARE—Continued.

Time.		Compound.	Blood pressure.
H.	*m.*		
	40	Choline (methyl iodide process) ..... 1 per cent, 1 c. c.	Rose 72 mm. (104 to 176).
	43	Choline (Kahlbaum) ..... 1 per cent, 1 c. c.	0.
	46	Choline (Grübler) ..... 1 per cent, 1 c. c.	0.
	51	Choline (methyl iodide process) ..... 1 per cent, 1 c. c.	Rose 60 mm. (102 to 162).
1	3	$ClN\begin{matrix}\equiv(C_2H_5)_3\\ \diagdown CH_2CH_2OH\end{matrix}$ ..... 0.87 per cent, 1 c. c.	Rose 8 mm. (96 to 104).
	7	$ClN\begin{matrix}\equiv(C_3H_7)_3\\ \diagdown CH_2CH_2OH\end{matrix}$ ..... 0.79 per cent, 1 c. c.	Rose 7 mm. (103 to 110).
	16	$ClN\begin{matrix}//(CH_3)_2\\ —C_5H_{11}\text{ (iso)}\\ \diagdown CH_2CH_2OH\end{matrix}$ ..... 0.87 per cent, 1 c. c.	0.
	21	Choline (methyl iodide process) ..... 1 to 100, 1 c. c.	0.
	32	Homorenon ..... 1 to 1,000, 0.4 c. c.	Rose 59 mm. (97 to 156).

EXPERIMENT 264.—CAT, 2.04 K. ETHER; CURARE.

10	57	Choline (chlorhydrine method) ..... 1 to 1,000, 1 c. c.	Fell 18 mm. (135 to 117).
	59	Choline (egg) ..... 1 to 1,000, 1 c. c.	Fell 15 mm. (135 to 120).
11	5	Choline (methyl iodide method) ..... 1 to 1,000, 1 c. c.	Fell 13 mm. (131 to 118).
	18	$ClN\begin{matrix}\equiv(C_3H_7)_3\\ \diagdown CH_2CH_2OH\end{matrix}$ ..... 1 to 1,000, 1 c. c.	Fell 10 mm. (132 to 122).
	22	$ClN\begin{matrix}\equiv(C_2H_5)_3\\ \diagdown CH_2CH_2OH\end{matrix}$ ..... 1 to 1,000, 1 c. c.	Fell 10 mm. (124 to 114).
12	1	$ClN\begin{matrix}\equiv(C_5H_{11})_3\\ \diagdown CH_2CH_2O(CH_3CO)\end{matrix}$ ..... 1 to 1,000, 1 c. c.	0.
	13	$ClN\begin{matrix}\equiv(C_5H_{11})_3\\ \diagdown CH_2CH_2OH\end{matrix}$ ..... 1 to 1,000, 1 c. c.	Fell 6 mm. (84 to 78).
	21	$ClN\begin{matrix}//(CH_3)_2\\ —C_5H_{11}\text{(iso)}\\ \diagdown CH_2CH_2O(CH_3CO)\end{matrix}$ ..... 1 to 1,000, 1 c. c.	Fell 17 mm. (81 to 64); rose 24 mm. (81 to 105). Heart slowed from 26½ to 22½ beats in 10 sec.
	38	Acetyl-choline ..... 1 to 10,000,000, 1 c. c.	Fell 24 mm. (74 to 50).
1	4	Acetyl-choline ..... 1 to 100,000, 1 c. c.	Fell 28 mm. (82 to 54). Heart slowed from 25 to 21 beats in 10 sec.
	20	$ClN\begin{matrix}\equiv(C_5H_{11})_3\\ \diagdown CH_2CH_2O(CH_3CO)\end{matrix}$ ..... 1 to 200, 1 c. c.	Fell 17 mm. (80 to 63). Rose 26 mm. (80 to 106).

EXPERIMENT 264.—CAT, 2.04 K. ETHER; CURARE—Continued.

Time.	Compound.	Blood pressure.
H. m.		
33	Acetyl-choline 1 to 100,000, 1 c. c.	Fell 45 mm. (100 to 55).
38	Acetyl-choline 1 to 10,000, 1 c. c. subcut.	Fell 24 mm. (96 to 72). (Returned slowly.)
45	Atropine sulph. 5 mg.	
48	Acetyl-choline 1 to 100,000, 1 c. c.	0.
54	$ClN\begin{cases}\equiv(C_5H_{11})_3\\ CH_2CH_2O(CH_3CO)\end{cases}$ 1 to 200, 1 c. c.	Rose 6 mm. (63 to 69).
2 2	Choline (egg) 1 to 200, 1 c. c.	Rose 5 mm. (78 to 83).
5	Choline (methyl iodide process) 1 to 200, 1 c. c.	Rose 42 mm. (78 to 120).
12	Choline (chlorhydrine process) 1 to 200, 1 c. c.	Rose 6 mm. (80 to 86).
16	Choline (methyl iodide process) 1 to 200, 1 c. c.	Rose 35 mm. (79 to 114).
2 27	$ClN\begin{cases}//(CH_3)_2\\ —C_5H_{11}(iso)\\ CH_2CH_2O(CH_3CO)\end{cases}$ 0.4 per cent, 1 c. c.	0.
29	$ClN\begin{cases}\equiv(C_2H_5)_3\\ CH_2CH_2OH\end{cases}$ 0.87 per cent, 1 c. c.	0.
33	$ClN\begin{cases}//(CH_3)_2\\ —C_5H_{11}(iso)\\ CH_2CH_2OH\end{cases}$ 0.87 per cent, 1 c. c.	0.

EXPERIMENT 266.—DOG, 11 K. MORPHINE AND CHLORETONE; VAGI CUT. ATROPINE.

11 23	Benzoyl-choline 1 to 500, 1 c. c.	Rose 46 mm. (112 to 158).
11 47	Choline (egg) 1 to 200, 2 c. c.	Rose 20 mm. (83 to 103).
49	Choline (methyl iodide process) 1 to 200, 2 c. c.	Rose 72 mm. (82 to 154).
12 00	Choline (egg) 1 to 100, 3 c. c.	Rose 46 (118 to 164).
3½	Choline (methyl iodide process) 1 to 200, 1 c. c.	Rose 27 mm. (112 to 139).

EXPERIMENT 267.—CAT, 3.17 K. ETHER; CURARE.

10 41	Choline (egg) 1 to 1,000, 1 c. c.	Fell 17 mm. (137 to 120). Heart increased from 34½ to 37 in 10 sec.
43	Choline (by methyl iodide process) 1 to 1,000, 1 c. c.	Fell 19 mm. (137 to 118). Heart increased from 34½ to 37½ in 10 sec.
54	$ClHN\begin{cases}//(CH_3)_2\\ CH_2CH_2OH\end{cases}$ 1 to 100, 1 c. c.	0.

EXPERIMENT 267.—CAT, 3.17 K. ETHER; CURARE—Continued.

Time.	Compound.	Blood pressure.
H. m.		
11 21	Benzoyl-choline (methyl iodide process) 1 to 500, 1 c. c.	Fell 31 mm. (108 to 77). Heart slowed from 34 to 18 beats in 10 sec.
25	Benzoyl-choline (egg) 1 to 500, 1 c. c.	Fell 19 mm. (112 to 93). Heart slowed from 33 to 25 beats in 10 sec.
28	$ClN\begin{cases}(C_2H_5)_3\\CH_2CH_2OH\end{cases}$ 0.65 per cent, 1 c. c.	Fell 31 mm. (117 to 86).
38	$ClN\begin{cases}(C_2H_5)_3\\CH_2CH_2O(C_6H_5CO)\end{cases}$ 0.844 per cent, 1 c. c.	Fell 10 mm. (115 to 105).
45	$ClN\begin{cases}(C_3H_7)_3\\CH_2CH_2OH\end{cases}$ 0.81 per cent, 1 c. c.	0.
48	$ClN\begin{cases}(C_3H_7)_3\\CH_2CH_2O(C_6H_5CO)\end{cases}$ 0.71 per cent, 1 c. c.	0.
12 4	$ClN\begin{cases}(C_5H_{11})_3\\CH_2CH_2OC_6H_5CO\end{cases}$ 1 to 500, 1 c. c.	Fell 45 mm. (123 to 78). Fell very slowly and remained low; heart slowed from 28½ to 20½.
11	Atropine, 5 mg	
19	$ClN\begin{cases}(C_2H_5)_3\\CH_2CH_2OH\end{cases}$ 0.65 per cent, 1 c. c.	0.
27	$ClN\begin{cases}(C_3H_7)_3\\CH_2CH_2OH\end{cases}$ 0.81 per cent, 1 c. c.	Fell 20 mm. (106 to 86).
1 4	Choline (methyl iodide process) 1 to 100, 1 c. c.	Rose 16 mm. (140 to 156).
12	$HClN\begin{cases}CH_3\\CH_3\\CH_2CH_2OH\end{cases}$ 1 to 100, 1 c. c.	0.
23	$ClN\begin{cases}(C_2H_5)_3\\CH_2CH_2OC_6H_5CO\end{cases}$ 0.844 per cent, 1 c. c.	Fell 17 mm. (160 to 143).
26	$ClN\begin{cases}(C_2H_5)_3\\CH_2CH_2OH\end{cases}$ 0.65 per cent, 1 c. c.	Fell 25 mm. (165 to 140).
33	$ClN\begin{cases}(C_3H_7)_3\\CH_2CH_2OH\end{cases}$ 0.81 per cent, 1 c. c.	Fell 36 mm. (170 to 134).
34	$ClN\begin{cases}(C_3H_7)_3\\CH_2CH_2O(C_6H_5CO)\end{cases}$ 0.707 per cent, 1 c. c.	Rose 8 mm. (170 to 178).
53	$ClN\begin{cases}(CH_3)_2\\C_5H_{11}\\CH_2CH_2O(C_6H_5CO)\end{cases}$ 0.666 per cent, 1 c. c.	Fell 20 mm. (162 to 142).

EXPERIMENT 267.—CAT. 3.17 K. ETHER; CURARE—Continued.

Time.	Compound.	Blood pressure.
H. *m.* 54	$ClN\begin{cases}=(CH_3)_2\\ -C_5H_{11}\\ -CH_2CH_2OH\end{cases}$ 0.86 per cent, 1 c. c.	0.
58	$ClN\begin{cases}\equiv(C_5H_{11})_3\\ -CH_2CH_2OH\end{cases}$ 1 to 100, 1 c. c.	0.
2 3	$ClN\begin{cases}\equiv(C_5H_{11})_3\\ -CH_2CH_2O(C_6H_5CO)\end{cases}$ 0.93 per cent, 1 c. c.	Fell 31 mm. (164 to 133).

EXPERIMENT 268 (FEBRUARY 6, 1909).—CAT, 2.82 K. ETHER; CURARE.

[In this experiment the effects of solutions prepared a few minutes before their injection were compared with those of solutions made 2 days (February 4) and 21 days (January 16) previously.]

12	27	Choline (egg), fresh 1 to 1,000, 1 c. c.	Fell 20 mm. (110 to 90).
	29	Choline (egg), old (1/16) 1 to 1,000, 1 c. c.	Fell 18 mm. (110 to 92).
	37	Choline (methyl iodide), old (1/16) 1 to 1,000, 1 c. c.	Fell 19 mm. (99 to 80).
	39	Choline (methyl iodide), fresh 1 to 1,000, 1 c. c.	Fell 19 mm. (104 to 85).
	42	Choline (methyl iodide) (2/4) 1 to 1,000, 1 c. c.	Fell 10 mm. (100 to 90); rose 22 mm. (100 to 122); slight movements.
12	43	Curare	
	47	Choline (methyl iodide) (2/4) 1 to 1,000, 1 c. c.	Fell 12 mm. (94 to 82).
	49	Choline (chlorhydrine), fresh 1 to 1,000, 1 c. c.	Fell 9 mm. (103 to 94).
	57	Choline (Grübler's), old (1/16) 1 to 1,000, 1 c. c.	Fell 12 mm. (100 to 88).
	58	Choline (Kahlbaum's), old (1/16) 1 to 1,000, 1 c. c.	Fell 5 mm. (101 to 96); rose 7 mm. (100 to 107).
1	00	Choline (egg), fresh 1 to 1,000, 1 c. c.	Fell 5 mm. (104 to 99).
	11	Choline (methyl iodide), fresh 1 to 1,000, 1 c. c.	Fell 11 mm. (97 to 86).
	13	Choline (chlorhydrine), fresh 1 to 1,000, 1 c. c.	Fell 6 mm. (95 to 89).
1	23	$ClN\begin{cases}\equiv(C_3H_7)_3\\ -CH_2CH_2OH\end{cases}$ 0.81 per cent, 1 c. c.	Fell slowly 9 mm. (82 to 73).
1	28	$ClN\begin{cases}\equiv(C_3H_7)_3\\ -CH_2CH_2O(C_6H_5CO)\end{cases}$ 0.71 per cent, 1 c. c.	Fell slowly 16 mm. (90 to 74).
1	38	$ClN\begin{cases}=(CH_3)_2\\ -C_5H_{11}(iso)\\ -CH_2CH_2O(C_6H_5CO)\end{cases}$ 0.67 per cent, 1 c. c.	Rose 17 mm. (76 to 93).
	41	$ClN\begin{cases}=(CH_3)_2\\ -C_5H_{11}(iso)\\ -CH_2CH_2OH\end{cases}$ 1 to 1,000, 1 c. c.	Fell 11 mm. (72 to 61).

EXPERIMENT 268 (FEBRUARY 6, 1909).—CAT, 2.82 K. ETHER; CURARE—Continued.

Time.	Compound.	Blood pressure.
H. m. 49	$ClN\begin{cases}=(CH_3)_2\\-C_5H_{11}\\-CH_2CH_2O(CH_3CO)\end{cases}$ 1 to 1,000, 1 c. c.	Fell 22 mm. (89 to 67).
56	$ClN\begin{cases}\equiv(C_5H_{11})_3\\-CH_2CH_2O(CH_3CO)\end{cases}$ 1 to 500, 1 c. c.	Fell 28 mm. (105 to 85).
2 4	Atropine sulphate 5 mg.	
9	Choline (egg), fresh 1 to 200, 1 c. c.	0.
11	Choline (methyl iodide), fresh 1 to 200, 1 c. c.	Rose 21 mm. (60 to 81).
15	Choline (chlorhydrine), fresh 1 to 200, 1 c. c.	0.
26	Choline (methyl iodide), (2/4) 1 to 200, 1 c. c.	Rose 34 mm. (70 to 104).
30	Choline (egg), fresh 1 to 200, 1 c. c.	0.
55	$ClN\begin{cases}\equiv(C_3H_7)_3\\-CH_2CH_2OH\end{cases}$ 0.81 per cent, 1 c. c.	Fell 12 mm. (92 to 80).
57	$ClN\begin{cases}\equiv(C_3H_7)_3\\-CH_2CH_2O(C_6H_5CO)\end{cases}$ 0.71 per cent, 1 c. c.	0.
3 3	$ClN\begin{cases}=(CH_3)_2\\-C_5H_{11}(iso)\\-CH_2CH_2O(C_6H_5CO)\end{cases}$ 0.67 per cent, 1 c. c.	Fell 11 mm. (88 to 77).

EXPERIMENT 269.—CAT, 3.04 K. ETHER; CURARE.

2 32	$ClN\begin{cases}\equiv(C_5H_{11})_3\\-CH_2CH_2O(CH_3CO)\end{cases}$ 1 to 500, 1 c. c.	Fell 24 mm. (109 to 85).
34	$ClN\begin{cases}=(CH_3)_2\\-C_5H_{11}\ (iso)\\-CH_2CH_2O(C_6H_5CO)\end{cases}$ 1 to 500, 1 c. c.	0.
38	$ClN\begin{cases}=(CH_3)_2\\-C_5H_{11}\ (iso)\\-CH_2CH_2O(C_6H_5CO)\end{cases}$ 0.67 per cent, 1 c. c.	Rose 54 mm. (116 to 170). Heart slowed from 40 to 28 in 10 sec.
1 8	$ClN\begin{cases}\equiv(CH_3)_3\\-CH_2CH_2CH_2OH\end{cases}$ 1 to 1,000, 1 c. c.	Fell 22 mm. (106 to 84).
10	$ClN\begin{cases}\equiv(CH_3)_3\\-CH_2CHOHCH_3\end{cases}$ 1 to 1,000, 1 c. c.	Fell 10 mm. (108 to 98).
14	Choline 1 to 1,000, 1 c. c.	Fell 14 mm. (104 to 90).
16	$ClN\begin{cases}\equiv(CH_3)_3\\-CH_2CH_2CH_2OH\end{cases}$ 1 to 1,000, 1 c. c.	Fell 22 mm. (99 to 77).

EXPERIMENT 269.—CAT, 3.04 K. ETHER; CURARE—Continued.

Time.	Compound.	Blood pressure.
H. m. 19	$ClN\langle\equiv(CH_3)_3$, $CH_2CHOHCH_3$ — 1 to 1,000, 1 c. c.	(Irregular).
25	$ClN\langle\equiv(CH_3)_3$, $CH_2CHOHCH_3$ — 1 to 100, 1 c. c.	Fell 22 mm. (98 to 76).
37	$ClN\langle\equiv(C_2H_5)_3$, $CH_2CHOHCH_3$ — 1 to 1,000, 1 c. c.	Fell 12 mm. (94 to 82).
38	$ClN\langle\equiv(C_2H_5)_3$, $CH_2CHOHCH_3$ — 1 to 100, 1 c. c.	Fell 28 mm. (88 to 60). Heart slowed from 33½ to 25 beats in 10 sec.
49	$ClN\langle\equiv(CH_3)_3$, $CH_2CHOHCH_2Cl$ — 1 to 1,000, 1 c. c.	Fell 20 mm. (99 to 79).
55	$ClN\langle\equiv(C_2H_5)_3$, $CH_2CHOHCH_2Cl$ — 1 to 1,000, 1 c. c.	Fell 7 mm. (105 to 98).
57	$ClN\langle\equiv(C_3H_7)_3$, $CH_2CHOHCH_2Cl$ — 1 to 1,000, 1 c. c.	0.
59	$ClN\langle\equiv(C_2H_5)_3$, $CH_2CHOHCH_2Cl$ — 1 to 100, 1 c. c.	Fell 35 mm. (105 to 70). Heart slowed from 35 to 27 beats in 10 sec.
2 7	$ClN\langle\equiv(C_5H_{11})_3$, $CH_2CHOHCH_2Cl$ — 1 to 100, 1 c. c.	Fell 13 mm. (119 to 106).
13	$ClN\langle\equiv(C_3H_7)_3$, $CH_2CHOHCH_2Cl$ — 1 to 100, 1 c. c.	Rose 6 mm. (100 to 106).
18	$ClN\langle(CH_3)_2$, $-C_5H_{11}$ (iso), $CH_2CH_2O(C_6H_5CO)$ — 0.67 per cent, 1 c. c.	Rose 12 mm. (100 to 112).
22	Atropine — 5 mg.	
41	$ClN\langle\equiv(CH_3)_3$, $CH_2CHOHCH_3$ — 1 to 100, 1 c. c.	Rose 19 mm. (124 to 143).
45	Choline — 1 to 100, 1 c. c.	Rose 16 mm. (130 to 146).
46	$ClN\langle\equiv(CH_3)_3$, $CH_2CH_2CH_2OH$ — 1 to 100, 1 c. c.	Rose 8 mm. (132 to 140).
3 9	$ClN\langle\equiv(C_2H_5)_3$, $CH_2CHOHCH_3$ — 1 to 100, 1 c. c.	Rose 6 mm. (152 to 158).
17	$ClN\langle\equiv(C_3H_7)_3$, $CH_2CHOHCH_2Cl$ — 1 to 100, 1 c. c.	Rose 6 mm. (146 to 152).

EXPERIMENT 269.—CAT, 3.04 K. ETHER; CURARE—Continued.

Time.	Compound.	Blood pressure.
H. m. 19	$ClN\langle^{(C_5H_{11})_3}_{CH_2CHOHCH_2Cl}$ 1 to 100, 1 c. c.	Fell 18 mm. (146 to 128).
31	$ClN\langle^{(C_5H_{11})_3}_{CH_2CH_2O(C_6H_5CO)}$ 0.93 per cent, 1 c. c.	Fell 56 mm. (133 to 77). Returned very slowly.

EXPERIMENT 270.—CAT, 3.47 K. ETHER; CURARE.

11 58	$ClN\langle^{(CH_3)_3}_{CH_2CH_2O(C_6H_5CO)}$ (methyl iodide process) 1 to 1,000, 0.4 c. c.	Fell 19 mm. (102 to 83).
12 7	$ClN\langle^{(CH_3)_3}_{CH_2CH_2CH_2O(C_6H_5CO)}$ 1.2 per cent, 1 c. c.	Fell 17 mm. (102 to 85).
16	$ClN\langle^{(CH_3)_3}_{CH_2CH_2CH_2OH}$ 1 to 1,000, 1 c. c.	Fell 25 mm. (97 to 72).
26	$ClN\langle^{(CH_3)_3}_{CH_2CHOH\ CH_3}$ 1 to 200, 1 c. c.	Fell 18 mm. (94 to 76).
28	Choline 1 to 1,000, 1 c. c.	Fell 15 mm. (92 to 77).
36	$ClN\langle^{(CH_3)_3}_{CH_2CHOHCH_2Cl}$ 1 to 1,000, 1 c. c.	Fell 17 mm. (88 to 71).
42	$ClN\langle^{(C_5H_{11})_3}_{CH_2CHOHCH_3}$ 0.69, 1 c. c.	Fell 14 mm. (84 to 70); fell slowly. Heart slowed from 25 to 21 beats in 10 sec.
48	$ClN\langle^{(C_5H_{11})_3}_{CH_2CHOHCH_2Cl}$ 1 to 200, 1 c. c.	Fell 20 mm. (86 to 66); remained low. Heart slowed from 20½ to 16.
1 13	Benzoyl-choline (methyl-iodide process) 0.92 per cent, 1 c. c.	Rose 81 mm. (43 to 124). Heart rate increased from 11 to 16 in 10 sec.
23	Choline (egg) 1 to 100, 1 c. c.	Fell 9 mm. (42 to 33).
28	Benzoyl-choline (methyl-iodide process) 0.92 per cent, 2 c. c. subcut.	0.
32	Benzoyl-choline (methyl-iodide process) 0.92 per cent, 1 c. c.	Rose 117 mm. (40 to 157). Heart rate increased from 10 to 16½ in 10 sec.
40	Choline (methyl-iodide process) 1 to 100, 1 c. c.	Fell 6 mm. (46 to 40); rose 10 mm. (46 to 56).
49	Benzoyl-choline (methyl-iodide process) 0.92 per cent, 1 c. c.	Rose 37 mm. (47 to 84).
51	Atropine 5 mg.	
2 4	Benzoyl-choline (egg) 1.3 per cent, 1 c. c.	Rose 12 mm. (40 to 52). No effect on heart rate.
10	Benzoyl-choline (methyl-iodide process) 0.92 per cent, 1 c. c.	Rose 12 mm. (40 to 52). No change in heart rate.

EXPERIMENT 271.—CAT, 2.43 K. ETHER; CURARE.

Time.	Compound.	Blood pressure.
H. *m.* 12 28	$ClN\langle^{(CH_3)_3}_{CH_2CHO(C_6H_5CO)CH_3}$ 1 to 100, 1 c. c.	Fell 39 mm. (132 to 93). Heart rate increased from 41⅛ to 44⅛ in 10 sec.
31	$ClN\langle^{(CH_3)_3}_{CH_2CHOHCH_3}$ 1 to 200, 1 c. c.	Fell 43 mm. (132 to 89). Heart slowed from 43 to 40⅛ beats in 10 sec.
46	$ClN\langle^{(CH_3)_3}_{CH_2CHOHCH_3}$ 1 to 1,000, 1 c. c.	Fell 24 mm. (114 to 90).
55	$ClN\langle^{(CH_3)_3}_{CH_2CH_2CH_2OH}$ 1 to 1,000, 1 c. c.	Fell 39 mm. (110 to 71).
1 5	Choline 1 to 1,000, 1 c. c.	Fell 22 mm. (90 to 68).
7	Benzoyl-choline 1 to 1,000, 1 c. c.	Fell 16 mm. (96 to 80). Heart rate increased from 37 to 40 in 10 sec.
9	$ClN\langle^{(C_2H_5)_3}_{CH_2CHO(C_6H_5CO)CH_3}$ 1 to 1,000, 1 c. c.	Fell slowly 22 mm. (94 to 72.) Remained low for some time. Heart rate slowly fell from 38 to 32 in 10 sec.
31	$ClN\langle^{(C_3H_7)_3}_{CH_2CHOHCH_3}$ 1 to 200, 1 c. c.	Rose 5 mm. (78 to 83).
35	$ClN\langle^{(CH_3)_3}_{CH_2CHO(CH_3CO)CH_3}$ 1 to 1,000,000, 1 c. c.	Fell 26 mm. (72 to 46).
38	Atropine sulphate 5 mg.	
40	$ClN\langle^{(CH_3)_3}_{CH_2CHO(CH_3CO)CH_3}$ 1 to 1,000,000, 1 c. c.	0.
48	$ClN\langle^{(CH_3)_3}_{CH_2CHOHCH_3}$ 1 to 200, 1 c. c.	0.
50	$ClN\langle^{(CH_3)_3}_{CH_2CHO(C_6H_5CO)CH_3}$ 1 to 200, 1 c. c.	Fell 7 mm. (70 to 63).
56	$ClN\langle^{(CH_3)_3}_{CH_2CH_2CH_2OH}$ 1 to 100, 1 c. c.	0.
2 4	$ClN\langle^{(CH_3)_3}_{CH_2CH_2CH_2O(C_6H_5CO)}$ 1.22 per cent, 1 c. c.	0.
8	Benzoyl-choline (methyl iodide process) 0.92 per cent, 1 c. c.	Rose 33 mm. (69 to 102).
22	$ClN\langle^{(C_3H_7)_3}_{CH_2CHOHCH_2Cl}$ 1 to 100, 1 c. c.	Rose 6 mm. (78 to 84).
24	$ClN\langle^{(C_3H_7)_3}_{CH_2CHOHCH_3}$ 1 to 100, 1 c. c.	Rose 8 mm. (82 to 90).
31	$ClN\langle^{(C_2H_5)_3}_{CH_2CHOHCH_3}$ 1 to 100, 1 c. c.	0.

EXPERIMENT 271.—CAT, 2.43 K. ÉTHER; CURARE—Continued.

Time.	Compound.	Blood pressure.
H. m.		
33	$ClN\begin{matrix}\equiv(C_2H_5)_3\\ \diagdown CH_2CHOHCH_2Cl\end{matrix}$ 1 to 100, 1 c. c.	Fell 6 mm. (84 to 78).
49	$ClN\begin{matrix}\equiv(C_5H_{11})_3\\ \diagdown CH_2CHOHCH_3\end{matrix}$ 1 to 1,000, 1 c. c.	Rose 11 mm. (79 to 90).
52	$ClN\begin{matrix}\equiv(C_5H_{11})_3\\ \diagdown CH_2CHOHCH_2Cl\end{matrix}$ 1 to 1,000, 1 c. c.	0.
3 17	$ClN\begin{matrix}\equiv(C_2H_5)_3\\ \diagdown CH_2CHO(C_6H_5CO)CH_3\end{matrix}$ 1.95 per cent, 1 c. c.	Rose 9 mm. (80 to 89).
21	$ClN\begin{matrix}\equiv(C_5H_{11})_3\\ \diagdown CH_2CHOHCH_3\end{matrix}$ 0.69 per cent, 1 c. c	Fell 17 mm. (77 to 60).
24	$ClN\begin{matrix}\equiv(C_5H_{11})_3\\ \diagdown CH_2CHOHCH_2Cl\end{matrix}$ 1 to 100, 1 c. c.	Fell 16 mm. (76 to 60).
28	$ClN\begin{matrix}\equiv(CH_3)_3\\ \diagdown CH_2CHO(CH_3CO)CH_3\end{matrix}$ 1 to 1,000, 1 c. c.	Fell 31 mm. (76 to 45).
30	Vagus stimulated	0.
33	Atropine sulphate 5 mg.	
36	$ClN\begin{matrix}\equiv(CH_3)_3\\ \diagdown CH_2CHO(CH_3CO)CH_3\end{matrix}$ 1 to 1,000, 1 c. c.	Fell 24 mm. (64 to 40).
38	Atropine 7 mg.	
40	$ClN\begin{matrix}\equiv(CH_3)_3\\ \diagdown CH_2CHO(CH_3CO)CH_3\end{matrix}$ 1 to 1,000, 1 c. c.	Fell 9 mm. (58 to 49).
42	Atropine 5 mg.	
48	$ClN\begin{matrix}\equiv(CH_3)_3\\ \diagdown CH_2CHO(CH_3CO)CH_3\end{matrix}$ 1.25 per cent, 1 c. c.	Fell 18 mm. (52 to 34).
53	Atropine 5 mg.	
4 00	Atropine 5 mg.	
8	Atropine 5 mg.	
9	$ClN\begin{matrix}\equiv(CH_3)_3\\ \diagdown CH_2CHO(CH_3CO)CH_3\end{matrix}$ 1.25 per cent, 1 c. c.	Fell 17 mm. (53 to 35).
13	$ClN\begin{matrix}\equiv(C_5H_{11})_3\\ \diagdown CH_2CH_2O(C_6H_5CO)\end{matrix}$ 1 to 200, 1.5 c. c.	Fell 10 mm. (50 to 40).

EXPERIMENT 272.—CAT, 3.25 K. ETHER; CURARE.

Time.	Compound.	Blood pressure.
H. m.		
11 38	Choline[a] 1 to 100, 1 c. c.	Fell 9 mm. (114 to 105).
50	$ClN\begin{matrix}(CH_3)_3\\ CH_2CHO(C_6H_5CO)CH_3\end{matrix}$ 1 to 200, 0.6 c. c.	Fell 13 mm. (126 to 113); rose 37 mm. (126 to 163).
12 2	$ClN\begin{matrix}(CH_3)_3\\ CH_2CH_2CH_2O(C_6H_5CO)\end{matrix}$ 1.2 per cent, 0.6 c. c.	Rose 16 mm. (127 to 143); fell 12 mm. (127 to 115).
4	$ClN\begin{matrix}(CH_3)_3\\ CH_2CHO(C_6H_5CO)CH_3\end{matrix}$ 1 to 200, 1 c. c.	Rose 46 mm. (127 to 173).
6	$ClN\begin{matrix}(CH_3)_3\\ CH_2CH_2CH_2O(C_6H_5CO)\end{matrix}$ 1.2 per cent, 1 c. c.	Rose 8 mm. (126 to 134); fell 14 mm. (126 to 112).
15	$ClN\begin{matrix}(CH_3)_3\\ CH_2CH_2CH_2OH\end{matrix}$ 1 to 500, 1 c. c.	Fell 16 mm. (137 to 121).
16	$ClN\begin{matrix}(CH_3)_3\\ CH_2CHOHCH_3\end{matrix}$ 1 to 500, 1 c. c.	Fell 28 mm. (132 to 104).
25	$ClN\begin{matrix}(C_2H_5)_3\\ CH_2CHO(CH_3CO)CH_3\end{matrix}$ 1 to 1,000, 1 c. c.	Fell 10 mm. (128 to 118).
31	$ClN\begin{matrix}(C_2H_5)_3\\ CH_2CHO(C_6H_5CO)CH_3\end{matrix}$ 1 to 2,000, 1 c. c.	Fell 15 mm. (117 to 102).
19	$ClN\begin{matrix}(C_2H_5)_3\\ CH_2CHO(CH_3CO)CH_3\end{matrix}$ 0.72 per cent, 2 c. c. subcut.	Fell (slowly) 20 mm. (96 to 76). Returned slowly.
54	$ClN\begin{matrix}(C_2H_5)_3\\ CH_2CHO(C_6H_5CO)CH_3\end{matrix}$ 1 to 1,000, 1 c. c.	Fell (slowly) 24 mm. (96 to 72).
1 5	$ClN\begin{matrix}(C_2H_5)_3\\ CH_2CHO(CH_3CO)CH_3\end{matrix}$ 1 to 500, 1 c. c.	Fell (slowly) 27 mm. (91 to 64).
30	$ClN\begin{matrix}(C_2H_5)_3\\ CH_2CHOHCH_2Cl\end{matrix}$ 1 to 500, 1 c. c.	Fell 17 mm. (104 to 87); rose 46 mm. (104 to 150).
32½	$ClN\begin{matrix}(C_2H_5)_3\\ CH_2CHOHCH_3\end{matrix}$ 1 to 500, 1 c. c.	Fell 17 mm. (103 to 86).
42	$ClN\begin{matrix}(CH_3)_3\\ CH_2CHO(CH_3CO)CH_3\end{matrix}$ 1 to 1,000,000, 1 c. c.	Fell 24 mm. (116 to 92).
43	Atropine 5 mg.	
51	Choline (fresh) 1 to 100, 1 c. c.	Rose 19 mm. (105 to 124).

[a] Solution made three years previously; kept in dark room at 15° C.

EXPERIMENT 272.—CAT, 3.25 K. ETHER; CURARE—Continued.

Time.	Compound.	Blood pressure.
H. m.		
53	Choline (3 years old) 1 to 100, 1 c. c.	Rose 11 mm. (103 to 114).
2 3	$ClN\begin{Bmatrix}(CH_3)_3\\CH_2CH_2CH_2O(C_6H_5CO)\end{Bmatrix}$ 1 to 500, 2 c. c.	0.
5	$ClN\begin{Bmatrix}(CH_3)_3\\CH_2CHO(C_6H_5CO)CH_3\end{Bmatrix}$ 1 to 500, 1 c. c.	Rose 28 mm. (86 to 114).
37	$ClN\begin{Bmatrix}(C_2H_5)_3\\CH_2CHO(CH_3CO)CH_3\end{Bmatrix}$ 0.72 per cent, 1 c. c.	0.
46	$ClN\begin{Bmatrix}(C_2H_5)_3\\CH_2CHO(C_6H_5CO)CH_3\end{Bmatrix}$ 1 to 200, 1 c. c.	0.
56	$ClN\begin{Bmatrix}(C_2H_5)_3\\CH_2CHOHCH_3\end{Bmatrix}$ 1 to 100, 1 c. c.	0.
58	$ClN\begin{Bmatrix}(C_2H_5)_3\\CH_2CHOHCH_2Cl\end{Bmatrix}$ 1 to 100, 1 c. c.	0.
3 9	$ClN\begin{Bmatrix}(CH_3)_3\\CH_2CHO(CH_3CO)CH_3\end{Bmatrix}$ 1 to 100,000, 1 c. c.	Fell 23 mm. (134 to 111).
19	Benzoyl-choline (egg) 1.3 per cent, 1 c. c.	Fell 31 mm. (119 to 88); rose 26 mm. (119 to 145).
25	Vagus stimulated	0.
37	$ClN\begin{Bmatrix}(CH_3)_3\\CH_3CHO(CH_3CO)CH_3\end{Bmatrix}$ 1 to 10,000, 1 c. c.	Fell 41 mm. (100 to 59).
47	Benzoyl-choline (egg) 1.3 per cent, 1 c. c.	Rose 32 mm. (107 to 139).
53	Benzoyl-choline (methyl iodide process) 0.92 per cent, 1 c. c.	Rose 42 mm. (96 to 138).
1 1	$ClN\begin{Bmatrix}(C_5H_{11})_3\\CH_2CHO(CH_3CO)CH_2Cl\end{Bmatrix}$ 1 to 100, 1 c. c.	Rose 20 mm. (108 to 128); returned slowly
16	$ClN\begin{Bmatrix}(CH_3)_3\\CH_2CHO(CH_3CO)CH_3\end{Bmatrix}$ 1 to 10,000, 1 c. c.	Fell 48 mm. (110 to 62).
17	Atropine 5 mg.	
19	$ClN\begin{Bmatrix}(CH_3)_3\\CH_2CHO(CH_3CO)CH_3\end{Bmatrix}$ 1 to 10,000, 1 c. c.	0.
24	$ClN\begin{Bmatrix}(CH_3)_3\\CH_2CHO(CH_3CO)CH_3\end{Bmatrix}$ 1 to 1,000, 1 c. c.	Fell 42 mm. (101 to 59).
35	$ClN\begin{Bmatrix}(CH_3)_3\\CH_2CH_2CH_2O(CH_3CO)\end{Bmatrix}$ 1 to 1,000, 1 c. c.	Fell 16 mm. (98 to 82).

EXPERIMENT 272.—CAT, 3.25 K. ETHER; CURARE—Continued.

Time.	Compound.	Blood pressure.
H. m. 47	$ClN\begin{cases}(CH_3)_3\\CH_2CH_2O(CH_3CO)\end{cases}$ 0.94 per cent, 1 c. c.	Fell 46 mm. (97 to 51).
52	Atropine 5 mg.	
56	$ClN\begin{cases}(CH_3)_3\\CH_2CH_2CH_2O(CH_3CO)\end{cases}$ 0.94 per cent, 1 c. c.	Fell 31 mm. (99 to 68).
59+	$ClN\begin{cases}(CH_3)_3\\CH_2CHO(CH_3CO)CH_3\end{cases}$ 1 to 1,000, 1 c. c.	Fell 29 mm. (95 to 66).
5 2	Atropine 10 mg.	
5	$ClN\begin{cases}(CH_3)_3\\CH_2CHO(CH_3CO)CH_3\end{cases}$ 1 to 1,000, 1 c. c.	Fell 11 mm. (95 to 84).
9	$ClN\begin{cases}(CH_3)_3\\CH_2CHO(CH_3CO)CH_3\end{cases}$ 1.25 per cent, 1 c. c.	Fell 55 mm. (98 to 43).
12	$ClN\begin{cases}(CH_3)_3\\CH_2CH_2CH_2O(CH_3CO)\end{cases}$ 0.94 per cent, 1 c. c.	Fell 11 mm. (94 to 83).
17	Atropine 12 mg.	
19	$ClN\begin{cases}(CH_3)_3\\CH_2CHO(CH_3CO)CH_3\end{cases}$ 1.25 per cent, 1 c. c.	Fell 6 mm. (90 to 84).
22	Atropine 25 mg.	Fell 23 mm. (87 to 64).
23	$ClN\begin{cases}(CH_3)_3\\CH_2CHO(CH_3CO)CH_3\end{cases}$ 1.25 per cent, 1 c. c.	Fell 19 mm. (57 to 38).
30	$ClN\begin{cases}(CH_3)_3\\CH_2CH_2CH_2O(CH_3CO)\end{cases}$ 0.94 per cent, 1 c. c.	Fell 3 mm. (70 to 67).
33	$ClN\begin{cases}(CH_3)_3\\CH_2CHO(CH_3CO)CH_3\end{cases}$ 1.25 per cent, 1 c. c.	Fell 31 mm. (75 to 44).

EXPERIMENT 273.—CAT, 4.52 K. ETHER; CURARE.

1	43	$ClN\begin{cases}(CH_3)_3\\CH_2CHO(CH_3CO)CH_2Cl\end{cases}$ 1 to 10,000, 1 c. c.	Fell slightly.
	44	$ClN\begin{cases}(CH_3)_3\\CH_2CH_2CH_2O(CH_3CO)\end{cases}$ 1 to 100,000, 1 c. c.	Fell 29 mm. (122 to 93).
	47	$ClN\begin{cases}(CH_3)_3\\CH_2CHO(CH_3CO)CH_2Cl\end{cases}$ 1 to 100,000, 1 c. c.	0.

EXPERIMENT 273.—CAT, 4.52 K. ETHER; CURARE—Continued.

Time.	Compound.	Blood pressure.
H. m. 49	$ClN\langle^{(CH_3)_3}_{CH_2CHO(CH_3CO)CH_2Cl}$ 1 to 1,000, 1 c. c.	Fell 21 mm. (138 to 117).
57	$ClN\langle^{(CH_3)_3}_{CH_2CHO(CH_3CO)CH_3}$ 1 to 100,000, 1 c. c.	Fell 45 mm. (145 to 100).
2 11	$ClN\langle^{(CH_3)_3}_{CH_2CHO(CH_3CO)CH_2Cl}$ 0.89 per cent, 1 c. c.	Fell 41 mm. (156 to 115).
17	$ClN\langle^{(CH_3)_3}_{CH_2CHO(CH_3CO)CH_3}$ 1 to 100,000, 1 c. c.	Fell 63 mm. (169 to 106).
25	$ClN\langle^{(C_5H_{11})_3}_{CH_2CHO(C_6H_5CO)CH_2Cl}$ 1 to 1,000, 1 c. c.	0.
26	$ClN\langle^{(C_5H_{11})_3}_{CH_2CHO(C_6H_5CO)CH_2Cl}$ 0.75 per cent, 1 c. c.	Fell (in 4 min.) 86 mm. (170 to 84). Heart slowed from 38 to 25 beats in 10 sec. (Remained slow.)
33	$ClN\langle^{(C_5H_{11})_3}_{CH_2CHO(CH_3CO)CH_2Cl}$ 1 to 1,000, 1 c. c.	Fell 6 mm. (84 to 78).
42	$ClN\langle^{(C_5H_{11})_3}_{CH_2CHOHCH_3}$ 1 to 200, 1 c. c.	0.
44	$ClN\langle^{(C_5H_{11})_3}_{CH_2CHOHCH_2Cl}$ 1 to 200, 1 c. c.	Fell 21 mm. (110 to 89).
48	$ClN\langle^{(C_3H_7)_3}_{CH_2CHO(C_6H_5CO)CH_2Cl}$ 1 to 100, 0.5 c. c.	Fell (slowly) 27 mm. (106 to 79).
57	$ClN\langle^{(CH_3)_3}_{CH_2CHO(CH_3CO)CH_2Cl}$ 1 to 1,000, 1 c. c.	Fell 14 mm. (88 to 74).
3 00	$ClN\langle^{(CH_3)_3}_{CH_2CH_2CH_2O(CH_3CO)}$ 1 to 100,000, 1 c. c.	Fell 34 mm. (89 to 55).
2	$ClN\langle^{(CH_3)_3}_{CH_2CHO(CH_3CO)CH_2Cl}$ 1 to 1,000, 1 c. c.	Fell 8 mm. (82 to 74).
4	$ClN\langle^{(CH_3)_3}_{CH_2CH_2CH_2O(CH_3CO)}$ 1 to 100,000, 1 c. c.	Fell 22 mm. (76 to 54).
8	$ClN\langle^{(CH_3)_3}_{CH_2CHO(CH_3CO)CH_3}$ 1 to 100,000, 1 c. c.	Fell 24 mm. (74 to 50).
11	$ClN\langle^{(CH_3)_3}_{CH_2CHO(CH_3CO)CH_2Cl}$ 1 to 1,000, 1 c. c.	Fell 4 mm. (80 to 76).

EXPERIMENT 273.—CAT, 4.52 K. ETHER; CURARE—Continued.

Time.	Compound.	Blood pressure.
H. m. 18	$ClN\langle^{(C_5H_{11})_3}_{CH_2CHO(CH_3CO)CH_2Cl}$ 1 to 100, 1 c. c.	Rose 20 mm. (89 to 109).
22	$ClN\langle^{(C_5H_{11})_3}_{CH_2CHO(C_6H_5CO)CH_2Cl}$ 0.75 per cent, 1 c. c.	Rose 22 mm. (84 to 106); afterwards fell to 46 mm.
37	$ClN\langle^{(C_5H_{11})_3}_{CH_2CHOHCH_3}$ 0.69 per cent, 1 c. c.	Fell 14 mm. (46 to 32).

EXPERIMENT 275.—CAT, 2.44 K. ETHER; CURARE.

3	9	$ClN\langle^{(C_3H_7)_3}_{CH_2CHO(C_6H_5CO)CH_2Cl}$ 1 to 200, 1 c. c.	Fell 26 mm. (94 to 68).
	12	$ClN\langle^{(C_3H_7)_3}_{CH_2CHOHCH_2Cl}$ 1 to 200, 1 c. c.	0.
	16	$ClN\langle^{(C_3H_7)_3}_{CH_2CHOHCH_3}$ 1 to 200, 1 c. c.	0.
	20	$ClN\langle^{(C_3H_7)_3}_{CH_2CH_2OH}$ 1 to 200, 1 c. c.	Fell 13 mm. (79 to 66).
	22	$ClN\langle^{(CH_3)_3}_{CH_2CHO(C_6H_5CO)CH_2Cl}$ 1 to 200, 1 c. c.	Rose 9 mm. (84 to 93).
	33	$ClN\langle^{(C_5H_{11})_3}_{CH_2CHO(C_6H_5CO)CH_2Cl}$ 1 to 200, 1 c. c.	Rose 15 mm. (103 to 118).
	37	$ClN\langle^{(C_5H_{11})_3}_{CH_2CHO(CH_3CO)CH_2Cl}$ 1 to 200, 1 c. c.	Rose 7 mm. (87 to 94).
	48	$ClN\langle^{(CH_3)_3}_{CH_2CHO(CH_3CO)CH_3}$ 1 to 1,000,000, 1 c. c.	Fell 26 mm. (76 to 50).
	52	$ClN\langle^{(CH_3)_3}_{CH_2CHO(CH_3CO)CH_2Cl}$ 1 to 10,000, 1 c. c.	Fell 14 mm. (70 to 56).
	55	$ClN\langle^{(CH_3)_3}_{CH_2CH_2CH_2O(CH_3CO)}$ 1 to 1,000,000, 1 c. c.	Fell 20 mm. (72 to 52).
	59½	$ClN\langle^{(C_3H_7)_3}_{CH_2CHO(CH_3CO)CH_2Cl}$ 1 to 1,000, 1 c. c.	Fell 8 mm. (78 to 70).
4	1	$ClN\langle^{(CH_3)_3}_{CH_2CHO(C_6H_5CO)CH_2Cl}$ 1 to 200, 1 c. c.	Fell 11 mm. (80 to 69).
	9	Atropine, 5 mg.	

EXPERIMENT 275.—CAT, 2.44 K. ETHER; CURARE—Continued.

Time.	Compound.	Blood pressure.
m. 12	$ClN\langle^{(C_3H_7)_3}_{CH_2CHO(CH_3CO)CH_2Cl}$ 1.54 per cent, 1 c. c.	Rose slightly.
16	$ClN\langle^{(C_3H_7)_3}_{CH_2CHO(C_6H_5CO)CH_2Cl}$ 1 to 100, 1 c. c.	Rose 5 mm. (92 to 97).
21	$ClN\langle^{(C_3H_7)_3}_{CH_2CHOHCH_2Cl}$ 1 to 200, 1 c. c.	0.
22	$ClN\langle^{(C_3H_7)_3}_{CH_2CHOHCH_3}$ 1 to 200, 1 c. c.	0.
28	$ClN\langle^{(C_3H_7)_3}_{CH_2CH_2O(C_6H_5CO)}$ 0.7 per cent, 1 c. c.	Rose 11 mm. (88 to 99).
29	$ClN\langle^{(C_3H_7)_3}_{CH_2CH_2OH}$ 0.81 per cent, 1 c. c.	0.
39	$ClN\langle^{(C_5H_{11})_3}_{CH_2CHO(C_6H_5CO)CH_2Cl}$ 1 to 200, 1 c. c.	Rose 12 mm. (87 to 99).
42	$ClN\langle^{(C_5H_{11})_3}_{CH_2CHO(CH_3CO)CH_2Cl}$ 1 to 200, 1 c. c.	0.
50	$ClN\langle^{(CH_3)_3}_{CH_2CH_2CH_2O(CH_3CO)}$ 1 to 100,000, 1 c. c.	0.
52	$ClN\langle^{(CH_3)_3}_{CH_2CH_2CH_2O(CH_3CO)}$ 1 to 10,000, 1 c. c.	Fell 9 mm. (53 to 44).
58	$ClN\langle^{(CH_3)_3}_{CH_2CHO(CH_3CO)CH_3}$ 1 to 10,000, 1 c. c.	Fell 7 mm. (36 to 29).

EXPERIMENT 276.—CAT, 2.72 K. ETHER; CURARE.

44	$ClN\langle^{(C_2H_5)_3}_{CH_2CHO(C_6H_5CO)CH_2Cl}$ 0.45 per cent. Subcut.	Fell (in 5 min.) 20 mm. (118 to 98; returned slowly.
00	$ClN\langle^{(C_2H_5)_3}_{CH_2CHOHCH_2OH}$ 1 to 200, 1 c. c.	Fell 14 mm. (98 to 84).
6	$ClN\langle^{(C_2H_5)_3}_{CH_2CHO(C_6H_5CO)CH_2Cl}$ 1 to 2,000, 1 c. c.	Fell 18 mm. (86 to 68).
9	$ClN\langle^{(C_2H_5)_3}_{CH_2CHOHCH_2OH}$ 1.28 per cent, 1 c. c.	Fell 12 mm. (91 to 79).

EXPERIMENT 276.—CAT, 2.72 K. ETHER; CURARE—Continued.

Time.	Compound.	Blood pressure.
H. *m.* 25	$ClN\begin{cases}(C_2H_5)_3\\CH_2CHO(C_6H_5CO)CH_3\end{cases}$ 1 to 200, 1 c. c.	Fell (slowly) 24 mm. (94 to 70).
35	$ClN\begin{cases}(C_2H_5)_3\\CH_2CHO(CH_3CO)CH_3\end{cases}$ 1 to 1,000, 1 c. c.	Fell 13 mm. (92 to 79).
2 1	$ClN\begin{cases}(C_2H_5)_3\\CH_2CHOHCH_3\end{cases}$ 1 to 100, 1 c. c.	Fell 8 mm. (66 to 58).
3	$ClN\begin{cases}(C_2H_5)_3\\CH_2CHOHCH_2Cl\end{cases}$ 1 to 100, 1 c. c.	Fell 9 mm. (67 to 58).
13	$ClN\begin{cases}(C_2H_5)_3\\CH_2CHOHCH_2OH\end{cases}$ 1.28 per cent, 1 c. c.	0.
1	$ClN\begin{cases}(C_2H_5)_3\\CH_2CHO(C_6H_5CO)CH_2Cl\end{cases}$ 0.45 per cent, 1 c. c.	0.
19	Atropine 5 mg.	
20	$ClN\begin{cases}(C_2H_5)_3\\CH_2CHO(C_6H_5CO)CH_2Cl\end{cases}$ 0.45 per cent, 1 c. c.	0.
21+	$ClN\begin{cases}(C_2H_5)_3\\CH_2CHOHCH_2OH\end{cases}$ 1.28 per cent, 1 c.c.	0.
28	$ClN\begin{cases}(C_2H_5)_3\\CH_2CHO(C_6H_5CO)CH_3\end{cases}$ 1 per cent, 1 c. c.	0.
30	$ClN\begin{cases}(C_2H_5)_3\\CH_2CHO(CH_3CO)CH_3\end{cases}$ 0.172 per cent, 1 c. c.	0.
41	$ClN\begin{cases}(C_2H_5)_3\\CH_2CHOHCH_3\end{cases}$ 1 to 100, 1 c. c.	0.
43	$ClN\begin{cases}(C_2H_5)_3\\CH_2CHOHCH_2Cl\end{cases}$ 1 to 100, 1 c. c.	0.
48	Choline 1 to 100, 1 c. c.	0.
50	Benzoyl-choline 1.32 per cent, 1 c. c.	0.
57	$ClN\begin{cases}(CH_3)_3\\CH_2CHOHCH_2OH\end{cases}$ 1 to 100, 1 c. c.	0.
3 3	$ClN\begin{cases}(CH_3)_3\\CH_2CHO(CH_3CO)CH_3\end{cases}$ 1 to 1,000, 1 c. c.	Fell 20 mm. (68 to 48).
10	$ClN\begin{cases}(CH_3)_3\\CH_2CH_2CH_2O(CH_3CO)\end{cases}$ 1 to 1,000, 1 c. c.	Fell 19 mm. (76 to 57).

EXPERIMENT 276.—CAT, 2.72 K. ETHER; CURARE—Continued.

Time.	Compound.	Blood pressure.
H. *m.* 37	$ClN\langle^{(CH_3)_3}_{CH_2CH_2CH_2O(CH_3CO)}$ 0.94 per cent, 1 c. c.	Fell 20 mm. (62 to 42).
44	Atropine 15 mg.	
48	$ClN\langle^{(CH_3)_3}_{CH_2CH_2CH_2O(CH_3CO)}$ 0.94 per cent, 1 c. c.	Fell 13 mm. (78 to 65).
54	$ClN\langle^{(CH_3)_3}_{CH_2CHO(CH_3CO)CH_2Cl}$ 0.89 per cent, 1 c. c.	0.
58	$ClN\langle^{(CH_3)_3}_{CH_2CHO(CH_3CO)CH_3}$	Fell 17 mm. (80 to 63).
4 00	$ClN\langle^{(CH_3)_3}_{CH_2CH_2CH_2O(CH_3CO)}$ 1 to 1,000, 1 c. c.	0.
20	$ClN\langle^{(CH_3)_3}_{CH_2CHO(CH_3CO)CH_2O(CH_3CO)}$ 1.22 per cent, 1 c. c.	0.

EXPERIMENT 277.—CAT, 2.55 K. ETHER; CURARE.

1	23	$ClN\langle^{(C_2H_5)_3}_{CH_2CHO(C_6H_5CO)CH_2O(C_6H_5CO)}$ 0.178 per cent, 0.5 c. c.	Fell (slowly) 46 mm. (118 to 72). Heart gradually slowed from 33 to 26½ beats in 10 sec.
	28	$ClN\langle^{(C_2H_5)_3}_{CH_2CHO(C_6H_5CO)CH_2O(C_6H_5CO)}$ 1 to 1,000, 1 c. c.	Fell (slowly) 29 mm. (115 to 86).
	51	$ClN\langle^{(CH_3)_3}_{CH_2CHOHCH_2OH}$ 1 to 100, 1 c. c.	Fell 44 mm. (136 to 92).
	52	$ClN\langle^{(C_2H_5)_3}_{CH_2CHOHCH_2OH}$ 1.28 per cent, 1 c. c.	0.
2	1	$ClN\langle^{(CH_3)_3}_{CH_2CHO(CH_3CO)CH_2O(CH_3CO)}$ 1.22 per cent, 1 c. c.	Fell 27 mm. (85 to 58).
	14	$ClN\langle^{(C_2H_5)_3}_{CH_2CHO(C_6H_5CO)CH_2O(C_6H_5CO)}$ 0.178 per cent, 1 c. c.	Fell (slowly) 37 mm. (102 to 65).
	21	$ClN\langle^{(C_2H_5)_3}_{CH_2CHO(CH_3CO)CH_2O(CH_3CO)}$ 1 to 200, 1 c. c.	0.
	24	$ClN\langle^{(C_2H_5)_3}_{CH_2CHO(CH_3CO)CH_2O(CH_3CO)}$ 0.93 per cent, 1 c. c.	Fell 18 mm. (116 to 98).
	43	$ClN\langle^{(CH_3)_3}_{CH_2CHO(C_6H_5CO)CH_2Cl}$ 1 to 200, 1 c. c.	0.

EXPERIMENT 277.—CAT, 2.55 K. ETHER; CURARE—Continued.

Time.	Compound.	Blood pressure.
H. m. 45	$ClN\langle\equiv\rangle(CH_3)_3$ / $CH_2CHO(C_6H_5CO)CH_3$ — 1 to 500, 1 c. c.	Rose 57 mm. (87 to 144).
3 1	$ClN\langle\equiv\rangle(CH_3)_3$ / $CH_2CH_2CH_2O(C_6H_5CO)$ — 1 to 500, 1 c. c.	Rose 21 mm. (109 to 130).
12	$ClN\langle\equiv\rangle(CH_3)_3$ / $CH_2CHO(C_6H_5CO)CH_2Cl$ — 1.27 per cent, 1 c. c.	0.
31	$ClN\langle\equiv\rangle(C_2H_5)_3$ / $CH_2CHO(C_6H_5CO)CH_3$ — 1 to 2,000, 1 c. c.	Fell 12 mm. (138 to 126).
34	$ClN\langle\equiv\rangle(C_2H_5)_3$ / $CH_2CHO(C_6H_5CO)CH_2Cl$ — 1 to 2,000, 1 c. c.	Fell 6 mm. (120 to 114).
48	$ClN\langle\equiv\rangle(C_2H_5)_3$ / $CH_2CHO(C_6H_5CO)CH_3$ — 1 to 200, 1 c. c.	Fell 22 mm. (132 to 110).
50	$ClN\langle\equiv\rangle(C_2H_5)_3$ / $CH_2CHO(C_6H_5CO)CH_2Cl$ — 0.45 per cent, 1 c. c.	Fell 11 mm. (128 to 117).
58	$ClN\langle\equiv\rangle(C_3H_7)_3$ / $CH_2CHO(CH_3CO)CH_2Cl$ — 1 to 200, 1 c. c.	Fell 10 mm. (147 to 137).
4 2	$ClN\langle\equiv\rangle(CH_3)_3$ / $CH_2CHO(C_6H_5CO)CH_2O(C_6H_5CO)$ — 1 to 1,000, 1 c. c.	Fell 12 mm. (134 to 122).
4	$ClN\langle\equiv\rangle(CH_3)_3$ / $CH_2CHO(C_6H_5CO)CH_2O(C_6H_5CO)$ — 1 to 100, 1 c. c.	Fell 53 mm. (128 to 75).
12	Atropine — 5 mg.	
16	$ClN\langle\equiv\rangle(CH_3)_3$ / $CH_2CHO(C_6H_5CO)CH_2O(C_6H_5CO)$ — 1.04 per cent, 1 c. c.	Fell 32 mm. (92 to 60).
18	$ClN\langle\equiv\rangle(C_2H_5)_3$ / $CH_2CHO(C_6H_5CO)CH_2O(C_6H_5CO)$ — 0.178 per cent, 1 c. c.	Rose slightly.
24	$ClN\langle\equiv\rangle(C_2H_5)_3$ / $CH_2CHOHCH_2OH$ — 1.28 per cent, 1 c. c.	Rose 15 mm. (62 to 77).
30	$ClN\langle\equiv\rangle(CH_3)_3$ / $CH_2CHOHCH_2OH$ — 1 to 100, 1 c. c.	0.
41	$ClN\langle\equiv\rangle(CH_3)_3$ / $CH_2CHO(CH_3CO)CH_2O(CH_3CO)$ — 1.22 per cent, 1 c. c.	Rose 5 mm. (66 to 71).
43	$ClN\langle\equiv\rangle(C_2H_5)_3$ / $CH_2CHO(CH_3CO)CH_2O(CH_3CO)$ — 0.93 per cent, 1 c. c.	0.

EXPERIMENT 277.—CAT, 2.55 K. ETHER; CURARE—Continued.

Time.	Compound.	Blood pressure.
H. m. 50	$ClN\langle^{(CH_3)_3}_{CH_2CH_2CH_2O(C_6H_5CO)}$ 1 to 500, 1 c. c.	0.
51	$ClN\langle^{(CH_3)_3}_{CH_2CHO(C_6H_5CO)CH_2Cl}$ 1.27 per cent, 1 c. c.	Rose 6 mm. (60 to 66).
54	$ClN\langle^{(CH_3)_3}_{CH_2CHO(C_6H_5CO)CH_3}$ 1 to 100, 1 c. c.	0.

EXPERIMENT 278.—CAT, 3.02 K. URETHANE AND CHLORAL; CURARE.

11	58	$ClN\langle^{(C_2H_5)_3}_{CH_2CHO(C_6H_5CO)CH_3)}$ 1 to 500, 1 c. c.	Fell 20 mm. (103 to 83). Heart slowed from 37¼ to 30 beats in 10 sec.
12	2	$ClN\langle^{(C_2H_5)_3}_{CH_2CHO(C_6H_5CO)CH_2Cl}$ 1 to 500, 1 c. c.	Fell 25 mm. (95 ,to 70); returned very slowly.
	14	$ClN\langle^{(C_2H_5)_3}_{CH_2CHO(C_6H_5CO)CH_2Cl}$ 0.45 per cent, 1 c. c. subcut.	0.
	24	$ClN\langle^{(C_2H_5)_3}_{CH_2CHOHCH_3}$ 1 to 200, 1 c. c.	Fell 27 mm. (92 to 65).
	31	$ClN\langle^{(C_2H_5)_3}_{CH_2CHOHCH_2Cl}$ 1 to 200, 1 c. c.	Fell 13 mm. (90 to 77).
	44	$ClN\langle^{(C_2H_5)_3}_{CH_2CHO(C_6H_5CO)CH_2O(C_6H_5CO)}$ 0.178 per cent, 1 c. c.	0.
	49	$ClN\langle^{(C_2H_5)_3}_{CH_2CHO(CH_3CO)CH_2O(CH_3CO)}$ 0.93 per cent, 1 c. c.	0.
1	8	$ClN\langle^{(C_3H_7)_3}_{CH_2CHO(C_6H_5CO)CH_3}$ 0.79 per cent, 1 c. c.	Fell (slowly) 16 mm. (59 to 43).
	18	$ClN\langle^{(CH_3)_3}_{CH_2CHO(CH_3CO)CH_3}$ 1 to 1,000,000, 1 c. c.	Fell 19 mm. (58 to 39).
	24	$ClN\langle^{(CH_3)_3}_{CH_2CH_2CH_2O(CH_3CO)}$ 1 to 1,000,000, 1 c. c.	Fell 16 mm. (56 to 40).
	36	$ClN\langle^{(CH_3)_2}_{(CH_2CH_2OH)_2}$ 1 to 100, 1 c. c.	Fell 7 mm. (62 to 55).
	52	Nicotine 5 mg.	Rose 109 (57 to 166). Heart increased from 18½ to 30 in 10 sec.
2	5	$ClN\langle^{(CH_3)_3}_{CH_2CHO(CH_3CO)CH_3}$ 1 to 1,000,000, 1 c. c.	Fell 17 mm. (55 to 38).

EXPERIMENT 278.—CAT, 3.02 K. URETHANE AND CHLORAL; CURARE—Continued.

Time.	Compound.	Blood pressure.
H. m. 11	$ClN\langle\begin{matrix}(CH_3)_3\\ CH_2CH_2CH_2O(CH_3CO)\end{matrix}\rangle$ 1 to 1,000,000, 1 c. c.	Fell 22 mm. (75 to 53).
14	$ClN\langle\begin{matrix}(CH_3)_2\\ (CH_2CH_2OH)_2\end{matrix}\rangle$ 1 to 100, 1 c. c.	0.
18	Nicotine 5 mg.	Rose 57 mm. (66 to 123). Heart increased from 18 to 25½ beats in 10 sec.
24	Acetyl-choline (old solution) 1 to 100,000, 1 c. c.	Fell 34 mm. (68 to 34).
28	$ClN\langle\begin{matrix}(CH_3)_3\\ CH_2CHO(CH_3CO)CH_3\end{matrix}\rangle$ 1 to 1,000,000, 1 c. c.	Fell 22 mm. (76 to 54).
33	$ClN\langle\begin{matrix}(CH_3)_3\\ CH_2CH_2CH_2O(CH_3CO)\end{matrix}\rangle$ 1 to 1,000,000, 1 c. c.	Fell 22 mm. (76 to 54).
35	Nicotine 5 mg.	Rose 18 mm. (75 to 93). No change in heart rate.
40	$ClN\langle\begin{matrix}(CH_3)_3\\ CH_2CH_2CH_2O(CH_3CO)\end{matrix}\rangle$ 1 to 1,000,000, 1 c. c.	Fell 25 mm. (78 to 53).
42	Nicotine 5 mg.	0.
45	$ClN\langle\begin{matrix}(CH_3)_3\\ CH_2CH_2CH_2O(CH_3CO)\end{matrix}\rangle$ 1 to 1,000,000, 1 c. c.	Fell 26 mm. (80 to 54).
49	Choline 1 to 100, 1 c. c.	Fell 23 mm. (78 to 45).
55	$ClN\langle\begin{matrix}(CH_3)_2\\ (CH_2CH_2OH)_2\end{matrix}\rangle$ 1 to 100, 1 c. c.	Fell 10 mm. (86 to 76).
11	$ClN\langle\begin{matrix}(C_2H_5)_3\\ CH_2CHO(C_6H_5CO)CH_2Cl\end{matrix}\rangle$ 0.45 per cent, 1 c. c.	0.
13	$ClN\langle\begin{matrix}(C_2H_5)_3\\ CH_2CHO(C_6H_5CO)CH_3\end{matrix}\rangle$ 1 to 100, 1 c. c.	0.
18	$ClN\langle\begin{matrix}(C_2H_5)_3\\ CH_2CHOHCH_3\end{matrix}\rangle$ 1 to 100, 1 c. c.	0.
20	$ClN\langle\begin{matrix}(C_2H_5)_3\\ CH_2CHOHCH_2Cl\end{matrix}\rangle$ 1 to 100, 1 c. c.	Fell 13 mm. (97 to 84).
25	$ClN\langle\begin{matrix}(C_2H_5)_3\\ CH_2CHO(C_6H_5CO)CH_2O(C_6H_5CO)\end{matrix}\rangle$ 0.178 per cent, 1 c. c.	0.
27	$ClN\langle\begin{matrix}(C_2H_5)_3\\ CH_2CHO(CH_3CO)CH_2O(CH_3CO)\end{matrix}\rangle$ 0.93 per cent, 1 c. c.	0.

EXPERIMENT 278.—CAT, 3.02 K. URETHANE AND CHLORAL; CURARE—Continued.

Time. H. m.	Compound.	Blood pressure.
34	Choline 1 to 100, 1 c. c.	Fell 32 mm. (90 to 58).
38	$ClN\begin{matrix}\equiv(CH_3)_3\\ \diagdown CH_2CHO(CH_3CO)CH_3\end{matrix}$ 1 to 1,000,000, 1 c. c.	Fell 28 mm. (92 to 64).
40	$ClN\begin{matrix}\equiv(CH_3)_3\\ \diagdown CH_2CHO(CH_3CO)CH_3\end{matrix}$ 1 to 1,000,000, 1 c. c.	Fell 11 mm. (94 to 83).
51	$ClN\begin{matrix}\equiv(C_3H_7)_3\\ \diagdown CH_2CHO(C_6H_5CO)CH_3\end{matrix}$ 0.79 per cent, 1 c. c.	Rose 7 mm. (100 to 107).
4 4	$ClN\begin{matrix}\equiv(C_3H_7)_3\\ \diagdown CH_2CHO(CH_3CO)CH_3\end{matrix}$ 1.2 per cent, 1 c. c.	0.
7	Atropine 5 mg.	
15	$ClN\begin{matrix}\equiv(C_2H_5)_3\\ \diagdown CH_2CHOHCH_2Cl\end{matrix}$ 1 to 200, 1 c. c.	0.
22	$ClN\begin{matrix}\equiv(CH_3)_2\\ \diagdown(CH_2CH_2OH)_2\end{matrix}$ 1 to 100, 1 c. c.	0.
24	Choline 1 to 100, 1 c. c.	0.
29	$ClN\begin{matrix}\equiv(CH_3)_3\\ \diagdown CH_2CH_2CH_2O(CH_3CO)\end{matrix}$ 1 to 100,000, 1 c. c.	0.
35	$ClN\begin{matrix}\equiv(CH_3)_3\\ \diagdown CH_2CHO(CH_3CO)CH_3\end{matrix}$ 1 to 100,000, 1 c. c.	0.
47	$ClN\begin{matrix}\equiv(CH_3)_3\\ \diagdown CH_2CH_2CH_2O(CH_3CO)\end{matrix}$ 1 to 1,000, 1 c. c.	0.
49	$ClN\begin{matrix}\equiv(CH_3)_3\\ \diagdown CH_2CHO(CH_3CO)CH_3\end{matrix}$ 1 to 1,000, 1 c. c.	Fell 25 mm. (73 to 48).
56	Atropine 20 mg.	
58	$ClN\begin{matrix}\equiv(CH_3)_3\\ \diagdown CH_2CHO(CH_3CO)CH_3\end{matrix}$ 1 to 1,000, 1 c. c.	0.
5 2	$ClN\begin{matrix}\equiv(CH_3)_3\\ \diagdown CH_2CHO(CH_3CO)CH_3\end{matrix}$ 1.25 per cent, 1 c. c.	Fell 25 mm. (66 to 41).
5	$ClN\begin{matrix}\equiv(CH_3)_3\\ \diagdown CH_2CH_2CH_2O(CH_3CO)\end{matrix}$ 0.94 per cent, 1 c. c.	0.
13	$ClN\begin{matrix}\equiv(C_2H_5)_3\\ \diagdown CH_2CHO(C_6H_5CO)CH_3\end{matrix}$ 1.95 per cent, 1 c. c.	Rose 10 mm. (82 to 92).

EXPERIMENT 278.—CAT, 3.02 K. URETHANE AND CHLORAL; CURARE—Continued.

Time.	Compound.	Blood pressure.
H. *m.* 18	Choline 1 to 100, 1 c. c.	Rose 9 mm. (86 to 95).
31	$ClN\begin{matrix}(CH_3)_2\\(CH_2CH_2OH)_2\end{matrix}$ 1 to 100, 1 c. c.	Rose 20 mm. (70 to 90).

EXPERIMENT 281.—CAT, 2.26 K. URETHANE AND CHLORAL; CURARE.

11	43	$ClN\begin{matrix}(C_5H_{11})_3\\CH_2CHO(CH_3CO)CH_3\end{matrix}$ 1 to 200, 1 c. c.	Rose 5 mm. (131 to 136).
	47	$ClN\begin{matrix}(CH_3)_2\\(CH_2CH_2O(CH_3CO))_2\end{matrix}$ 1 to 1,000, 0.6 c. c.	Fell 65 mm. (123 to 58). Heart irregular.
	50	$ClN\begin{matrix}(CH_3)_3\\CH_2CHO(CH_3CO)CH_2O(CH_3CO)\end{matrix}$ 1 to 1,000, 1 c. c.	Fell 31 mm. (124 to 93).
	52	$ClN\begin{matrix}(CH_3)_2\\(CH_2CH_2O(CH_3CO))_2\end{matrix}$ 1 to 1,000, 1 c. c.	Fell 83 mm. (117 to 34). Heart irregular.
12	12	$ClN\begin{matrix}(C_2H_5)_3\\CH_2CHO(CH_3CO)CH_2O(CH_3CO)\end{matrix}$ (Old solution) 1 to 200, 1 c. c.	Fell 21 mm. (119 to 98).
	16	$ClN\begin{matrix}(CH_3)_3\\CH_2CHOHCH_2OH\end{matrix}$ 1 to 200, 1 c. c.	Fell 37 mm. (114 to 77).
	19	$ClN\begin{matrix}(CH_3)_2\\(CH_2CH_2OH)_2\end{matrix}$ 1 to 200, 1 c. c.	Fell 14 mm. (118 to 104).
	26	Choline (chlorhydrine process) 1 to 200, 1 c. c.	Fell 32 mm. (110 to 78).
	34	$ClN\begin{matrix}(C_5H_{11})_3\\CH_2CHO(C_6H_5CO)CH_2Cl\end{matrix}$ 1 to 500, 1 c. c.	Fell (slowly) 56 mm. (104 to 48); remained low.
	58+	$ClN\begin{matrix}(C_5H_{11})_3\\CH_2CHO(C_6H_5CO)CH_2Cl\end{matrix}$ 1 to 500, 1 c. c.	Fell (slowly) 18 mm. (43 to 25).
1	10	Homorenon 1 to 1,000, 0.6 c. c.	Rose 117 mm. (23 to 140).
	33	Nicotine 5 mg.	Fell 21 mm. (52 to 31); rose 24 mm. (52 to 76).
	43	$ClN\begin{matrix}(C_3H_7)_3\\CH_2CHO(CH_3CO)CH_3\end{matrix}$ 1 to 200, 1 c. c.	0.
2	13	$ClN\begin{matrix}(CH_3)_2\\(CH_2CH_2O(CH_3CO))_2\end{matrix}$ 1 to 100, 1 c. c.	Fell 15 mm. (64 to 49).
	15	Atropine sulphate 5 mg.	

EXPERIMENT 281.—CAT, 2.26 K. URETHANE AND CHLORAL; CURARE—Continued.

Time.	Compound.	Blood pressure.
H. *m.* 19	$ClN\begin{cases}(CH_3)_2\\(CH_2CH_2O(CH_3CO))_2\end{cases}$ 1 to 100, 1 c. c.	0.
25	Homorenon 1 to 1,000, 1 c. c.	Rose 25 mm. (63 to 88).
30	$ClN\begin{cases}(C_5H_{11})_3\\CH_2CHO(C_6H_5CO)CH_2Cl\end{cases}$ 0.75 per cent, 1 c. c.	Fell (slowly) 36 mm. (76 to 40).
34	Homorenon 1 to 1,000, 1 c. c.	Rose 12 mm. (44 to 56).

EXPERIMENT 282.—CAT, 2.27 K. URETHANE AND CHLORAL; CURARE.

12	45	$ClN\begin{cases}(C_5H_{11})_3\\CH_2CHO(CH_3CO)CH_3\end{cases}$ 1 to 200, 1 c. c.	Rose 9 mm. (155 to 164).
	51	$ClN\begin{cases}(C_5H_{11})_3\\CH_2CHO(CH_3CO)CH_2Cl\end{cases}$ 1 to 100, 1 c. c.	Fell very slowly (in 10 min.) from 147 mm. to 31 mm.; in 39 min, had risen to 128 mm. Heart slowed from 36½ to 22 beats in 10 sec.
1	32	$ClN\begin{cases}(C_5H_{11})_3\\CH_2CHO(CH_3CO)CH_3\end{cases}$ 1 to 200 1 c. c.	Fell slowly from 128 mm. to 92 mm.
	47	$ClN\begin{cases}(CH_3)_2\\(CH_2CH_2O(CH_3CO))_2\end{cases}$ 1 to 10,000, 1 c. c.	Fell 37 mm. (107 to 70).
	54	$ClN\begin{cases}(CH_3)_3\\CH_2CHO(CH_3CO)CH_2O(CH_3CO)\end{cases}$ 1 to 1,000, 1 c. c.	Fell 35 mm (124 to 89).
	56	$ClN\begin{cases}(CH_3)_2\\(CH_2CH_2O(CH_3CO))_2\end{cases}$ 1 to 100,000, 1 c. c.	Fell 25 mm. (123 to 98).
2	3	$ClN\begin{cases}(CH_3)_3\\CH_2CHOHCH_2OH\end{cases}$ 1 to 200, 1 c. c.	Fell 43 mm. (138 to 95).
	5	$ClN\begin{cases}(CH_3)_2\\(CH_2CH_2OH)_2\end{cases}$ 1 to 200, 1 c. c.	Fell 10 mm. (130 to 120).
	16	$ClN\begin{cases}(C_5H_{11})_3\\CH_2CHO(C_6H_5CO)CH_3\end{cases}$ 1 to 1,000, 1 c. c.	Fell slowly (in 25 min.) from 101 mm. to 24 mm. and remained low for some time.
2	48	$\begin{cases}CH_2N(CH_3)_3Cl\\CHO(C_6H_5CO)\\CH_2N(CH_3)_3Cl\end{cases}$ 1 to 1,000, 1 c. c.	Rose 35 mm. (26 to 61). Heart slowed from 20 to 17 beats in 10 sec.
	52	Benzoyl-choline (methyl iodide process) 1 to 200, 1 c. c.	Rose 58 mm. (38 to 96).
3	2	Benzoyl-choline (methyl iodide process) 1 to 200, 1 c. c.	Rose 51 mm. (46 to 97). Heart rate increased from 21 to 24 beats in 10 sec.

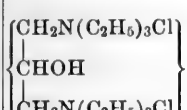
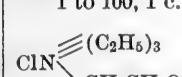
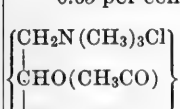
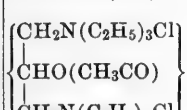
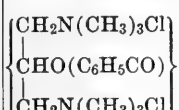
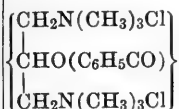
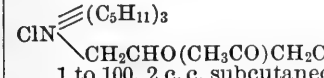
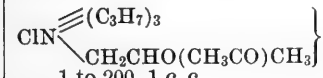
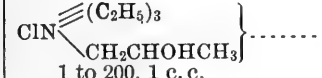
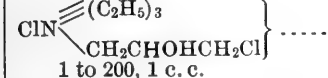
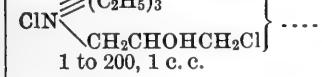
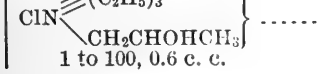
EXPERIMENT 282.—CAT, 2.27 K. URETHANE AND CHLORAL; CURARE—Continued.

Time.	Compound.	Blood pressure.
H. m.		
22	$\left.\begin{matrix}CH_2N(CH_3)_3Cl\\ \vert\\ CHOH\\ \vert\\ CH_2N(CH_3)_3Cl\end{matrix}\right\}$ 1 to 1,000, 1 c. c.	Rose 14 mm. (37 to 51).
26	$\left.\begin{matrix}CH_2N(CH_3)_3Cl\\ \vert\\ CHO(CH_3CO)\\ \vert\\ CH_2N(CH_3)_3Cl\end{matrix}\right\}$ 1 to 1,000, 1 c. c.	Rose 6 mm. (31 to 37).
33	$\left.\begin{matrix}CH_2N(C_2H_5)_3Cl\\ \vert\\ CHOH\\ \vert\\ CH_2N(C_2H_5)_3Cl\end{matrix}\right\}$ 1 to 100, 1 c. c.	Rose 19 mm. (30 to 49).
43	Benzoyl-choline (methyl iodide process) 1 to 200, 1 c. c.	Rose 24 mm. (21 to 45). Heart increased from 16 to 20 beats in 10 sec.
47	$\left.\begin{matrix}CH_2N(CH_3)_3Cl\\ \vert\\ CHO(C_6H_5CO)\\ \vert\\ CH_2N(CH_3)_3Cl\end{matrix}\right\}$ 1 to 200, 1 c. c.	0.
3 52	$\left.\begin{matrix}CH_2N(CH_3)_3Cl\\ \vert\\ CHO(C_6H_5CO)\\ \vert\\ CH_2N(CH_3)_3Cl\end{matrix}\right\}$ 0.99 per cent, 1 c. c.	Fell 6 mm. (18 to 12).
55	Benzoyl-choline 1 to 200, 1 c. c.	0.
4 19	Adrenalin 1 to 10,000, 1 c. c.	Rose 119 mm. (23 to 142).
23	$ClN\begin{matrix}\equiv(C_5H_{11})_3\\ \diagdown CH_2CHO(C_6H_5CO)CH_2Cl\end{matrix}$ 1 to 500, 2 c. c.	Fell slowly from 85 to 49 mm.
29	$ClN\begin{matrix}\equiv(C_5H_{11})_3\\ \diagdown CH_2CHO(C_6H_5CO)CH_2Cl\end{matrix}$ 0.75 per cent, 1 c. c.	Fell 24 mm. (49 to 25).
31	Adrenalin 1 to 10,000, 1 c. c.	Rose 49 mm. (31 to 80).
36	Adrenalin 1 to 10,000, 1 c. c.	0. (Blood pressure 53 mm.)
38	Adrenalin 1 to 10,000, 2 c. c.	0. (Blood pressure 58 mm.)
40	Adrenalin 1 to 10,000, 3 c. c.	0. (Blood pressure 56 mm.)

EXPERIMENT 283.—CAT, 2.9 K. URETHANE AND CHLORAL; CURARE.

12	28	$\left.\begin{matrix}CH_2N(CH_3)_3Cl\\ \vert\\ CHOH\\ \vert\\ CH_2N(CH_3)_3Cl\end{matrix}\right\}$ 1 to 500, 1 c. c.	Fell 13 mm. (89 to 76).
	30	Choline (chlorhydrine process) 1 to 500, 1 c. c.	Fell 24 mm. (88 to 64).

EXPERIMENT 283.—CAT, 2.9 K. URETHANE AND CHLORAL; CURARE—Continued.

Time.	Compound.	Blood pressure.
H. m.		
47	 1 to 100, 1 c. c.	Fell 24 mm. (90 to 66).
49	 0.65 per cent, 1 c. c.	Fell 22 mm. (89 to 67).
57	 1 to 1,000, 1 c. c.	Fell 22 mm. (90 to 68).
1 2	 0.92 per cent, 1 c. c.	Fell 8 mm. (96 to 88).
8	Acetyl-choline (old solution) 1 to 100,000, 1 c. c.	Fell 31 mm. (91 to 60).
13	 1 to 1,000, 1 c. c.	Fell 7 mm. (97 to 90).
29	 1 to 200, 1 c. c.	Rose 47 mm. (92 to 139).
33	Benzoyl-choline 1 to 200, 1 c. c.	Rose 37 mm. (93 to 130).
38	 1 to 100, 2 c. c. subcutaneously.	0.
2 26	 1 to 200, 1 c. c.	Fell 18 mm. (85 to 67).
37	 1 to 200, 1 c. c.	Fell 15 mm. (81 to 66),
40	 1 to 200, 1 c. c.	Fell 21 mm. (84 to 63).
42	Nicotine 5 mg.	Rose 101 mm. (82 to 183). Heart rate increased from 17½ to 26 beats in 10 sec.
3 1	 1 to 200, 1 c. c.	Fell 17 mm. (81 to 64).
6	 1 to 100, 0.6 c. c.	Fell 21 mm. (86 to 65).

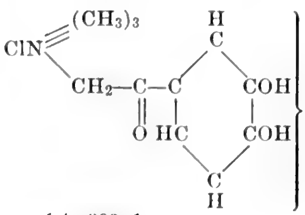
EXPERIMENT 283.—CAT, 2.9 K. URETHANE AND CHLORAL; CURARE—Continued.

Time.	Compound.	Blood pressure.
H. m.		
18	$\left.\begin{array}{l}CH_2N(CH_3)_3Cl\\ \vert\\ CHOH\\ \vert\\ CH_2N(CH_3)_3Cl\end{array}\right\}$ 1 to 100, 1 c. c.	Fell 19 mm. (88 to 69).
24	Choline 1 to 200, 1 c. c.	Fell 31 mm. (94 to 63).
30	$\left.\begin{array}{l}CH_2N(C_2H_5)_3Cl\\ \vert\\ CHOH\\ \vert\\ CH_2N(C_2H_5)_3Cl\end{array}\right\}$ 1 to 100, 1 c. c.	Fell 7 mm. (101 to 94).
33	$ClN\begin{array}{l}\equiv(C_2H_5)_3\\ \diagdown CH_2CH_2OH\end{array}\Big\}$ 0.65 per cent, 1 c. c.	Rose 14 mm. (98 to 112).
44½	$\left.\begin{array}{l}CH_2N(CH_3)_3Cl\\ \vert\\ CHO(CH_3CO)\\ \vert\\ CH_2N(CH_3)_3Cl\end{array}\right\}$ 1 to 1,000, 1 c. c.	Fell 30 mm. (104 to 74).
51	$\left.\begin{array}{l}CH_2N(C_2H_5)_3Cl\\ \vert\\ CHO(CH_3CO)\\ \vert\\ CH_2N(C_2H_5)_3Cl\end{array}\right\}$ 0.92 per cent, 1 c. c.	0.
4 5	$\left.\begin{array}{l}CH_2N(CH_3)_3Cl\\ \vert\\ CHO(C_6H_5CO)\\ \vert\\ CH_2N(CH_3)_3Cl\end{array}\right\}$ 1 to 200, 1 c. c.	Fell 26 mm. (110 to 84).
10	Benzoyl-choline (egg) 1 to 200, 1 c. c.	Rose 12 mm. (106 to 118).
12	$\left.\begin{array}{l}CH_2N(CH_3)_3Cl\\ \vert\\ CHO(C_6H_5CO)\\ \vert\\ CH_2N(CH_3)_3Cl\end{array}\right\}$ 1 to 200, 1 c. c.	Fell 26 mm. (112 to 86).
14	Atropine 5 mg.	
16	$\left.\begin{array}{l}CH_2N(CH_3)_3Cl\\ \vert\\ CHO(C_6H_5CO)\\ \vert\\ CH_2N(CH_3)_3Cl\end{array}\right\}$ 1 to 200, 1 c. c.	0.
19	$\left.\begin{array}{l}CH_2N(CH_3)_3Cl\\ \vert\\ CHOH\\ \vert\\ CH_2N(CH_3)_3Cl\end{array}\right\}$ 1 to 100, 1 c. c.	0.
24	Choline (chlorhydrine process) 1 to 100, 1 c. c.	0.
30	$\left.\begin{array}{l}CH_2N(C_2H_5)_3Cl\\ \vert\\ CHOH\\ \vert\\ CH_2N(C_2H_5)_3Cl\end{array}\right\}$ 1 to 100, 1 c. c.	Fell 9 mm. (106 to 97).

EXPERIMENT 283.—CAT, 2.9 K. URETHANE AND CHLORAL; CURARE—Continued.

Time.	Compound.	Blood pressure.
H. m. 37	$ClN\begin{cases}(C_2H_5)_3\\CH_2CH_2OH\end{cases}$ 0.65 per cent, 1 c. c.	Fell 9 mm. (116 to 107); rose 8 mm. (116 to 124).
47	$\begin{cases}CH_2N(CH_3)_3Cl\\CHO(CH_3CO)\\CH_2N(CH_3)_3Cl\end{cases}$ 1 to 200, 1 c. c.	Fell 6 mm. (111 to 105).
48½	$\begin{cases}CH_2N(C_2H_5)_3Cl\\CHO(CH_3CO)\\CH_2N(C_2H_5)_3Cl\end{cases}$ 0.92 per cent, 1 c. c.	Rose 7 mm. (111 to 118).
00	$ClN\begin{cases}(C_3H_7)_3\\CH_2CHO(CH_3CO)CH_3\end{cases}$ 1.2 per cent, 1 c. c.	0.
6	$ClN\begin{cases}(C_2H_5)_3\\CH_2CHOHCH_2Cl\end{cases}$ 1 to 200, 1 c. c.	0.
9	Acetyl-choline 1 to 10,000, 0.6 c. c.	Fell 31 mm. (110 to 79).
11	$ClN\begin{cases}(C_2H_5)_3\\CH_2CHOHCH_3\end{cases}$ 1 to 100, 1 c. c.	Rose 3 mm. (106 to 109).
13	Atropine sulphate 7 mg.	
14	Acetyl-choline 1 to 10,000, 1 c. c.	0.
16	$ClN\begin{cases}(C_5H_{11})_3\\CH_2CHO(CH_3CO)CH_2Cl\end{cases}$ 1 to 100, 0.6 c. c.	Rose 21 mm. (108 to 129). (Remained high.)
29	$ClN\begin{cases}(C_5H_{11})_3\\CH_2CHO(CH_3CO)CH_3\end{cases}$ 1 to 200, 0.6 c. c.	Rose 9 mm. (123 to 132).
36	$\begin{cases}CH_2N(C_2H_5)_3Cl\\CHOH\\CH_2N(C_2H_5)_3Cl\end{cases}$ 1 to 100, 1 c. c.	Fell 5 mm. (124 to 119).
40	$\begin{cases}CH_2N(CH_3)_3Cl\\CHO(CH_3CO)\\CH_2N(CH_3)_3Cl\end{cases}$ 1.3 per cent, 1 c. c.	0.
48	$ClN\begin{cases}(C_5H_{11})_3\\CH_2CHO(CH_3CO)CH_2Cl\end{cases}$ 1 to 100, 2 c. c.	0.
50	Suprarenalin 1 to 5,000 (Old solution), 1 c. c.	Rose 39 mm. (110 to 149).

EXPERIMENT 284.—CAT, 2.49 K. URETHANE AND CHLORAL; CURARE.

Time.	Compound.	Blood pressure.
H. m. 11 24	$CH_2N(C_2H_5)_3Cl$ $\vert$ $CHOH$ $\vert$ $CH_2N(C_2H_5)_3Cl$ 1 to 100, (old solution) 1 c. c.	Fell 6 mm. (70 to 64).
27	$CH_2N(C_2H_5)_3Cl$ $\vert$ $CHO(CH_3CO)$ $\vert$ $CH_2N(C_2H_5)_3Cl$ 0.92 per cent. 1 c. c.	0.
36	$CH_2N(CH_3)_3Cl$ $\vert$ $CHO(CH_3CO)$ $\vert$ $CH_2N(CH_3)_3Cl$ 1 to 200, 1 c. c.	Fell 8 mm. (60 to 52); rose 18 mm. (60 to 78).
49	Choline (chlorhydrine process) 1 to 200, 1 c. c.	Fell 7 mm. (65 to 58).
53	$CH_2N(CH_3)_3Cl$ $\vert$ $CHOH$ $\vert$ $CH_2N(CH_3)_3Cl$ 1 to 100, 1 c. c.	Fell 8 mm. (70 to 62).
57	Choline 1 to 100, 1 c. c.	Fell 12 mm. (70 to 58); rose 9 mm (70 to 79).
12 11	$ClN \begin{cases} (CH_3)_2 \\ (CH_2CH_2O(C_6H_5CO))_2 \end{cases}$ 1 to 100, 1 c. c.	Rose 11 mm. (62 to 73).
16	Benzoyl-choline (egg) 1 to 200, 1 c. c.	Rose 50 mm. (60 to 110). Heart rate increased from 26 to 37 in 10 sec.
27	Benzoyl-choline (egg) 1.18 per cent, 2 c. c. subcut.	0.
49	$ClN \begin{cases} \equiv (C_2H_5)_3 \\ CH_2CHO(C_6H_5CO)CH_2O(C_6H_5CO) \end{cases}$ 0.178 per cent, 1 c. c.	Rose 9 mm. (68 to 77).
1 8½	Benzoyl-choline (egg) 1 to 200, 1 c. c.	Rose 17 mm. (73 to 90). Heart rate increased from 23 to 27½ beats in 10 sec.
13	$CH_2N(CH_3)_3Cl$ $\vert$ $CHO(C_6H_5CO)$ $\vert$ $CH_2N(CH_3)_3Cl$ 1 to 200, 1 c. c.	Rose 28 mm. (77 to 105). No change in heart rate.
18	Benzoyl-choline (egg) 1 to 200, 1 c. c.	Rose 13 mm. (82 to 95). Heart rate increased from 24 to 27 beats in 10 sec.
30	$ClN \begin{cases} \equiv (C_3H_7)_3 \\ CH_2CHO(C_6H_5CO)CH_2O(C_6H_5CO) \end{cases}$ 1 to 100, 1 c. c.	Fell 37 mm. (67 to 30). Heart slowed from 28 to 15 in 10 sec.
40	 1 to 200, 1 c. c.	Rose 36 mm. (48 to 84). Heart increased from 13 to 26 beats in 10 sec.

EXPERIMENT 284.—CAT, 2.49 K. URETHANE AND CHLORAL; CURARE—Continued.

Time.	Compound.	Blood pressure.
h. m.		
25	$ClN\equiv(C_3H_7)_3$ / $CH_2CHO(CH_3CO)CH_2O(CH_3CO)$ — 1.17 per cent, 1 c. c.	Rose 5 mm. (72 to 77); fell 8 mm. (72 to 64).
30	Benzoyl-choline — 1 to 200, 1 c. c.	Rose 17 mm. (66 to 83).
33	$CH_2N(CH_3)_3Cl$ / $CHO(C_6H_5CO)$ / $CH_2N(CH_3)_3Cl$ — 1 to 200, 1 c. c.	Fell 10 mm. (66 to 56); rose 11 mm. (66 to 77).
36	Nicotine — 5 mg.	Rose 9 mm. (77 to 86); fell to 10 mm.; returned to 58.
53	$CH_2N(CH_3)_3Cl$ / $CHO(C_6H_5CO)$ / $CH_2N(CH_3)_3Cl$ — 1 to 200, 1 c. c.	Fell 4 mm. (58 to 62).
56	Benzoyl-choline — 1 to 200, 1 c. c.	Rose 15 mm. (65 to 80).
6	$ClN\equiv(C_3H_7)_3$ / $CH_2CHO(C_6H_5CO)CH_2O(C_6H_5CO)$ — 1 to 100, 1 c. c.	Fell 46 mm. (88 to 42). Fell slowly. Heart rate increased from 21 to 24 in 10 sec.
39	Choline (chlorhydrine process) — 1 to 100, 1 c. c.	Fell 25 mm. (88 to 63).
43	Atropine — 5 mg.	
49	Choline — 1 to 100, 1 c. c.	Rose 7 mm. (97 to 104).
50	$ClN\equiv(C_3H_7)_3$ / $CH_2CHO(C_6H_5CO)CH_2O(C_6H_5CO)$ — 1 to 100, 1 c. c.	Fell 21 mm. (97 to 76).
6	$ClN\equiv(C_3H_7)_3$ / $CH_2CHOHCH_2OH$ — 1 to 100, 1 c. c.	Fell 4 mm. (96 to 92).
35	$ClN\equiv(CH_3)_3$ / CH_2-C-C(H)(COH) / O HC(COH) — 1 to 200, 1 c. c.	Rose 23 mm. (89 to 112).
43	Atropine — 5 mg.	
45	$ClN\equiv(C_3H_7)_3$ / $CH_2CHO(C_6H_5CO)CH_2O(C_6H_5CO)$ — 1 to 100, 1 c. c.	Fell 14 mm. (95 to 81).
48	Atropine — 10 mg.	
49	$ClN\equiv(C_3H_7)_3$ / $CH_2CHO(C_6H_5CO)CH_2O(C_6H_5CO)$ — 1 to 100, 1 c. c.	Fell 16 mm. (86 to 70).

EXPERIMENT 286.—CAT, 3.25 K. URETHANE AND CHLORAL; CURARE.

Time.	Compound.	Blood pressure.
H. m. 11 45	$ClN\langle^{(C_3H_7)_3}_{CH_2CHO(C_6H_5CO)CH_2O(C_6H_5CO)}$ 1 to 200, 1 c. c.	Fell 27 mm. (132 to 105); returned, then fell slowly to 90 mm.
51	$ClN\langle^{(C_3H_7)_3}_{CH_2CHO(C_6H_5CO)CH_2O(C_6H_5CO)}$ 1 to 100, 1 c. c. subcutaneously.	0.
57	$ClN\langle^{(CH_3)_3}_{CH_2CHO(C_6H_5CO)CH_2O(C_6H_5CO)}$ 1 to 1,000, 1 c. c.	Fell 5 mm. (138 to 133).
59	$ClN\langle^{(CH_3)_3}_{CH_2CHO(C_6H_5CO)CH_2O(C_6H_5CO)}$ 1 to 100, 1 c. c.	Rose 18 mm. (133 to 151). (Slight movements.)
12 2	Benzoyl-choline (egg) 1 to 200, 1 c. c.	Rose 50 mm. (131 to 181). Heart slowed from 38 to 34 beats in 10 sec.
15	$ClN\langle^{(CH_3)_3}_{CH_2CHO(CH_3CO)CH_2O(CH_3CO)}$ 1 to 200, 1 c. c.	Fell 36 mm. (107 to 71).
18	$ClN\langle^{(C_2H_5)_3}_{CH_2CHO(CH_3CO)CH_2O(CH_3CO)}$ 1 to 200, 0.5 c. c.	Fell (slowly) 21 mm. (122 to 101).
28	$ClN\langle^{(C_2H_5)_3}_{CH_2CHO(CH_3CO)CH_2O(CH_3CO)}$ 1 to 200, 1 c. c.	Fell 18 mm. (126 to 198). Heart slowed from 37 to 31 beats in 10 sec.
31	$ClN\langle^{(CH_3)_2}_{(CH_2CH_2O(CH_3CO))_2}$ 1 to 100,000, 1 c. c.	Fell 14 mm. (122 to 108).
35	$ClN\langle^{(CH_3)_2}_{(CH_2CH_2O(CH_3CO))_2}$ 1 to 10,000, 1 c. c.	Fell 33 mm. (122 to 89).
39	$ClN\langle^{(CH_3)_3}_{CH_2CHOHCH_2OH}$ 1 to 500, 1 c. c.	Fell 22 mm. (118 to 96).
42	$ClN\langle^{(CH_3)_3}_{CH_2CH_2CH_2OH}$ 1 to 1,000, 1 c. c.	Fell 27 mm. (115 to 88).
45	$ClN\langle^{(CH_3)_3}_{CH_2CHOHCH_3}$ 1 to 1,000, 1 c. c.	Fell 7 mm. (116 to 109).
1 3	$ClN\langle^{(CH_3)_3}_{CH_2CHOHCH_3}$ 1 to 200, 1 c. c.	Fell 13 mm. (101 to 88).
5	$ClN\langle^{(CH_3)_3}_{CH_2CH_2CH_2OH}$ 1 to 500, 1 c. c.	Fell 28 mm. (101 to 73).
12	Choline (chlorhydrine process) 1 to 500, 1 c. c.	Fell 18 mm. (122 to 104).
21	$ClN\langle^{(CH_3)_3}_{CH_2-C(=O)-C(H)-C(OH)-C(H)(OH)}$ 1 to 200, 1 c. c.	Rose 54 mm. (135 to 189).

EXPERIMENT 286.—CAT, 3.25 K. URETHANE AND CHLORAL; CURARE—Continued.

Time.	Compound.	Blood pressure.
m. 45	$ClN\begin{Bmatrix}(C_3H_7)_3\\CH_2CHOHCH_2OH\end{Bmatrix}$ 1 to 100, 1 c. c.	Fell 16 mm. (142 to 126).
47	$ClN\begin{Bmatrix}(C_3H_7)_3\\CH_2CHO(CH_3CO)CH_2O(CH_3CO)\end{Bmatrix}$ 1.2 per cent, 1 c. c.	0.
7	Atropine 5 mg.	
16	$ClN\begin{Bmatrix}(C_3H_7)_3\\CH_2CHO(C_6H_5CO)CH_2O(C_6H_5CO)\end{Bmatrix}$ 1 to 200, 1 c. c.	Fell 68 mm. (135 to 67).
26	$ClN\begin{Bmatrix}(C_3H_7)_3\\CH_2CHO(CH_3CO)CH_2O(CH_3CO)\end{Bmatrix}$ 1.2 per cent, 1 c. c.	Fell 6 mm. (103 to 97).
28	$ClN\begin{Bmatrix}(C_3H_7)_3\\CH_2CHOHCH_2OH\end{Bmatrix}$ 1 to 100, 1 c. c.	Fell 11 mm. (115 to 104).
35	$ClN\begin{Bmatrix}(CH_3)_3\\CH_2CHO(CH_3CO)CH_2O(CH_3CO)\end{Bmatrix}$ 1.2 per cent, 1 c. c.	0.
37½	$ClN\begin{Bmatrix}(C_2H_5)_3\\CH_2CHO(CH_3CO)CH_2O(CH_3CO)\end{Bmatrix}$ 0.93 per cent, 1 c. c.	Fell (slowly) 32 mm. (113 to 81).
15	$ClN\begin{Bmatrix}(CH_3)_3\\CH_2CHOHCH_2OH\end{Bmatrix}$ 1 to 100, 1 c. c.	0.
16	$ClN\begin{Bmatrix}(CH_3)_2\\(CH_2CH_2O(CH_3CO))_2\end{Bmatrix}$ 1 to 100, 1 c. c.	Fell 18 mm. (44 to 26).
20	$ClN\begin{Bmatrix}(CH_3)_3\\CH_2CHOHCH_3\end{Bmatrix}$ 1 to 100, 1 c. c.	Fell 4 mm. (41 to 37).
24	$ClN\begin{Bmatrix}(CH_3)_3\\CH_2CH_2CH_2OH\end{Bmatrix}$ 1 to 100, 1 c. c.	Fell 6 mm. (40 to 34).
48	$ClN\begin{Bmatrix}(C_3H_7)_3\\CH_2CHO(C_6H_5CO)CH_2O(C_6H_5CO)\end{Bmatrix}$ 1 to 100, 1 c. c.	Fell (slowly) 14 mm. (61 to 47).

EXPERIMENT 289.—CAT, 3.82 K. URETHANE AND CHLORAL; CURARE.

58	$ClN\begin{Bmatrix}(C_5H_{11})_3\\CH_2CHO(CH_3CO)CH_2O(CH_3CO)\end{Bmatrix}$ 1 to 1,000, 1 c. c.	Fell 10 mm. (146 to 136).
00	$ClN\begin{Bmatrix}(C_5H_{11})_3\\CH_2CHOHCH_2OH\end{Bmatrix}$ 1 to 200, 1 c. c.	Rose 8 mm. (145 to 153).
8	$ClN\begin{Bmatrix}(C_5H_{11})_3\\CH_2CHOHCH_2OH\end{Bmatrix}$ 1 to 100, 1 c. c.	Fell 20 mm. (120 to 100); rose 20 mm. (120 to 140).

EXPERIMENT 289.—CAT, 3.82 K.—URETHANE AND CHLORAL; CURARE—Continued.

Time.	Compound.	Blood pressure.
H. m. 14	$ClN\equiv(C_5H_{11})_3$, $CH_2CHO(CH_3CO)CH_2O(CH_3CO)$ 1 to 200, 1 c. c.	Fell 26 mm. (139 to 113). (Returned very slowly.)
19	$ClN\equiv(C_5H_{11})_3$, $CH_2CHOHCH_2OH$ 1 to 100, 1 c. c.	Rose 7 mm. (117 to 124); fell 21 mm. (117 to 96); rose to 135 mm.
33	$ClN\equiv(C_5H_{11})_3$, $CH_2CHO(CH_3CO)CH_2O(CH_3CO)$ 1 to 100, 1 c. c.	Rose 15 mm. (133 to 148); fell (slowly) 52 mm. (133 to 81). Returned slowly.
49	$ClN\equiv(C_5H_{11})_3$, $CH_2CHOHCH_2OH$ 1 to 100, 1 c. c.	Fell 30 mm. (133 to 103).
55	$ClN\equiv(C_5H_{11})_3$, $CH_2CHO(CH_3CO)CH_2O(CH_3CO)$ 1.4 per cent, 1 c. c. subcut.	0.

EXPERIMENT 290.—CAT, 3.25 K. URETHANE AND CHLORAL; CURARE.

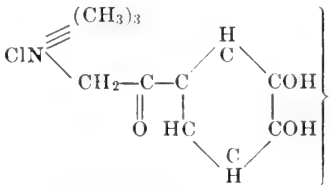
2	8	$ClN\equiv(CH_3)_3$, CH_2OH 1 to 1,000, 1 c. c.	Fell 23 mm. (66 to 43); rose 14 mm. (66 to 80).
	13	$ClN\equiv(CH_3)_3$, CH_2OCH_3 1 to 1,000, 1 c. c.	Fell 10 mm. (60 to 50); rose 6 mm. (60 to 66).
	19	$ClN\equiv(CH_3)_3$, CH_2OCH_3 1 to 500, 1 c. c.	Fell 13 mm. (70 to 57); rose 14 mm. (70 to 84).
	24	$ClN\equiv(CH_3)_3$, CH_2OH 1 to 1,000, 1 c. c.	Fell 26 mm. (65 to 39); rose 13 mm. (65 to 78).
	40	"Choline" [a] from $ClN\equiv(CH_3)_3$, $CH_2CH_2OC_2H_5$ 1 to 2,000, 1 c. c.	Fell 37 mm. (64 to 27). Heart slowed from 34 to 12 beats in 10 sec.
	54	Choline (egg) 1 to 500, 1 c. c.	Fell 6 mm. (64 to 58).
3	2	$ClN\equiv(C_5H_{11})_3$, $CH_2CHOHCH_2OH$ 1 to 200, 1 c. c.	Rose 16 mm. (61 to 77).
	6	$ClN\equiv(C_5H_{11})_3$, $CH_2CHO(CH_3CO)CH_2O(CH_3CO)$ 1 to 200, 1 c. c.	Fell 6 mm. (66 to 60); rose 7 mm. (66 to 73). Heart rate increased from 36 to 40 beats in 10 sec.
	14	$ClN\equiv(CH_3)_3$, $CH_2CHOHCH_2OH$ 1 to 500, 1 c. c.	Fell 11 mm. (57 to 46).
	17	$ClN\equiv(C_2H_5)_3$, $CH_2CHOHCH_2OH$ 1 to 200, 1 c. c.	Rose 10 mm. (56 to 66).

[a] The platinum double salt of this contained 31.33 per cent platinum (31.63 required for choline; 28.65 for the ethyl ether); it was doubtless contaminated with the ethyl ether from which it resulted. The latter is a much more poisonous substance than choline (Meyer, Liebig's Annalen, 1904, 337, p. 50).

EXPERIMENT 290.—CAT, 3.25 K. URETHANE AND CHLORAL; CURARE—Continued.

Time.	Compound.	Blood pressure.
H. m.		
34	Betaine ..... 1 to 100, 1 c. c.	0.
40	$ClN\begin{matrix}\equiv(CH_3)_3\\ \diagdown CH_2OH\end{matrix}$ ..... 1 to 1,000, 1 c. c.	Rose 16 mm. (72 to 88); fell 26 mm. (72 to 46).
43	$ClN\begin{matrix}\equiv(CH_3)_3\\ \diagdown CH_2OCH_3\end{matrix}$ ..... 1 to 500, 1 c. c.	Rose 20 mm. (71 to 91).
54	$ClN\begin{matrix}\equiv(CH_3)_3\\ \diagdown CH_2OCH_3\end{matrix}$ ..... 1 to 1,000, 1 c. c.	Fell 7 mm. (82 to 75); rose 14 mm. (82 to 96).
57	Choline (egg) ..... 1 to 500, 1 c. c.	Rose 8 mm. (77 to 85).
59	"Choline" from $ClN\begin{matrix}\equiv(CH_3)_3\\ \diagdown CH_2CH_2OC_2H_5\end{matrix}$ ..... 1 to 2,000, 1 c. c.	Fell 35 mm. (80 to 45).
4 1	Atropine ..... 5 mg.	
3	$ClN\begin{matrix}\equiv(CH_3)_3\\ \diagdown CH_2OH\end{matrix}$ ..... 1 to 1,000, 1 c. c.	0.
6	$ClN\begin{matrix}\equiv(CH_3)_3\\ \diagdown CH_2OH\end{matrix}$ ..... 1 to 100, 1 c. c.	Rose 25 mm. (83 to 108).
9	"Choline" from $ClN\begin{matrix}\equiv(CH_3)_3\\ \diagdown CH_2CH_2OC_2H_5\end{matrix}$ ..... 1 to 1,000, 1 c. c.	Rose 28 mm. (86 to 114).
16	Choline (egg) ..... 1 to 100, 1 c. c.	Rose 19 mm. (90 to 109).
23	$ClN\begin{matrix}\equiv(CH_3)_3\\ \diagdown CH_2OCH_3\end{matrix}$ ..... 1 to 500, 1 c. c.	Rose 11 mm. (94 to 105).
25	$ClN\begin{matrix}\equiv(C_5H_{11})_3\\ \diagdown CH_2CHO(CH_3CO)CH_2O(CH_3CO)\end{matrix}$ ..... 1 to 200, 1 c. c.	Fell 23 mm. (96 to 73).
34	$ClN\begin{matrix}\equiv(CH_3)_3\\ \diagdown CH_2OCH_3\end{matrix}$ ..... 1 to 100, 1 c. c.	Rose 26 mm. (80 to 106).
41	$ClN\begin{matrix}\equiv(C_5H_{11})_3\\ \diagdown CH_2CHOHCH_2OH\end{matrix}$ ..... 1 to 100, 1 c. c.	Fell 11 mm. (77 to 66).
48	$ClN\begin{matrix}\equiv(CH_3)_3\\ \diagdown CH_2CHOHCH_2OH\end{matrix}$ ..... 1 to 200, 1 c. c.	0.
51	$ClN\begin{matrix}\equiv(C_5H_{11})_3\\ \diagdown CH_2CHO(CH_3CO)CH_2O(CH_3CO)\end{matrix}$ ..... 1 to 200, 1 c. c.	Fell 21 mm. (87 to 66).
53	Atropine ..... 10 mg.	Fell.

EXPERIMENT 290.—CAT, 3.25 K. URETHANE AND CHLORAL; CURARE—Continued.

Time.	Compound.	Blood pressure.
H. m. 56	$ClN\equiv(C_5H_{11})_3$ / $CH_2CHO(CH_3CO)CH_2O(CH_3CO)$ — 1 to 200, 1 c. c.	Fell 7 mm. (56 to 49).
59	Atropine — 10 mg.	
5 00	$ClN\equiv(C_5H_{11})_3$ / $CH_2CHO(CH_3CO)CH_2O(CH_3CO)$ — 1 to 200, 1 c. c.	Fell 8 mm. (54 to 46).
4	Betaine — 1 to 100, 2 c. c.	0.
10	Chloraceto-pyrocatechine — 1 to 100, 1 c. c.	Fell 27 mm. (52 to 25). (Fell slowly.)
16	 — 1 to 200, 1 c. c.	Rose 23 mm. (57 to 80).

EXPERIMENT 296.—KITTEN, 1.1 K. CHLORAL; URETHANE; SOME CURARE.

12 41	$ClHN(CH_3)(C_2H_4OC_2H_4OH)$ — 1 to 2,500, 1 c. c.	0.
43	$ClHN(CH_3)(C_2H_4OC_2H_4OH)$ — 1 to 250, 1 c. c.	0.
46	Benzoyl derivative of $ClHN(CH_3)(C_2H_4OC_2H_4OH)$ — 1 to 4,000, 1 c. c.	0.
48	Benzoyl derivative of $ClHN(CH_3)(C_2H_4OC_2H_4OH)$ — 2.5 per cent. 1 c. c.	Fell 38 mm. (83 to 45).
53	Acetyl derivative of $ClHN(CH_3)(C_2H_4OC_2H_4OH)$ — 1 to 10,000, 1 c. c.	0.
58	Acetyl derivative of $ClHN(CH_3)(C_2H_4OC_2H_4OH)$ — 1 to 1,000, 1 c. c.	Fell 16 mm. (80 to 64).
1 00	Benzoyl derivative of $ClHN(CH_3)(C_2H_4OC_2H_4OH)$ — 2.5 per cent, 1 c. c.	Fell 23 mm. (80 to 47).
3	Atropine sulphate — 2 mg.	

EXPERIMENT 296.—KITTEN, 1.1 K. CHLORAL; URETHANE; SOME CURARE—Continued.

Time.	Compound.	Blood pressure.
H. m. 4	Benzoyl derivative of $ClHN(CH_3)(H)(C_2H_4OC_2H_4OH)$ 2.5 per cent, 1 c. c.	Fell 49 mm. (69 to 20). Heart slowed.
8	Acetyl derivative of $ClHN(CH_3)(H)(C_2H_4OC_2H_4OH)$ 1 to 1,000, 1 c. c.	0.
9	Acetyl derivative of $ClHN(CH_3)(H)(C_2H_4OC_2H_4OH)$ 1 per cent, 1 c. c.	0.
1 33	Benzoyl derivative of $ClHN(CH_3)(H)(C_2H_4OC_2H_4OH)$ 2.5 per cent, 1 c. c.	Fell 32 mm. (47 to 15). Heart slowed from 35 to 22 beats in 10 sec.

EXPERIMENT 297.—CAT, 1.8 K. CHLORAL; URETHANE; CURARE.

12 56	Benzoyl derivative of $ClHN(CH_3)(H)(C_2H_4OC_2H_4OH)$ 2.5 per cent, 1 c. c.	Fell 8 mm. (98 to 90); rose 12 mm. (98 to 110); fell slowly 15 mm. (98 to 83).
1 1	Acetyl derivative of $ClHN(CH_3)(H)(C_2H_4OC_2H_4OH)$ 1 to 1,000, 1 c. c.	Fell 25 mm. (85 to 60).
3	Choline (egg) 1 to 200, 1 c. c.	Fell 24 mm. (94 to 70).
7	Palmityl-choline 1 to 3,330, 1 c. c.	Fell 35 mm. (104 to 69).
9	Choline 1 per cent, 1 c. c.	Fell 18 mm. (104 to 86); rose 10 mm. (104 to 114).
22	Atropine sulphate 4.5 mg.	
1 22	Palmityl-choline 1 to 3,330, 1 c. c.	0.
24	Benzoyl derivative of $ClHN(CH_3)(H)(C_2H_4OC_2H_4OH)$ 2.5 per cent, 1 c. c.	Fell 10 mm. (84 to 74).
26	Acetyl derivative of $ClHN(CH_3)(H)(C_2H_4OC_2H_4OH)$ 1 to 1,000, 1 c. c.	0.
32	α-Brom-iso-capronyl-choline 1 to 185, 1 c. c.	Rose 54 mm. (80 to 134).
35	Choline 1 per cent, 1 c. c.	Rose 28 mm. (87 to 115).
2 19	α-Brom-iso-capronyl-choline 1 to 185, 1 c. c.	Rose 34 mm. (76 to 110).
23	Palmityl-choline 1 to 330, 1 c. c.	Rose 8 mm. (73 to 81).
25	Choline 1 per cent, c. c.	Rose 21 mm. (79 to 100).

EXPERIMENT 298.—CAT, 3.8 K. ETHER; NO CURARE.

Time.	Compound.	Blood pressure.
H. m.		
9 52	α-Brom-iso-capronyl-choline 1 to 1,850, 1 c. c	0.
54	α-Brom-iso-capronyl-choline 1 to 185, 1 c. c.	Rose 48 mm. (127 to 175); fell 23 mm. (127 to 104).
59	Palmityl-choline 1 to 3,330, 1 c. c.	Fell 22 (120 to 98).
10 3	Palmityl-choline 1 to 1,000, 1 c. c.	Fell 31 mm. (120 to 89).
6	Choline (egg) 1 to 100, 1 c. c.	Fell 36 mm. (136 to 100).
8	Acetyl derivative of $ClHN\begin{cases}CH_3\\ H\\ C_2H_4OC_2H_4OH\end{cases}$ 1 to 1,000, 1 c. c.	Fell 39 mm. (141 to 102).
9	Atropine sulphate 4 mg.	
11	α-Brom-iso-capronyl-choline 1 to 185, 1 c. c.	Rose 12 mm. (106 to 118).
14	α-Brom-iso-capronyl-choline 2.7 per cent, 1 c. c.	Rose 82 mm. (95 to 177). Heart rate increased from 37 to 42.
17	Palmityl-choline 1 to 1,000, 1 c. c.	0.
19	Choline 1 to 100, 1 c. c.	Rose 10 mm. (92 to 102).
20	Acetyl derivative of $ClHN\begin{cases}CH_3\\ H\\ C_2H_4OC_2H_4OH\end{cases}$ 1 to 1,000, 1 c. c.	0.
21	Benzoyl derivative of $ClHN\begin{cases}CH_3\\ H\\ C_2H_4OC_2H_4OH\end{cases}$ 2.5 per cent, 1 c. c.	Fell 20 mm. (92 to 72).

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 ulicis* n. g., n. sp.) in American mosquitoes (*Culex sollicitans*); by Ch. Wardell Stiles. The type species of the cestode genus *Hymenolepis;* by Ch. Wardell Stiles.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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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.

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

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

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

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

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

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No. 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 (*Ielaps echidnimus*). By W. W. Miller.

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

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

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No. 72.—I. Report on an Outbreak of Typhoid Fever at Omaha, Nebr. (1909-1910), by L. L. Lumsden. II. The Water Supply of Williamson, W. Va., and its Relation to an Epidemic of Typhoid Fever. By W. H. Frost.

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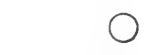

TREASURY DEPARTMENT
Public Health and Marine-Hospital Service of the United States

HYGIENIC LABORATORY—BULLETIN No. 74
JANUARY, 1911

DIGITALIS STANDARDIZATION AND THE VARIABILITY OF CRUDE AND OF MEDICINAL PREPARATIONS

By

WORTH HALE

WASHINGTON
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United States Public Health and Marine-Hospital Service.

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IGITALIS STANDARDIZATION AND THE VARIABILITY OF CRUDE AND OF MEDICINAL PREPARATIONS.[1]

By Worth Hale,

ssistant Pharmacologist, Division of Pharmacology, Hygienic Laboratory, United States Public Health and Marine-Hospital Service, Washington, D. C.

The value of any drug depends upon its having the effect which xperience and experiments have shown to be beneficial in the dis-sed condition. However, it is by no means sufficient that a given rug be shown to possess valuable medicinal properties; these must e present in the commercial preparations of a drug in constant and finite amounts to secure the best therapeutic results. Yet drug ants and chemical substances used as drugs have long been known vary either in the amount of their active constituents or in purity, metimes because of natural and unpreventable causes; sometimes ecause of accidental or intended contamination or adulteration. his has resulted in a serious condition of affairs as neither the doctor or the patient, who is really the most interested in the matter, could epend upon a remedy to produce a definite effect as a result of a ven dosage.[2]

To provide against such a condition of affairs there were incorpo-ted in the last revision of the United States Pharmacopœia a number standards and purity tests to which the pharmacopœial prepara-ons must conform. Especially is this true in the case of a number important drugs, such as opium, belladonna, nux vomica, etc., in hich the chief active constituents are capable of quantitative isola-on. With a considerable number of drugs this is not possible, at ast by chemical methods, for the reason that their active constitu-ts are not known or can not be isolated quantitatively by any the known chemical methods.[3] By far the most important drug

Manuscript submitted for publication November 29, 1910.

Since submitting this paper for publication my attention has been called to a case of poisoning, as fol-s: The patient, who suffered from chronic heart disease, had been receiving tincture of strophanthus in ertain dose. The prescription was refilled and the medication continued. After the second dose from new strophanthus tincture poisonous symptoms developed, although fortunately ultimate recovery ued after two days of serious illness. An assay made at this laboratory of the strophanthus showed t it was an exceedingly active preparation, and whereas the patient was supposed to be receiving the ie dose as of the old he actually received an amount of the active principle in this new preparation equal bout three times that of the strophanthus tincture which he had formerly been taking.

In this group may be included the digitalis group of heart tonics—digitalis, strophanthus, squills, con-laria, apocynum, and suprarenal extract, ergot, cannabis indica, and aconite.

of this class is digitalis on account of its almost universal use in certain forms of heart disease. Digitalis, however, is a notoriously variable drug, its activity varying from 100 to 400 per cent, due to a large number of different factors.

The conditions modifying the activity of digitalis are briefly as follows:

THE CRUDE DRUG.[1]

The locality in which the plants are grown has long been known to alter the amount of their active principles, a variation due not only to climatic conditions but also to variations in the soil. This factor is thought to be of considerable importance in the case of digitalis, and at the present time the English leaves are considered to be the best, and these, as well as those from Germany, are believed to be superior to the American-grown leaves. Parenthetically it is to be noted that Bohemian leaves are said to be so toxic that it is necessary in therapeutics to materially reduce the usual amounts given in order to avoid poisonous effects. It has been shown, however, that leaves from the same locality may vary widely in activity not only at different harvests, but, what is equally important, leaves of the same locality of the same harvest. As an example of these factors, Duffield,[2] using a chemical method, stated that American leaves are more toxic than English, and these in turn more toxic than German leaves. Edmunds,[3] using a biological method, examined three pharmacopœial tinctures made from German leaves and three from English leaves. According to his results the German leaves gave values of 8, 18, and 25; the English leaves values of 11, 20, and 29. Results so discordant as these certainly throw considerable doubt upon the value of the place of origin of the crude drug as a criterion of its activity, although other factors to be considered later probably played an important role in this variability.

A somewhat more generally established dictum is that digitalis leaves gathered from wild-growing plants are more potent than those growing in gardens, an opinion first promulgated by William Withering,[4] the English physician, who introduced digitalis into regular medicine. This view is generally accepted at the persent time although certain pharmacopœias recognize the cultivated leaf as official. Almost equally well established also is the opinion that leaves gathered from second-year plants are the more active, and that these should be gathered just at the beginning of the flowering season.

[1] For a more extensive bibliography of the literature relating to the various factors influencing the activity of digitalis the reader is referred to Bulletin 48, The Standardization of Digitalis, Hyg. Lab., U. S. P. H. and Mar. Hosp. Service, 1909.

[2] Duffield: Am. J. of Pharm., 1869, **41**, 55.

[3] Edmunds: Journ. Am. Med. Ass., 1907, **48**, 1744.

[4] Withering: An account of the foxglove and some of its medicinal uses. 1785.

Subsequent to this the activity of the leaves is thought to be somewhat lessened because of the energy used in the formation of the flower and the seed.[1] Hart[2] believed, as the result of his experiments, that the first-year leaves were more toxic than the second year's growth, but this is not the commonly accepted view, and the International Convention recommends leaves of the second year's growth gathered as the plant begins to flower.

A great deal has been said about the deleterious action of certain enzymes which supposedly break up the unstable digitalis glucosides, the process being said to be especially rapid in the presence of heat and moisture. Because of this great care is usually observed in drying the leaves, since the deleterious enzyme action is supposed to be especially active at this time. As early as 1807, Hamilton,[3] from his clinical observations, warned physicians against the use of improperly dried digitalis leaves, and a somewhat similar observation was made by Tourdes[4] in 1867, who accounted for the greater potency of the digitalis obtained about Strassburg, from the fact that after careful selection the leaves were dried in the shade and later in an oven at a temperature of not over 40° C. (102° F.). The value of such procedure by which the leaves are carefully and thoroughly dried has been emphasized by a number of more recent investigations.[5] In most instances moderate heat is used, and it is also suggested that vacuum drying should be used, and that the leaves should be dried over lime,[6] the idea being to reduce the moisture content to such a degree (1 to 2 per cent instead of 10 to 15 per cent, as in the ordinary methods) that the enzyme action would become so slight as to be practically of no importance. And having thus carefully dried the leaves, it has been suggested that they be kept in dark, air-tight containers to prevent further absorption of moisture. It would seem fairly well established that the leaves should be carefully and quickly dried, but certain workers have found that preparations several years old and quite open to the influence of the moisture of the air were well preserved. Others, however, were very weak.[7] But in spite of this evidence the careful drying and storing of leaves would seem to be not without importance, although possibly of somewhat less importance than is ascribed to the method by the firms which exploit such carefully dried leaves.

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[1] Focke: Arch. d. Pharm., 1903, **241**, 128.
[2] Hart: Pharm. Journ. and Tr., 1908, **26**, 440.
[3] Hamilton: Observations on Fox Glove, 1807, p. 7.
[4] Tourdes: Gaz. med. de Strasb., 1867, **27**, p. 191.
[5] Focke Archiv d. Pharm., 1903, **241**, 128.
[6] Bachem: Therap. Monatsh., 1908, **22**, 303.
[7] At the University of Michigan an assay of digitalis leaves at least eight years old, which had been stored in an ordinary paper bag, showed a high degree of activity and were of about the same value as recently purchased leaves.

The digitalis dialysates have been suggested as a means of securing preparations in which no enzyme action was possible, and therefore uniform and stable compounds; but biological and chemical investigations have shown that great variation was still to be found, possibly to be *ascribed to seasonal* variation in the drug plant.

MEDICINAL PREPARATIONS.

The various methods which are used in preparing the crude drug for medicinal purposes, and the variability in the keeping quality of these, are also important factors in lessening the chance of securing uniform digitalis products. Little need be said in this place about the pharmacopœial preparations. The official tincture and infusion answer the requirements of the physician, while the fluid extract, which is rarely if ever used as such, seems superfluous. Its most important and about its only use is in the manufacture of what may be termed artificial pharmacopœial tinctures or infusions. No one will deny that the method is simple, but the resulting preparation could never be called pharmacopœial, nor is it at all possible that the resulting pseudo-tinctures or infusions even approximately represent the regular pharmacopœial preparation of the same name. It is therefore somewhat surprising to find that some of the leading manufacturing houses are lending themselves to this practice by stating upon the labels of their fluid extracts, and even of preparations made according to special formulæ, the methods of dilution to secure a pharmacopœial tincture or a pharmacopœial infusion.

The manufacture of special digitalis products in the present unsatisfactory state of digitalis standardization is also a questionable procedure. Of course, a great deal of credit should be given to the manufacturer for discovering and perfecting the methods by which preparations are made more attractive, uniform, and with less secondary action. With a drug such as digitalis, however, it would seem desirable to decrease rather than increase the number of special formulæ, and the manufacturers would perform a real favor to those suffering from heart disease if they withheld such preparations from the market until careful experimentation and hosptial trial had shown them to have not only a real value over the older and official preparations, but also to remain unchanged as a result of ageing. At the present time a large part of their value lies in the greater profit to the firm exploiting them, not in a better result to the patient who uses them. In direct line with this it is worth while noting that several such preparations purchased from a retail pharmacist have recently been examined at this laboratory,[1] and while some were found to be more active than the pharmacopœial preparations, others

[1] Edmunds and Hale: Bulletin 48, U. S. P. H. and M. H. Service, Washington.

were much less potent; some had no digitalis action upon the heart. and one which had apparently deteriorated badly was directly depressant to it, a result which, to say the least, would be very distrous to anyone suffering from heart disease.

METHODS OF ASSAY.

The knowledge that the crude drugs as well as that the preparations of digitalis (or of the digitalis series) vary in strength is the result of a large number of investigations in which a number of different methods of determining the drug's activity have been used. These include clinical, chemical, and biological methods, but at the present time the clinical method has largely been supplanted by the other two, and it is to be noted also that the biological method is in much more general use than the chemical. Because these methods differ so widely and because of the many modifications in the manner in which they are carried out, it is not so surprising that a great many contradictory statements regarding this important group of heart tonics have been made. Nor should a very great degree of accuracy be expected when it is noted that the chemical method depends on the estimation of only one of a number of active substances, while conclusions drawn from biological investigations are based on extremely varied methods of experimentation.

THE CHEMICAL METHOD.

The chemical method as used at the present time depends upon the quantitative determination of the digitoxin content, the most active "pure" principle found in digitalis leaves. It must not be forgotten, however, that the chemistry of digitalis is very imperfectly understood; that digitalis owes its activity to at least two other principles, digitalein and digitalin, both of which exert a marked digitalis action upon the heart. There is also no evidence to show that these substances may not be present in varying proportions, so that the digitoxin content[1] might be relatively small and yet the given sample of leaves be very active on account of a high content of the other substances, a fact which the chemical method of assay would not demonstrate. The method is also questionable on account of the difficulty with which digitoxin is estimated. For example, Barger and Shaw,[2] two English investigators, after adding digitoxin to an artificial tincture, were able to recover only 25 per

[1] The claim is sometimes made by those favoring an assay of the digitoxin that this procedure is analgous to that of assaying opium by determining the morphine content and neglecting the presence of codeine. This, however, does not appear to be quite the correct view, since codeine occurs in only about one-thirtieth the amount in which morphine does and, moreover, it is about four times weaker in action. The amount of codeine therefore in the average specimen of opium would account for only about 1 per cent of its activity.

[2] Barger & Shaw: Pharm. Jour. 1904, **19** 249.

cent of the amount added. The estimation of the digitoxin content, on account of these uncertain factors, could give therefore but an incomplete idea of the actual value of a digitalis preparation.

As a defense of the chemical method, a number of experimenters have compared it with the biological method, but the agreement has been so slight as to give it little apparent value. The most concordant results were obtained by Reed and Vanderkleed,[1] but an analysis of their finding shows a very imperfect parallelism.[2] It may be said, therefore, in the present state of our knowledge of the chemistry of digitalis that its chemical assay is not of practical value.

BIOLOGICAL METHODS.

The comparative value of the drugs comprising the digitalis group has been more often studied by biological than by chemical methods. Like all methods, however, whether clinical, chemical, or biological, unless the various steps in such comparative assays are uniform and the same precautions are observed by all investigators, very variable results are sure to follow, even when the same preparation is being examined. And this is about the present condition of affairs, for biological methods are applied by nearly every investigator in a modified way, so that the results of such assays, although of relative value, do not admit of absolute comparisons.

In the large number of investigations that have been made since that by Koppe[3] in 1875, the methods have varied to such a degree that when classified according to their end reactions three distinct and separate type methods may be described. These type methods have undergone many modifications, so that under each several distinct submethods depending on animals used, methods of experimentation, time limits, temperature factors, etc., have been employed.

In a study of these type methods and certain of the more commonly-used submethods, Edmunds and Hale[4] reported that no very close agreement was found between the results obtained in an assay of a number of commercial preparations. However, it is noteworthy, as attesting the relative if not the absolute value of biological methods, that such preparations as tested weak by one method assayed weak by all, and those showing marked activity by one did so by all.

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[1] Reed and Vanderkleed: Am. Journ. of Pharm., 1908, **80**, 110; 1910, **82**, 453.

[2] It is of interest to note that nearly all investigators of the chemical method have used biological methods as a means of controlling the former. This is of course an acknowledgment even by those who urge chemical standardization that biological methods are probably accurate, and to-day this belief is becoming more and more general, so that a larger number of manufacturers are adopting some one of the several biological methods as a test of the activity of their preparations of digitalis.

[3] Koppe: Arch. f. Exper. Path. u. Pharm., 1875, **3**, 274.

[4] Edmunds and Hale: The Physiological Standardization of Digitalis, Bull. 48, Hygienic Laboratory, U. S. P. H. and M. H. S.

In this paper objections were urged against certain of the methods made use of partly upon the basis of experimental difficulties and partly on theoretical grounds.

In the general toxic method the drug is given in sufficient doses to cause death without reference to the organ or organs chiefly affected. The method is usually carried out, using mammals as experimental animals, and while this is believed by some to be a step in the right direction, on account of the closer relation between the higher animals, when compared to cold-blooded animals (frogs) and man, certain experimental evidence tends to show that this is not necessarily so. At least it is to be noted that death in such animals in certain cases apparently results from an action on the central nervous system while the heart, as recorded by blood-pressure tracings, is still in good condition. In other cases the respiratory movements are kept up some time after the heart has stopped beating and the blood pressure has fallen to zero.

In this connection it is further urged that a digitalis preparation with a high percentage of the saponinlike body, digitonin, would cause death from the action of this poison, which depresses both the heart and the central nervous system. Thus such a preparation would show a high value by a general toxic method, although the heart tonic principles might be present in relatively small amounts, or of two preparations with the same proportion of substances acting on the heart that with the larger amount of the saponinlike body would assay stronger. In the general toxic method in which frogs are used, as in Houghton's "12-hour method," this objection would naturally lose some weight on account of the ability of such animals to live a considerable time independently of the central nervous system, the action of the drug being therefore presumably upon the heart.

As a further objection it may be suggested also that a general toxic method would not show adulteration or accidental contamination of a digitalis preparation, since a lethal effect could easily be secured from an action of any of a number of poisons, or possibly also from substances formed in the deterioration which digitalis preparations are known to undergo. To be certain of a heart tonic, a digitalis action, such a method would need to be controlled by tests upon the heart itself.

The two other type methods are based on a general principle which it would seem worth while emphasizing—namely, that any biological assay method for any drug should take into account that action of the drug upon which its chief therapeutic usefulness rests and that in so far as practical difficulties did not especially interfere, this action should be made the basis of the biological method used in its assay. Such a principle would appear to be of much more importance than

that urged by certain investigators—namely, that mammals should be used rather than cold-blooded animals because of their closer biologic relation to man. The reaction of elemental tissues such as muscle and nerve is qualitatively alike without regard to species, the difference only one of degree.

In the case of the digitalis group it would seem of considerable importance that the end reaction should be one involving the characteristic effect of the group upon the circulatory apparatus and this, as a matter of fact, is the basis of the two type methods in one of which the effect is manifested upon the arterial pressure as controlled by the vasomotor center, the muscular wall of the arterioles and upon the output of the heart; in the second, the effect being manifested by changes in the heart muscle itself.

With a substance such as epinephrin, the blood-pressure method offers many advantages. The action from very small doses is marked, and yet is so transient and so without accumulation effects that many injections of the same or of other similar preparations may be made, using the same animal. In the case of digitalis, squills, convallaria, and strophanthus, however, the immediate action from even comparatively large doses is never marked, is prolonged and accumulative, so that a second injection is always modified by the first, thus making a series of observations on the same animal an impossibility.

The second type method of determining the comparative value of preparations of the digitalis series by noting their effects upon the heart, especially of the frog, is perhaps the oldest and at the present time the most widely used of all methods. On purely theoretical grounds such a type method would appear to be ideal on account of the close relation between the therapeutic use of the drug and the end reaction in this type of assay. On practical grounds also some of the widely used submethods are very simple (though not so much as Houghton's 12-hour method).

Three submethods may be described, although others are occasionally used; in one the isolated frogs' heart is perfused; in one the drug is given to the intact animal, which is subsequently operated on to note the condition of the heart; in the other the drug is given to the animal subsequent to the operative procedure in which the heart is exposed. The chief advocate of the latter method is Focke, who operates on the unpithed animal, exposing the heart, and then injects the drug into a lymph sac. The heart is then watched and the time noted at which complete systolic standstill takes place. The dose must be sufficiently large (usually one-fortieth of the animal's weight of a 10 per cent infusion) to bring on this end reaction in from 7 to 15 minutes, and the strength of the preparation "V" is determined by a formula in which the weight of the frog is divided by the dose multiplied by the time in minutes until the reaction is complete.

Focke's method seems open, however, to a number of objections, in spite of the fact that the action on the heart is taken as the basis of the end reaction. In the first place, Focke exposes the heart by cutting away the sternum in the unpithed frog. This he claims [1] causes no suffering, since the pain sense in the lower orders of animal life is very imperfectly, if at all, developed. Such a conclusion, however, seems based on purely theoretical grounds, and although the sense of pain is probably not highly developed in this animal, it would seem better to resort to pithing. If pithing or the slight loss of blood which follows would interfere with the accuracy of the method, it would appear better to discard the method.

From an experimental point of view the method presents further difficulties also. Several observers have commented on the fact that there is great difficulty in determining the exact moment at which the heart may be said to be in permanent systole. The modification of observing the cessation of the movement of the corpuscles in the web of the frog's foot is also very unsatisfactory. The difficulty of determining the definite end period undoubtedly brings about a certain amount of inaccuracy and might account for the variability in results which other writers have reported. Thus according to Edmunds and Hale [2] the results obtained with the same preparation on a series of frogs varied as much as 100 per cent, and according to Chevalier [3] who, however, did not observe the time limits suggested by Focke, as much as 400 to 600 per cent. Focke's [4] own figures, after eliminating certain results which, with the same dosage, fall outside the range of the established time limits, vary as much as 72 per cent.

In this method also the injection of large doses is required (0.5 to 0.8 c. c.) to bring on the end reaction within the specified time limits. Focke's [3] observations with Rana Temporaria is that such doses are quickly absorbed, but my own observations on Rana pipiens, the common grass frog of the section of the United States east of the Rocky Mountains, is that the absorption is often much delayed. Further, also very much smaller doses will cause systolic stoppage of the frog's heart by allowing a longer time for absorption, and accordingly it would seem very obvious that the end reaction is brought on by a fraction of the dose used.

The method of using the isolated frog's heart as a test object for digitalis and allied drugs, is based on the ground that the total amount of drug acts directly on the heart through the perfusion fluid without the question of imperfect or delayed absorption entering into the

[1] Focke: Archiv. der Pharm., 1910, **248**, 352.

[2] Edmunds and Hale: The Standardization of Digitalis. Bull. 48, Hygienic Laboratory, U. S. P. H. and M. H. Service, Washington, p. 46.

[3] Chevalier: Les Nouveaux Remèdes, 1910, **27**, 124.

[4] Focke: Archiv. der Pharm., 1910, **248**, 258.

problem. Edmunds and Hale [1] reported in 1909 that no very close agreement was to be found in the time in which the heart ceased beating, but Krailsheimer [2] and Schmiedeberg [3] have recently made a further study of this method, obtaining somewhat more concordant results. Their figures also show some wide variations, however, thus one heart stopped beating after 10, 14, and 20 minutes; in another case after 22 to 39 minutes. It seems likely that this lack of uniformity may be due to a possible greater or more variable precipitation by the perfusion fluid of the active digitalis principles in the same preparation and certainly in the case of different preparations. The temperature factor is also an important one, and to secure the best results, an absolutely uniform temperature of the perfusion fluid should be maintained.

So far as operative technique is concerned this method is one of the most complex of all those which have been proposed, and could only be employed by investigators having considerable experience in perfusion work if anything like comparative results were to be expected. In any case also certain results would need to be discarded arbitrarily, as has been done by Schmiedeberg, because such results seemed to be outside the usual limits for the drug in question.

In the third submethod, that of examining the heart at some time subsequent to the injection of the drug, the chief points of differences in the manner of experimentation is in the time at the end of which the heart of the frog should be found in complete systole. Time limits varying from 30 minutes to 2 hours have been used.

This method has been provisionally adopted at this laboratory for testing digitalis and allied preparations. The general method of procedure is essentially the same as that suggested by Famulener and Lyons,[4] in which the permanent systole of the frog's ventricle at the end of exactly 1 hour [5] is taken as the end reaction. Some slight modifications have been made, the chief of which is that the assays are all carried out at a constant temperature, as it is believed that this precaution results in greater accuracy. The drug is injected through the mouth into the anterior lymph sac of the intact frog (Rana pipiens) and at the end of an hour the animal is pithed (both brain and cord) and the thorax opened. The condition of the heart is then noted and a smaller or larger dose is injected as indicated. To hasten the assay a series of three frogs is usually given doses which vary quite widely and an approximate dose is estimated from these preliminary results. By successively narrowing the limits of

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[1] Edmunds and Hale: Bull. Hyg. Lab., No. 48, 1909.

[2] Krailsheimer: Arch. f. exper. Path. u. Pharm., 1910, **62**, 296.

[3] Schmiedeberg: *Ibid.*, p. 305.

[4] Famulener and Lyons: Proc. Am. Pharm. Ass., 1902, **50**, 415.

[5] The method of using the action of digitalis on the frog's heart as an assay method was first carried out under the direction and at the suggestion of Prof. A. R. Cushny.

dosage that amount just necessary to produce the end reaction may be determined by the use of from 8 to 12 frogs.

The method has the advantage of comparative simplicity so far as operative technique is concerned. The animals are always easily obtained and even if not obtainable in the immediate vicinity of the laboratory they can be easily shipped by express from long distances. In this connection it is worth while calling attention to the difficulty of obtaining a suitable number of mammals, especially cats, rabbits, or dogs, for carrying out such tests on warm-blooded animals when a large number of assays are to be made. The number of assays to be reported on in the following pages would have been absolutely impossible on this ground alone. Of less importance also is that of cost. Frogs for an assay would cost approximately 50 cents. If mammals are used, on the basis that at least three should be used for each assay, the cost would be from $2 to $3, while to this would be added the expense of food supplies, etc., which in the case of the higher animals is a considerable item. However, the question of expense is regarded as purely secondary.

Objections have been made against all biological methods as affording even approximately accurate results in the assay of digitalis. One very large manufacturer states as the position of his firm that they "do not think it right to control the effect of their medicines on man by experiments on the lower animals." [1] This does not seem to be the general or progressive view of the question, however, and Hatcher [2] has recently stated that by the use of a lethal dose method for cats assays may be made with an error of only 3 per cent.

Objections have been made specifically against the use of the frog for making assays of the digitalis group usually on the ground that the frog is notoriously variable in its reaction to the influence of the digitalis group. It seems, however, that the variability of frogs kept under proper conditions has been very much overstated. At least after a rather extended experience with one species, Rana pipiens, I am inclined to lay less stress upon the variation due to age, sex, size, and season than have certain other workers. The variability of their results have been ascribed to variation in the reaction of the frogs used whereas a number of other easily applied precautions, such as are necessary in any quantitative study, would possibly have yielded much more uniform results.

Frogs for assay purposes should be *healthy* and no difficulty has been experienced in maintaining such a condition. If the storage tanks be kept at a suitable temperature, 10° to 15° centigrade, frogs will live for months with absolutely no care excepting that they be supplied with running water. The temperature is a primary and important essential, however, as at the ordinary summer heat at

[1] Journal Am. Med. Ass., 1908, **51**, 2133.

[2] Hatcher: Journal Am. Med. Ass., 1910, **54**, 1050; also Am. Jour. of Pharm., 1910, **82**, 360.

this latitude it is not an unusual experience to remove daily from 1(
to 20 per cent of dead frogs from the tanks. At 10° to 15° the deatl
of a frog is a rare occurrence.

A second precaution in using frogs as assay animals is that the
experiments should be carried out at the same temperature. On
account of the fact that ordinary room temperature is most easily
maintained in the operating room all assays are carried on at 22° C
A slightly higher temperature might not be inconsistent with accu-
racy, but on account of the easy susceptibility of frogs to heat
preferably lower temperatures rather than higher should be used.

A simple apparatus makes constant temperatures easily possible.
At this laboratory a large galvanized-iron pan is kept partly filled
with water which is heated or cooled as is necessary and in this are
placed the small cages also made of galvanized iron containing the
frogs.

While a difference in the weight of the frogs does not seem to be a
cause of variability, it is probably much better to use frogs of medium
weight, 20 to 35 grams, rather than very small or very large frogs.
The dose should then be computed per gram of body weight.

The amount of liquid injected should bear some direct relation to
the size of the frog, and it is the custom not to use more than 0.015
c. c. per gram of body weight. The unknown should be so diluted
or concentrated that approximately such a dose may be given. On
this account, in making assays, of the crude drug, pharmacopœial
tinctures are made up rather than the more easily prepared official
infusion. The strength of the tincture is usually such that neither
much dilution nor concentration is necessary while the infusion is so
much less active that either large doses are required or concentration
by the use of heat or more slowly at room temperature is necessary.

The question of absorption is very important and in all cases where
it is incomplete the frog should be rejected as unfit for the assay. The
lymph sac should be entirely empty of all but traces of fluid and close
adherance to this rule is absolutely essential in securing accurate
results.

Just what conditions predispose to delayed absorption has not been
determined. It is fairly apparent, however, that frogs which are not
healthy are apt to show little absorption. This has been especially
noted in apparently healthy frogs taken from a lot, part of which were
dying with the so-called "red leg disease." Not only does this disease
fail to appear, however, but the general health and absorption of the
animals is apparently much better if a temperature of 10° to 15° C.
is maintained for the storage tanks.

While thus expressing my belief in the frog as a suitable and,
despite certain elemental precautions, a convenient animal for assay
purposes it has never been shown to what degree biological assays,
using either frogs or other animals, were accurate and especially as

showing the therapeutic potency of a given preparation. It is generally conceded, however, that the therapeutic efficiency of digitalis depends largely on its contained digitoxin, digitalin, and digitalein, of strophanthus on the presence of strophanthin, and of convallaria on the presence of convallamarin and that with increased amounts of these substances would come increased therapeutic effects in a direct proportion. It is granted, however, that the action might possibly be modified to a slight degree by the presence in the crude drug of organic acids, resins, and chiefly by the saponin like body, digitonin, as affecting their absorption from the alimentary tract. Nevertheless if by the use of biological methods these so-called pure principles could be assayed quantitatively and it were shown that two grams would have double the effect on frogs or other animals of half that amount the therapeutic effect might be expressed in terms of biological activity. Further, if combinations of two of these substances as in French and German digitalin showed by these tests merely a summation action the question of variation in the proportion of these substances would not alter the therapeutic value as shown by biological assay methods.

To this end a number of the so-called pure principles of digitalis, strophanthus, and convallaria were assayed according to the one hour frog heart method to determine their biologic activity. Stock solutions of crystalline digitoxin, French digitalin, German digitalin, digitalein, strophanthin, and convallamarin were made up using in all cases 25 per cent alcohol as a solvent on account of the insolubility of digitoxin and French digitalin in water, thus making possible comparisons of the biological activity of the various principles.

Digitoxin.—The assay of digitoxin was carried out in the manner already described using frogs of the same species (Rana pipiens) in this and in all subsequent assays of the other active principles which were kept under the same conditions both in the storage tanks and in the operating room. Ten milligrams of crystalline digitoxin were dissolved in 25 c. c. of 50 per cent alcohol and after complete solution was effected water was added to make 50 c. c. thus diluting the alcohol to 25 per cent.

The results of this assay are shown in the following table. The dose in this and all subsequent tables is given in grams per gram of body weight.

Weight of frog in grams.	Digitoxin dose.	Result.	Remarks.
28	0.000006	−	Beats.
27	0.000007	−	Do.
31	0.000007	−	Do.
38	0.000008	−	Do.
31	0.000008	+	Beats on stimulation.
26	0.000009	+	Past end.
32	0.000009	+	Do.
29	0.000010	+	No beat on stimulation.
36	0.000010	−[1]	Nonabsorption.

[1] The absorption of the injected drug should be complete in all cases to make the result of value. In case it is incomplete, note should always be made of the fact as many variations in results are to be thus explained.

From the above assay it was estimated that the least dose producing systole of the heart in one hour was approximately 0.0000085 grams per gram weight. It is of interest to note in this connection also that Famulener and Lyons [1] using approximately the same method and a digitoxin made by the same firm reported an assay value of 0.00034 gram per 40 grams (standard) frog or 0.0000085 grams per gram body weight.

Having thus determined the activity of the sample of digitoxin a chemist in the Division of Pharmacology was asked to prepare unknown solutions for examination.[2] Three such were made by diluting the stock solution used in the preliminary assay. The results of the assay of sample No. 1 were as follows:

Weight of frog in grams.	Dose.[1]	Result.	Remarks.
29	0.000008	−	Beats.
26	0.000010	−	Do.
35	0.000010	−	Do.
26	0.000011	+	Beats on stimulation.
32	0.000011	+	Do.
32	0.000012	+	No beat on stimulation.
25	0.000013	−	Nonabsorption.

[1] As the amount of drug per cubic centimeter was unknown, it was necessary to figure all doses on the basis of the milligrams per c. c. of the stock solution and then determine the amount of drug in the unknown by the ratio thus obtained between the two solutions.

From the above results the assay value was estimated at 0.000011 gram per gram of body weight. The ratio between this and the known solution therefore was 11 to 8.5, an inverse ratio since the larger dose represented the weaker solution. Figured in per cent therefore on the basis that 8.5 = 100 per cent according to the inverse proportion, 8.5:11:×:100 = 77.2 per cent. The unknown was 70 per cent of the known, thus indicating an error of 7.2 per cent.

A second unknown was assayed in like manner, giving the following results:

Weight of frog in grams.	Dose.[1]	Result.	Remarks.
26	0.000,009	−	Beats.
24	0.000,010	−	Do.
27	0.000,011	−	No beat; diastole.
30	0.000,0115	+	Corner base ventricle beats.
28	0.000,012	+	Beats on stimulation; systole.
28	0.000,013	+	No beat on stimulation.

[1] In terms of digitoxin in stock solution.

Judging from these results, the assay value of the unknown was estimated to be approximately 0.000,0115. The inverse ratio of the

[1] Famulener and Lyons: Proc. Am. Pharm. Ass., 1902, **50**, 415.

[2] In order that no bias should enter into the determination of the amount of drug in solution, the per cent of the stock solutions or the amount by weight were only learned upon reporting the result of the biological assay to the chemist who made up the solutions.

unknown to the stock solution, therefore, was 11.5 to 8.5, the percentage ratio according to the inverse proportion $8.5 \times 11.5 :: \times : 100$, being 100 to 73.8 per cent. The unknown being by weight 70 per cent of the known, the results show an error of 3.8 per cent.

A third unknown was assayed. This solution required 0.000,0095 in terms of the stock solution to produce the end reaction. The inverse ratio, therefore, was as 8.5 to 9.5, or in terms of per cent according to the inverse proportion, 89.3 per cent. As the unknown represented 90 per cent of the stock solution, the error was 0.7 per cent.

No estimations of unknown solutions made up by weight directly were carried out.

French digitalin.—This so-called pure principle is a mixture of glucosides, consisting mainly of digitalin verum, was also assayed to determine its comparative biologic value. Twenty-five per cent alcohol was used as a solvent. The results of this assay are given in the following table:

Weight of frog in grams.	Dose.[1]	Result.	Remarks.
32	0.000,010	−	Beats.
35	0.000,011	−	Do.
34	0.000,012	−	Do.
32	0.000,013	+	Beats on stimulation.
26	0.000,013	+	Systole corner base beats.
31	0.000,014	−	Nonabsorption.
25	0.000,015	+	No beat on stimulation.
26	0.000,016	+	Past end.

[1] Dose represents amount of digitalin per gram body weight.

From the above results it was estimated that the least dose necessary to produce systole in one hour was approximately 0.000,013 gram per gram body weight.

Famulener and Lyons's assay of a French digitalin, made eight years earlier, gave a value of 0.000,015 gram per gram of body weight.

An unknown solution made up from the above stock solution was assayed. The results were as follows:

Weight of frog in grams.	Digitalin dose.	Result.	Remarks.
23	0.000,013	−	Heart beats.
26	0.000,015	−	Do.
31	0.000,016	−	Do.
41	0.000,016	−	Stopped; diastole.
38	0.000,017	+	Corner base beats.
31	0.000,017	+	Beats on stimulation.
20	0.000,018	+	No beat on stimulation.
25	0.000,020	+	Do.

From the above results, the amount per gram of body weight required to produce systolic stoppage of the heart was estimated, in terms of the stock solution, at 0.000,017 in inverse ratio. The per-

centage of the unknown of the stock solution according to the inverse proportion 13:17:: × :100, was 76.4. The unknown was 75 per cent, hence an error of 1.6 per cent.

Another unknown solution was made up, using a weighed amount of digitalin (French) dissolved in 25 per cent alcohol. The dosage was figured on the basis that 1 c. c. equaled 1 milligram. The results were as follows:

Weight of frog in grams.	Digitalin dose.	Result.	Remarks.
22	0.000,0025	−	Heart beats.
23	0.000,0030	−	Do.
23	0.000,0035	+	Auricle beats; faint wave in ventricle.
24	0.000,0040	+	Beats on stimulation.
21	0.000,0050	+	Wave on stimulation.
20	0.000,0060	+	No beat on stimulation.

It was concluded from these results that the end reaction was produced by 0.000,0035 gram per gram weight. This was 3.71 times stronger, however, than the original stock solution (0.000,013 gram), figured on the basis that 1 c. c. equaled 1 milligram. Hence, the unknown was estimated to contain 3.71 milligrams per c. c. The actual amount by weight was 3.86 milligrams, hence an error of 3.8 per cent.

A third unknown, made up as the above from a weighed amount of the digitalin, gave a value of 0.000,0065 gram per gram weight, or twice as strong as the original stock solution assay. On the basis that 1 c. c. equaled 1 milligram, the unknown was therefore estimated to contain 2 milligrams per c. c. The amount by weight was 1.93 milligrams, or an error of 3.6 per cent.

German digitalin, a mixture of glucosides consisting largely of digitonin but also of some digitalin verum, was assayed to determine its activity on the frog's heart. The solvent was 25 per cent alcohol, thus making comparisons with the assay values of the insoluble (in water) digitoxin and French digitalin possible. The results of this assay were as follows:

Weight of frog in grams.	Dose.	Result.	Remarks.
27	0.000,02	−	Beats.
38	0.000,04	−	Do.
20	0.000,06	−	Do.
47	0.000,06	−	Do.
43	0.000,07	+	Beats on stimulation.
52	0.000,08	+	No beat on stimulation.
41	0.000,09	+	Do.

From the results as recorded above, the amount of German digitalin necessary to produce permanent systole was estimated at 0.000,07

gram per gram of frog weight. Famulener and Lyons,[1] using a German digitalin of a different manufacturer, reported a considerably smaller dose (0.000,0225 gram per gram weight) as sufficient to bring on the end reaction. On account of the wide variations from their results, a second assay was made to eliminate any possible error. In this case the activity was found to be the same as in the previous assay. The results were as follows:

Frog weight in grams.	Dose.	Result.	Remarks.
29	0.000,04	−	Beats.
20	0.000,05	−	Do.
32	0.000,06	−	Do.
26	0.000,07	+	Beats on stimulation.
16	0.000,07	+	Do.
30	0.000,08	+	No beat on stimulation.

Having thus established the amount of German digitalin necessary to produce the end reaction unknown solutions were assayed. Solution No. 1 assayed as follows:

Frog weight in grams.	Dose.	Result.	Remarks.
35	0.000,07	−	Beats.
25	0.000,07	−	Do.
48	0.000,08	+	Beats on stimulation.
21	0.000,08	+	Do.
43	0.000,09	−	Nonabsorption.
21	0.000,09	+	No beat on stimulation.

From these results the amount of the unknown necessary to produce systolic stoppage of the frog's heart was estimated at 0.000,08 or in terms of the stock solution an inverse ratio of 8 to 7. The percentage of the unknown in terms of the known solution therefore equaled (8:7::100:×) 87.5. The unknown was 90 per cent of the stock solution or an error of 2.5 per cent.

A second unknown made up by dissolving a weighed amount of digitalin (German) in 25 per cent alcohol assayed as follows:

Weight of frog in grams.	Digitalin dose.	Result.	Remarks.
19	0.000,030	−	Heart beats.
22	0.000,040	−	Nonabsorption.
20	0.000,045	−	Heart beats.
19	0.000,045	+	Wave in ventricle; auricle beats.
19	0.000,050	−	Heart beats; near end.
20	0.000,0525	−	Do.
20	0.000,055	+	Wave in ventricle on stimulation.
22	0.000,060	+	Beats on stimulation.
21	0.000,065	+	Do.

[1] Famulener and Lyons: Proc. Am. Pharm. Ass., 1902, **50**, 415.

As will be noted certain irregularities occur in this table, so that a little care was necessary in deciding upon that amount required to produce the typical end reaction. 0.000,055 gram was decided upon as the amount when figured on the basis that 1 c. c. = 4 milligrams. The original assay value of the stock solution had shown 0.000,07 gram per gram of body weight to be the amount required. Hence, $0.000,07 \div 0.000,055 \times 4$ gave 5.08 milligrams per c. c. of the unknown. The solutions contained 5.23 milligrams per c. c. or an error therefore of 2.8 per cent.

A third unknown containing 5.86 milligrams German digitalin per c. c. assayed 6.2 milligrams, an error of 5.9 per cent.

A fourth unknown containing 2 milligrams German digitalin per c. c. assayed 2 milligrams, an error represented by 0 per cent.

Digitalein.—Digitalein is generally regarded as a definite compound which occurs along with digitoxin and digitalin in digitalis leaves. It is easily soluble in water, but to make comparisons possible it was also dissolved in 25 per cent alcohol preliminary to its assay. The results were as follows:

Weight of frog in grams.	Dose.	Result.	Remarks.
22	0.000,021	−	Beats.
22	0.000,022	−	Do.
21	0.000,023	−	Half ventricle beats.
22	0.000,024	+	Beats on stimulation.
20	0.000,025	+	Do.
19	0.000,026	−	Nonabsorption.
21	0.000,026	+	Beats on stimulation.
17	0.000,028	−	Nonabsorption.

From these results the amount necessary to cause systolic stoppage of the heart was estimated at 0.000,024 gram per gram of body weight.[1]

Unknown solutions of digitalein were then made up. Solution No. 1 assayed as follows:

Weight of frog in grams.	Dose.	Result.	Remarks.
16	0.000,024	−	Near end.
16	0.000,025	−	Diastole; beat on stimulation.
15	0.000,026	+	Beat on stimulation.
14	0.000,027	−	Nonabsorption.
17	0.000,028	+	No beat on stimulation.
19	0.000,032	+	Do.

From these results the amount necessary to bring on the end reaction was estimated at 0.000,026 gram per gram weight on the assumption that each c. c. of the unknown represented 3 milligrams of drug. Hence $0.000,024 \div 0.000,026 \times 3$ gave 2.769 milligrams per c. c. or an error of 4.5 per cent.

[1] Famulener and Lyons (loc. cit.) gave an assay value of 0.000,0325 for digitalein.

In a second unknown in which the frogs reacted very irregularly it was finally decided, after using 15 frogs, that 0.000,024 gram per gram weight was the amount required to produce the end reaction. In estimating dosage each c. c. of the solution was estimated to contain 3 milligrams of the drug, hence 0.000,024 ÷ 0.000,024 × 3 gave 3 milligrams per c. c. By weight the amount was 2.89 milligrams, hence an error of 3.8 per cent.

A third solution made up from a stock solution to 70 per cent strength assayed 68.5 per cent.

A fourth unknown, also 70 per cent of the stock solution, assayed 64 per cent.

Digitalein proved to be the most difficult of all the digitalis principles to assay. With this drug considerable variation was noted in the absorption, so that the exactly necessary amount was somewhat difficult to determine. In this connection it is to be noted also that an assay made a year previously gave a value of 0.000,026 to 0.000,028. The above unknowns were estimated on the basis of 0.000,028 at first, No. 1 showing an error of 11.3+ per cent; No. 2, 21+ per cent; No. 3, 10+ per cent, and No. 4, 5.8+ per cent.

Strophanthin.—The strophanthin used in the assay experiments which follow was a Merck product, probably derived from strophanthus Kombe. A solution made up with 25 per cent alcohol, 0.5 milligrams to the c. c., assayed as follows:

Weight of frog in grams.	Strophanthin dose.	Result.	Remarks.
12	0.000,000,6	−	Beats.
12	0.000,000,8	−	Do.
17	0.000,000,9	−	Do.
18	0.000,001,0	−	Occasional beat.
19	0.000,001,1	+	Beats on stimulation.
11	0.000,001,1	+	Do.
11	0.000,001,2	+	Do.
10	0.000,001,3	+	No beat on stimulation.

From the results recorded in the above table 0.000,001,1 was estimated to be the assay value for this strophanthin. This value is slightly more than double than that given by Famulener and Lyons,[1] who found 0.000.000,5 gram per gram of body weight necessary to produce the end reaction according to the same method. As it is generally believed by pharmacologists[2] that strophanthin Kombe is of only about half the activity of stophanthin crystalline (ouabaine) the above results suggest at once that Famulener and Lyons probably worked with the stronger product.

It is important to point out here the danger from the clinical use of strophanthin as the most serious result could only result if ouabaine

[1] Famulener and Lyons: Proc. Am. Pharm. Ass. 1902, **50**, 415.

[2] Hatcher: Jour. Am. Med. Ass. 1910, **54**, 1050.

were substituted for the amorphous strophanthin Kombe; such danger could readily be obviated by the use of physiological tests.

Unknown solutions containing strophanthin dissolved in 25 per cent alcohol were assayed basing the dose in c. c. upon the arbitrary assumption that each c. c. contained 0.04 milligram. Solution No. 1 gave an assay value of 0.000,001,4 gram per gram of body weight. As the known assay value for this strophanthin was 0.000,001,1 the unknown equaled 11/14 of 0.04 milligram or 0.0314 milligram per c. c. The amount by weight was 0.032 milligram, hence an error of 1.8 per cent.

A second unknown was assayed giving a value of 0.000,001,7, hence was 11/17 of the assumed amount. 11/17 of 0.04 milligram equals 0.02588 milligram. The amount by weight was 0.02666 milligram, per c. c., or an error of 2.9 per cent.

Convallamarin.—This glucoside is found in convallaria and is recommended in the same general class of diseased conditions as digitalis. A solution containing a definite amount by weight in 25 per cent alcohol assayed as follows:

Weight of frog in grams.	Convallamarin dose.	Result.	Remarks.
26	[1] 0.000,004,25	−	Beats.
27	0.000,004,50	−	Beats (near end).
26	0.000,004,50	−	Beats.
25	0.000,004,75	−	Do.
27	0.000,004,75	+	No beat on stimulation.
21	0.000,005,00	+	Do.
26	0.000,005,25	−	Nonabsorption.
26	0.000,005,50	+	No beat on stimulation.

[1] The approximate dose had been figured out in experiments carried on at another time. It would have been necessary to have given doses of greater range if the approximate dose had been undetermined.

From these results the amount of convallamarin necessary to produce systole of the frog's heart was estimated at 0.000,004,75 gram per gram weight.

Unknown solutions of convallamarin in 25 per cent alcohol were made up and assayed. Solution No. 1 gave a value of 0.000,005,5 gram per gram of body weight when based on the arbitrary assumption that 1 c. c. = 1.25 milligrams. Hence the value of the known 0.000,004,75 ÷ 0.000,005,5, the value of the unknown, multiplied by 1.25 represents the amount of drug per c. c. of the unknown, or 1.078 milligrams. The amount by weight was 1.18, hence an error of 8.6 per cent.

A second unknown assayed 0.000,007,5 on the basis that 1 c. c. of the solution contained 0.5 milligram of the drug. Hence 0.000,004,75 ÷ 0.000,055 × 0.5 milligram equals 0.3165 milligram of convallamarin per c. c. of the unknown. The amount by weight was 0.354 milligram per c. c., hence an error of 10.6 per cent.

The results of the above assays are grouped together in the following table not only as a means of comparing the activities of the various so-called pure principles but also to show, in tabular form, the degree of error which is to be expected when using a frog-heart method of assay.

Drug.	1910.	1902.[1]	Unknown solutions; per cent error.			
			No. 1.	No. 2.	No. 3.	No. 4.
Strophanthin (Merck)	0.000,001.10	0.000,00,05	1.8	2.9	.....	.....
Convallamarin (Merck)	0.000,004,75	0.000,004,5	8.6	10.6	.....	.....
Digitoxin (Merck)	0.000,008,50	0.000,008,5	7.2	3.8	0.7	.....
French digitalin (Merck)	0.000,013,00	0.000,015	1.6	3.8	3.6	.....
Digitalein (Merck)	0.000,024,00	0.000,032,5	4.5	3.8	6.0	1.5
German digitalin (Grübler)	0.000,070,00	0.000,022,5	[2] (11.3)	(21)	(10)	(5.8)
			2.5	2.8	5.9	0.0

[1] Famulener & Lyons.
[2] Error in per cent as first reported. End dose figured as 0.000,028 on an assay which gave a value of 0.000,026 to 0.000,028. Later 0.000,024 was found to be a more nearly correct value.

From this summary it is apparent that strophanthin is the most active, the German digitalin (Digitalin purum Grübler) is the weakest, and that digitoxin, the most active of the digitalis glucosides, is about eight times less active than amorphous strophanthin. It is not urged on the basis of these experiments, however, that in the clinical use of these substances doses be given proportionate to these values. But in so far as secondary factors, such as rate of absorption and elimination, do not interfere proportionate doses would probably give like therapeutic results. Elemental tissues, such as the heart muscle, no matter from what animal species, react qualitatively the same. As pointed out by Cushny [1] the action of the digitalis group upon the frog's heart is strictly analogous to the action on the mammalian heart, with differences in action which give the three distinct stages of action in mammals dependent upon a greater vagus activity in the higher animal species. In the therapeutic use of the drug only the first stage, which consists of a minimal activity of the vagi and a definitely increased activity of the muscle substance itself, is desired. The action on the frog is almost purely a muscle action and it is upon such grounds that biological assay methods on the lower forms of animal life are believed to give results which are capable of direct utilization in therapeutics.

On theoretical grounds, therefore, there is no reason why assays using frogs will not give results which may be depended on by clinician and which may be used as a basis for dosage as safely as if such assays were carried out on guinea pigs, rabbits, cats, or dogs, and so far as the evidences of accuracy and totality of action go, far more safely than if carried out by estimating the single active con-

[1] Cushny: Pharmacology and Therapeutics, 1906, p. 466.

stituent digitoxin by chemical means. Assays, as is to be noted by the above summary, may be made using frogs (possibly also other animals) which will give an estimate of the amount of glucosides in a solution to within a few (1 to 10) per cent of absolute accuracy. Therefore if definite amounts by weight of the glucosides of the digitalis group may be depended on to produce definite and proportionate clinical results, the results of the frog heart method of assay as outlined above will indicate the amount of an unknown solution of such glucosides which will produce a definite and proportionate effect in man, and this, too, whether it be a simple alcoholic solution of the glucoside or the cruder galenical preparation.

In all cases the unknown factors of absorption, elimination, and individual susceptibility will persist in spite of all attempts at assaying the preparations of the digitalis group. But these factors would also exist in like degree as long as several differently active bodies and resinous substances are to be reckoned with, even if absolute chemical assays of not one but all the active glucosides of the group were possible. The frog method of assay gives all any assay method can possibly give; that is, a definite index of the amount of active substances, and what is especially important an index of the activity of *all substances* in the preparation which act upon the heart.

Certain factors regarding the reaction of frogs to the digitalis group of glucosides, or to other poisons, are of little importance aside from increasing to a very slight degree the complexity of the assay process. The whole question resolves itself into using a digitalis preparation of known activity and keeping quality (and for this purpose crystallized digitoxin or ouabain is suggested) to standardize the frogs at the same time that the unknown is assayed. In this way accurate results may be obtained without reference to season, age, sex, temperature conditions, or species of frogs used.

CRUDE DIGITALIS.

Having thus proven this method of frog assay to be reasonably accurate, an investigation was undertaken to determine the factors which affected the potency of crude digitalis.

It has been repeatedly stated that only second-year digitalis leaves should be used, and in recognition of this the pharmacopœias of Belgium, Switzerland, Japan, Austria, France, Germany, Holland, etc., all prescribe leaves of the second year's growth to be gathered at the time of flowering. It is difficult to trace the origin of this belief in the greater value of digitalis leaves of the second year's growth, but it is quite old and antidates any attempt to determine the activity of this drug on animals. Thus Tordes [1] in 1867 accounted

[1] Tordes: Gaz. med. de Strasb., 1867, 27, 191.

for the greater activity of the digitalis at Strassburg partly on the ground that second-year leaves were used. Focke[1] reported, however, when compared with digitalis leaves of the second year's growth gathered at the time of seeding that the first-year leaves were about 20 per cent more active, although at the time of flowering the order was reversed. This is ascribed to the energy used up in the formation of the seeds, but it seems somewhat strange that a poisonous constituent stored up in the leaves should decrease unless the digitalis glucosides act as plant food. Hart, in England, reported on the assay of one sample of first-year leaves which gave a value about 20 per cent higher than second-year leaves gathered at the time of flowering.

The experiments in this paper relate to three lots of first-year leaves, all of which were garden grown. One lot was grown at Madison, Wis., in 1908, and two in the Government drug garden at Arlington, Va., one lot of the 1907 harvest, the other of the harvest of 1909. In the early part of 1910 each lot of leaves was powdered to a number 60 powder and then made up into tinctures according to the U. S. P. VIII. The results of their assays were as follows:

Arlington, 1907, first-year leaves made up into a 10 per cent tincture according to the U. S. P. VIII.

Frog weight, in grams.	Dose per gram.	Result.	Remarks.
	c. c.		
18	0.003	−	Beats.
18	0.004	−	Do.
16	0.005	+	Slight wave in ventricle.
18	0.006	+	Auricle beats; not ventricle.
16	0.009	+	No beat.
18	0.012	+	Do.

Wisconsin, 1908, first-year leaves made up into a 10 per cent tincture according to the U. S. P. VIII.

Weight of frog, in grams.	Dose per gram.	Result.	Remarks.
	c. c.		
15	0.004	−	Beats.
16	0.005	−	Do.
32	0.005	−	Do.
34	0.005	+	No beat on stimulation.
31	0.006	+	Do.
32	0.007	+	Beats on stimulation.
26	0.009	+	No beat on stimulation.

[1] Focke: Arch d. Pharm., 1903, **241**, 128.

Arlington, 1909, first-year leaves made up into a 10 per cent tincture according to th U. S. P. VIII.

Weight of frog, in grams.	Dose per gram.	Result.	Remarks.
	c. c.		
17	0.003	−	
19	0.004	−	
16	0.005	−	Nonabsorption.
15	0.005	+	Spontaneous wave in base ventricle.
18	0.006	+	No beat on stimulation.
17	0.009	+	Do.
14	0.012	+	Do.

The dose per gram of body weight to produce the end reaction fo Arlington, 1907, was estimated at 0.005 c. c., for Wisconsin, 1908, a 0.0055 c. c., and for Arlington, 1909, at 0.005 c. c

For the purpose of comparison a tincture was made up at the sam time from selected English leaves of the second year's growth. The dose to produce the end reaction was estimated at 0.007 c. c., pe gram of body weight. In terms of per cent the first-year leaves grow at the Government drug farm were 40 per cent more active, the first-year leaves from Wisconsin were 28 per cent more active. As further proof of the high potency of these lots of first-year leaves it is also of interest to note that in only one other instance in the assay of commercial preparations has a tincture been found which showed as high values as these.[1]

In connection with these assays a further interesting fact is to be noted, namely, that the first-year leaves were all garden grown. The often-repeated statement that wild-growing plants furnish leaves of higher potency than leaves grown in gardens probably is true in many instances, but it seems doubtful if this alone has anything whatsoever to do with high activity. Nevertheless, the French Codex and the German Pharmacopœia make only wild-growing leaves official, and Focke,[2] among the recent investigators, found cultivated leaves only half as active as those from wild plants.

In further proof of the high potency of cultivated digitalis leaves, it is worth while noting that Allen's English leaves, which give high assay values as compared with a number of assays of tinctures made from leaves of unknown origin, are garden grown. However, it is recognized that the assay of five lots of cultivated leaves is not a sufficiently extensive investigation to prove invariably higher potency to carefully cultivated leaves. But it is contended from these assays that the question of cultivation by itself probably is of little importance.

[1] Recently garden-grown first-year leaves and wild-growing second-year leaves from Seattle, Wash., were examined. The first-year leaves assayed 0.006; the second-year leaves, 0.0085.

[2] Focke: Arch. d. Pharm., 1903, **241**, 128.

A point upon which practically all observers agree in securing digi-alis leaves of high value relates to the manner of drying. Hamilton [1] as early as 1807 from clinical observations warned physicians against mproperly dried leaves. This was more accurately established later by more exact observations on animals, and Focke [2] has especially nvestigated this point in this way. According to his observations he leaves should be dried within three days of gathering to a moisture ontent of 1.5 per cent. When thus dried and stored in air-tight con-ainers the loss in activity from year to year is practically negligible.

In the experiments carried out to determine the effect of storing in ordinary containers on the activity of digitalis, exact conclusions are mpossible owing to the fact that no assays were made of the several ots examined at the time of gathering. One lot had been stored in paper bag for eight years, a second lot was stored in a cloth bag for period of three years, a third lot was stored in a paper bag for two ears, and a fourth lot in a cloth bag for one year. The moisture con-ent was estimated at the time of making the assays. The results of hese assays are given in the following table, together with an assay f select English leaves which was assayed five months after being arvested:

Preparation.	Moisture in, per cent.	Amount of tincture per gram of frog weight.
ight-year-old leaves	9.1	0.007,5
hree-year-old leaves	5.8	0.005
wo-year-old leaves	7.8	0.005,5
ne-year-old leaves	9.4	0.005
nglish leaves	7.3	0.007

From this table it will be noted that all the different lots of leaves ad a high value and three of them a higher value than the control reparation examined in less than six months following the harvest. s is to be noted, the moisture content in all cases is much in excess of he amount advised by Focke. Thus there does not seem to be ecessarily any marked deterioration because of an ordinary amount f moisture.

If sufficient moisture be present to cause molding, however, there s a marked deterioration with age. Focke assayed a preparation hich gave a value of 4.36, but a year later, having become moldy, ave a value of only 1.6.

Only one sample of moldy leaves was examined. In August, 909, this assayed, when made into a tincture, 0.007 per gram of frog eight. A second assay, made April, 1910, gave a value of 0.012, or

[1] Hamilton: Observations on Fox Glove, 1807, p. 7.
[2] Focke: Archiv. d. Pharm., 1903, **241**, 128.

a loss in activity of approximately 90 per cent. In conclusion, therefore, it seems probable that careful and prompt drying is essential, but that is is not necessary to reduce the moisture content to as low as 1.5 per cent.

In this connection the use of heat as an aid to the drying process is an important consideration. Wolff [1] recommends vacuum drying; Focke,[2] drying with the use of heat up to 80° C. Hart,[3] on the other hand, found that high temperatures in drying reduced the activity of the leaves about 25 per cent.

To determine what effect heating would have on ordinarily dried digitalis leaves, several small lots from the same stock were submitted to temperatures ranging from 80° to 140° C. for two hours. These were then made up into tinctures and assayed. The results are given in the following table:

Series.	No heat.	Temperature.			
		80°	100°	120°	140°
I	0.009	0.009	0.010	0.009	0.014
II	0.0075	0.008	0.007	0.008	0.013
III	.....	0.009	0.008	.....	.....

Some variability is to be noted in the above table, which is probably due to differences in percolation. This is especially apparent in the column designated "no heat," since both tinctures were made from the same lot of leaves to which no heat had been applied. In the other assays the differences might be ascribed to partial deterioration due to the heating, but it seems more likely that it was due to differences in preparing the tinctures.

From this table it is apparent that no special deterioration due to heat occurs from the use of temperatures up to 120°, but that at 140° there is a decided lessening in activity. It seems evident, therefore, that the drying process might be hastened by the use of heat up to 100° without danger of destroying any of the active constituents of the leaves, but that degrees of heat above 100° should not be used.

Another point to be noted is that the use of heat in the above experiments was upon dried leaves, but which contained about 10 per cent of moisture. Whether any deterioration would result from the use of temperatures up to 100° C. in drying the fresh plant is not a possible conclusion on that account, but the probabilities are that the action of heat in such cases would not be different.

The use of heat (100°) in drying the fresh plant has a further very decided advantage, however, in that deterioration from age would probably be materially lessened. At any rate, the general view is that

[1] Wolff: Therap. d. Gegenwart, 1902, 43, 423.
[2] Focke: Arch. der Pharm., 1903, 241, 128.
[3] Hart: Pharm. Jour. and Tr., 1908, 26, 440.

much of the decrease in the activity of digitalis leaves is due to enzyme action, and the use of heat at 100° would be sufficient to destroy most, if not all, enzymes of vegetable origin.[1]

The results of the experiments using heat up to 120° without destroying the activity of the leaves would indicate that Bokay[2] had no real grounds for the introduction of a preparation of digitalis made by macerating the leaves in cold water because of possible deterioration due to the use of boiling water, as in the official infusion. They also indicate that the use of vacuum drying is an entirely unnecessary procedure.

It is generally believed that the stems are less active than the leaves. According to Hart,[3] however, tinctures prepared out of the whole leaves are more active than from leaves without the stalk and midrib.

Experiments were made to determine this point. The stems were sorted from the samples of first-year leaves investigated above and also from a sample of second-year leaves. These were ground to a No. 60 powder and then made up into 10 per cent tinctures according to the general directions of the U. S. P., VIII. The results of their assay and of the controls made from the leaf proper were as follows:

Preparation.	Dose of stem tincture.[1]	Dose of leaf tincture.
1907, first year	0.011	0.0050
1908, first year	0.009	0.0055
1909, first year	0.010	0.0050
Second-year leaves	0.016	0.0075

[1] Dose in cc. per gram weight of frog to produce end reaction.

From this table it will be noted that the stems appear to be only about 50 per cent as active as the leaves, the result supporting, therefore, the general belief in the lesser activity of the stems.

PREPARATIONS OF DIGITALIS.

The variability in crude digitalis necessarily appears in the finished product, so that these vary at least as widely as the crude drug in activity. The use of an assayed crude drug, however, does not insure a finished product of unvarying activity. This is due to a number of factors, chief among which are imperfect extraction of the active glucosides, which may be due to faulty manipulation or to the character of the solvents used. This point has been made by Vanderkleed and Bernegau[4] with reference to a number of official preparations made by various pharmacists from reputed assayed crude drugs, the variation from standard being as much as 250 per cent.

[1] Simon: Physiological Chemistry, 1901, p. 102.
[2] Bokay: Berl. Tierärzt. Wchnschr., 1907, **19**, 382.
[3] Hart: Pharm. Jour. and Tr., 1908, **26**, 440.
[4] Vanderkleed and Bernegau: Proc. Am. Pharm. Ass., 1908, p. 176.

This point was also brought out in the course of this investigation. Three tinctures were prepared from each of two lots of leaves, all the steps in their preparation being carried out in as nearly as possible a similar manner. They assayed as follows:

	Series.	Dose in cc. per gram of frog weight.
English leaves I	I	0.008
	II	0.007
	III	[1] 0.012
English leaves II	I	0.007
	II	0.0075
	III	0.009

[1] No explanation of the wide variation in this result can be offered, but undoubtedly was due to faulty manipulation at some point in the manufacture of the preparation.

The same point is again shown by the fact that a fluid extract made from the same leaves as in the above table (English I) assayed 0.0024, being only about three times instead of ten times as active as the tincture. This result is quite closely in accord also with the results of assays of commercial fluid extracts which have never assayed relatively as active as the commercial tinctures. On the grounds of incomplete extraction of the active principles these facts would indicate that the official fluid extract is not a desirable digitalis preparation. Furthermore, there does not seem to be a very extensive use of the fluid extract as such, but only as a stock solution, from which so-called official tinctures and infusions are made by the retail pharmacists according to the directions given by the manufacturer. Such preparations, however, even by the loosest interpretation, could not be called official or be said to represent them in activity, and the use of the fluid extract in this way should be discouraged.

Menstrua of different alcoholic strength than that official in the U. S. P. VIII are used to a considerable extent, 70 per cent alcohol being proposed in the international convention for the unification of potent remedies for the tincture of digitalis. To test the value of these in extracting the active digitalis glucosides, tinctures were prepared by the use of 35, 50, 70, and 99 per cent alcohol. The method of percolation was made as uniform as possible for the whole series. The finished preparations were then evaporated and the alcohol strength in each case made to 25 per cent, so that with corresponding doses the same amount of alcohol would be injected. The results of the assay of these preparations were as follows:

Leaves used.	Alcoholic strength.	Dose per gram body weight.
English I	35	0.0085.
Do	50	0.007; series II, 0.0075.
Do	70	0.007; series II, 0.0065.
Do	99	0.007.

These results are so nearly similar for all strengths of alcohol that no apparent advantage is to be seen in the use of the stronger alcohol as a menstruum. Two points of advantage, however, are to be found in the use of the stronger alcohol. The most important of these relates to the deterioration of the finished digitalis preparations with age and will be discussed later in the paper.

The other advantage is the reduction by the use of the stronger alcoholic menstruum of the total solids of the finished preparation without loss, or at least material loss, in activity. This is especially the case with the fluid extracts which were found to be precipitated by the addition of 99 per cent alcohol. Thus 5 c. c. of a fluid extract was taken, to which was added 10 c. c. of 99 per cent alcohol. An abundant gummy precipitate resulted, which was separated out by decanting the alcohol. It was then dried at 140° for three hours and weighed. Three fluid extracts were treated in this way with the following results:

Preparation.	Series.	Total solids in 5 c. c., average.	Total precipitate with 99 per cent alcohol.	Average per cent precipitate.
A	I	1.8651	0.6520	36.8
	II		0.6882	
B	I	1.4018	0.5108	37.4
	II		0.5252	
C	I	1.2322	0.4097	33.7
	II		0.4154	

The activity of the precipitate was tested to determine to what extent the active principles were carried down mechanically. The following protocol illustrates this point:

Frog weight, 22 grams. Pithed, heart exposed; precipitate dissolved in water.

230. Heart rate 58.
231. Injected 0.5 c. c.=0.25 c. c. fluid extract.
240. Heart rate 60.
245. Injected 0.5 c. c.=0.25 c. c. fluid extract.
250. Heart rate 54.
300. Heart rate 40.
330. Heart rate 20, irregular—prolonged diastole.
335. Injected 1 c. c.=0.5 c. c. fluid extract.
400. Heart rate 18—systole very complete.
440. Beating —.

From this protocol it is seen that the precipitate although having some digitalis action was very weak, an amount representing 1 c. c. of the fluid extract not producing permanent systole after two hours, although the original extract killed in one hour in a dose for the same sized frog of 0.0529 c. c.

The decanted alcohol from the precipitation experiments was also tested, the alcohol being reduced to 25 per cent to allow of comparisons. The results were as follows:

Preparation.	Original fluid extract, dose per gram weight.	Purified fluid extract, dose per gram weight.
A	0.0024	0.0025
B	0.0020	0.0020

Thus it will be seen that the loss in potency by precipitation of the gums is quite negligible.

The amount of solids in tinctures made by using 50, 70, and 99 per cent alcohol was also determined by evaporating 5 c. c. lots of the several tinctures and drying for three hours at 140°. The results are given in the following table:

Preparation.	Total solids in 5 c. c. in grams.	Per cent of official tincture.
50 per cent alcohol	0.158	100
70 per cent alcohol	0.121	76.6
99 per cent alcohol	0.115	72.7

Thus by the use of the alcohol of the international convention approximately 23 per cent of total solids were gotten rid of and by the use of 99 per cent alcohol 27 per cent, with no loss of activity in the resulting tinctures. The advantage in using a higher alcoholic menstruum, however, appears more especially related to the keeping qualities of the resulting preparations.

Deterioration.—Although differences in manipulation may cause variation in the finished product, the factor of subsequent deterioration is also to be considered. This has been especially noted in the case of the infusion of digitalis, but seems to be true for the alcoholic preparations as well. Houghton and Hamilton [1] have made a series of assays of digitalis preparations covering a period of 6 years. The extract of digitalis (11 samples) lost, during 5 years, 40 per cent of the original activity, or 8 per cent a year. The fluid extract of the U. S. P. VII (8 samples) during a period of 6 years lost 24 per cent, or 4 per cent a year. The fluid extract of the U. S. P. VIII (11 samples) lost, during 3½ years, a total of 35 per cent of the original activity, or 10 per cent a year. The tincture of digitalis (8 samples) during a period of 3 years lost 27 per cent of the original potency, a yearly loss of 9 per cent. Two very important facts are pointed out as a result of these experiments, namely, that the U. S. P. VII

[1] Houghton and Hamilton: Am. Jour. of Pharm., 1909, 81, 461.

samples of the fluid extract were 1.3 times more potent than those of the U. S. P. VIII, and, furthermore, the potency loss was much less in the former, being only 4 per cent as compared with 10 per cent for the U. S. P. VIII preparations. From this fact the conclusion is drawn that the greater percentage of alcohol lessened the deterioration as a result of age.

As has aready been pointed out in this paper, there seems to be no very marked difference, at least in original potency so far as the tinctures are concerned, dependent on the use of alcohol even down to 35 per cent strength. The time these preparations have been made, however, has not been sufficiently long to draw any definite conclusions regarding the rôle the percentage of alcohol plays in preventing subsequent deterioration.

Certain evidence has been established as the result of assaying old preparations of the tincture and fluid extracts of digitalis, which indicate that a high degree of potency may in certain instances be maintained for at least 8 years.

Two tinctures of digitalis made in 1902 with the 70 per cent alcohol of the international convention for the unification of potent remedies were assayed in 1910. One of these made from English leaves required 0.007 c. c., the other made from German leaves 0.0085 c. c. per gram of frog weight to produce an end reaction. These were compared with two tinctures made from English leaves, one made in 1908 and examined in 1910, and a second made in 1909, at which time it was assayed, both of which required 0.007 c. c. per gram of frog weight. These results are given in the following table for convenience in making comparisons:

Preparation.	Age.	Assay value.
1902, English	8 years	0.007
1902, German	do	0.0085
1908, English	3 years	0.007
1909, English	2 weeks	0.007

Whether these results, showing nearly similar potency, indicate an extraordinarily high original potency for the preparations made with 70 per cent alcohol or whether there was very little deterioration with age can not be decided, since no assays were made at the time of manufacture. The results, however, are suggestive and in that degree confirm the statement of Houghton and Hamilton that higher percentages lessen the rate of deterioration.

Further evidence regarding loss of potency with age is furnished by a number of assays of fluid extracts, tinctures, and other preparations of digitalis a year to a year and a half following the original assays. It is recognized that the time was too short to draw very

definite conclusions, yet a number of facts developed which are of interest.

In 1908 a number of assays were made [1] of digitalis preparations obtained on the open market. Part of these same samples were reassayed in 1909, approximately a year later, and all were assayed again in May, 1910, 22 months after the original assay. The results, as in Bulletin 48, are based on the dose, when diluted to tincture strength, sufficient to produce the end reaction. The results were as follows:

Preparation.[2]	Time of assay.			Deterioration, per cent.
	1908.	1909.	1910.	
Mulford	0.016	0.020	0.024	33.3
B., W. & Co.	0.020	.....	0.024	16.6
P. D. & Co.	0.021	0.020	0.023	8.7
N. B. & Co.	0.022	0.022	0.023	4.3
Merrell	0.024	.....	0.028	14.3
H. B. & W.	0.025	.....	0.030	16.7
S. & D.	0.027	.....	0.029	6.9
Lloyd	0.030	.....	0.036	16.7

[2] Burroughs, Wellcome & Co.=B., W. & Co.; Parke, Davis & Co.=P., D. & Co.; Nelson, Baker & Co.=N., B. & Co.; Hance Bro. & White=H. B. & W.; Sharp & Dohme=S. & D.

Part of these figures agree very closely with those in Houghton and Hamilton's investigation. However, a comparison of the column of per cent deterioration at once reveals that the various drugs fall into two general classes. The official fluid extracts of P., D. & Co., N., B. & Co., and S. & D. belong together, showing very little deterioration. The others which, with the exception of H. B. & W. are nonofficial, show a very much more rapid loss of potency, and this is especially to be noted in the case of digitol. This contains, according to the label, only 30 per cent alcohol, and although originally the most active of the nine preparations examined (Bull. 48) it shows the greatest deterioration. The physical appearance also indicated change, there being a fine flocculent precipitate formed during the two years the sample had been stored.

From these results of assays of preparations kept under similar conditions of light, temperature, etc., it would seem clear that manufacturers of special digitalis preparations should submit them to the most rigorous tests before placing them on the market, otherwise the physician would do well to use such preparations as seldom as possible, unless assured of their very recent manufacture. This would necessitate the dating of all such preparations at the time of manufacture and some method of calling such preparations from the market after a certain time.

The importance of this, so far as the patient is concerned, is especially suggested by an assay (previously reported) in which the deteri-

[1] Edmunds and Hale: Bull. 48, U. S. P. H. and M. H. S.

oration was of such a character that the special formula preparation was actually depressant to the circulation. Yet this preparation was obtained from a local jobber upon the order for an original bottle of this drug without specification as to age, manner of storage, etc.

PART II.

SPECIAL DIGITALIS PREPARATIONS.

Tablet triturates.—A large number of comparative tests of the value of commercial digitalis preparations have been made, but usually these have been upon the official drugs. A widely used class of digitalis preparations are the tablet triturates. These are made up to contain a certain amount of the fluid extract or the tincture together with some inert substance as excipient.

To test the potency of these triturates as sold in the open market, an order was placed with a local jobber, and nine different lots from different manufacturers were obtained. These were then dissolved in 25 per cent alcohol as follows: Sixty tablets of 1 minim each were first dissolved in 30 c. c. of the solvent. These were vigorously shaken several times during 24 hours and then filtered. The residue was further washed with 50 per cent alcohol. The second filtrate was then evaporated at about 90° C. to a gummy consistency, taken up with 25 per cent alcohol sufficient to make the total solution from the 60 tablets equal to 36.6 c. c. or a preparation of approximately tincture strength.

Marked differences in the color of the solutions from the different lots of tablets suggested that they might vary widely in potency. The relation of the color index to activity was not constant, however, although as a rule the darker preparations assayed the stronger.

The solutions thus prepared were assayed by the frog heart method and gave the comparative values as recorded in the following table:

Preparation.	Color order.[1]	Dose per gram frog weight.
Betz	8	0.011
Lilly	2	0.014
Stearns	1	0.019
S. and D.[1]	3	0.019
Schmid	7	0.021
Merrell	9	0.026
Ray	4	0.027
Warner	5	0.035
H. B. and W.[2]	6	0.040

[1] Number 1 the darkest; number 9 the lightest color.
[2] S. and D.=Sharpe and Dohme; H. B. and W.=Hance Bro. and White.

A wide variation in the potency of the different preparations is to be noted in the above results, the strongest preparation being about 360 per cent stronger than the weakest preparation. This corre-

sponds to the variation discovered by Edmunds[1] in an examination of seventeen official tinctures of digitalis and to the variations found by Edmunds and Hale[2] in an examination of official fluid extracts and preparations made up according to special formula. It indicates that the tablet triturates, although varying in activity so widely that serious harm could easily result from such variation, are no worse than the official or special preparations in this regard.

Hypodermic tablets.—A further study was made of the hypodermic tablets on the market sold under the name of the loosely used term digitalin. As this might mean either the true digitalin of Kiliani or the comparatively inactive German digitalin, although more probably the latter on account of its easy solubility in water, it was thought that wide differences in potency might be found.

The hypodermic tablets were purchased as were the tablet triturates through a local pharmacist. To prepare them for injection they were dissolved as follows: Fifty tablets of $\frac{1}{60}$ grain each, placed in stoppered cylinders, were shaken up with 10 c. c. of 25 per cent alcohol. After sedimentation the clear portion was passed through a small filter, the residue being further washed with 5 c. c. and, after decanting the clear portion, with a second 5 c. c. of 25 per cent alcohol. The residue was then shaken up with a small portion of 50 per cent alcohol, filtered and the filtrate evaporated nearly to dryness after which it was added together with sufficient alcohol to make 20 c. c., the solution corresponding therefore to approximately 2.5 mg. per c. c. Differences in the physical appearance of the solutions were noted, being as a rule slightly opalescent, but one was absolutely and another comparatively clear.

These solutions were then assayed on frogs and gave results as follows:

Preparation.	No. of frogs used.	Dose per gram frog weight.	Character of solution.
		Gram.	
Betz	13	0.000,020	Clear.
S. and D	17	0.000,025	Cloudy.
P. D. & Co	12	0.000,025	Do.
Mulford	13	0.000,032	Do.
Schieffelin	13	0.000,045	Do.
Wyeth	10	0.000,046	Almost clear.

These results indicate that there is considerable variation in the activity of preparations of digitalin as sold for hypodermic use.

The experiments were in some measure unsatisfactory, however, as in many instances the absorption was incomplete, thus causing some difficulty in arriving at a definite end reaction. This is illustrated by the unusually large number of frogs necessary in making

[1] Edmunds: Jour. Am. Med. Ass., 1907, 48, 1744.
[2] Edmunds and Hale: Bulletin 48, U. S. P. H. and M. H. S., p. 44.

these determinations. The same difficulty was also found in working with German digitalin in the experiments with the so-called pure principles and makes the frog method somewhat unsatisfactory for this substance. In contrast digitoxin (as are the other related glucosides), though adjudged as the least soluble of the pure principles, seems to be readily absorbed, and the same is true also of the crude preparations with the exception of the infusion.

Fluid glycerate of digitalis.—Although this extract of digitalis is distinctive from the official fluid extract only in the menstruum employed, it was thought best not to take it up in connection with the discussion of menstrua, page 32, as it so clearly is a wide departure from the usual digitalis preparations.

Beringer,[1] in view of the solvent properties of glycerin for alkaloids and glucosides and the lack of solubility of resins, fats, and fixed oils, proposed this substance as a solvent in preparing fluid glycerates of various drugs. These preparations are made up so that 1 c. c. of the finished product was obtained from 1 gram of crude drug. Various percentages of glycerin were used but finally 50 per cent strength in water was adopted as being most conducive to good keeping qualities.

Among the preparations suggested to be made up with 50 per cent glycerin as menstruum is digitalis. Rippetoe,[2] following the suggestion of Beringer, made up a 10 per cent preparation with 50 per cent alcohol, thus corresponding to the U. S. P. tincture, and a 10 per cent preparation using crude drug from the same lot but percolated with 50 per cent glycerin. These were both tested on frogs, method not given, determining the minimum lethal dose. The former produced death in a dose of 0.005 c. c. per gram, the latter in a dose of 0.025 c. c. In relative terms he found the alcoholic preparation five times as active as that made with glycerin.

A sample of the fluid glycerate and the official fluid extract [3] were examined, using frogs and mice as experimental animals. To make the conditions as nearly alike as possible a sufficient amount of glycerin was added to the fluid extract, when diluting it to make it suitable for injection, to correspond to the amount in the fluid glycerate when thus similarly diluted.

The fluid extract diluted with glycerin and water required 0.0022 c. c., calculated as fluid extract, per gram of frog weight; the fluid glycerate 0.005. Thus the fluid glycerate appeared to be approximately 2.25 times weaker than the alcoholic solution.

The experiments on mice were made by injecting the fluid glycerate and the official fluid extract diluted with glycerin under the loose skin of the back and noting the amount just necessary to produce death.

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[1] Beringer: Amer. Jour. Pharm., 1907, **79**, 410; Ibid., 1908, **80**, 525.

[2] Rippetoe: Am. Jour. Pharm., 1909, **81**, 84.

[3] I am indebted to Mr. Beringer for these preparations which were made up from the same lot of leaves in order to make comparisons possible.

As the duration of time the animals lived is of some value in making these comparisons a full protocol of these experiments is given in the following table:

FLUID EXTRACT DIGITALIS.

Mouse weight.	Fluid extract, dose in c. c. per gram.	Result.	Time till death.
Grams.			
13.52	0.003,0	—	—
15.91	0.003,5	—	—
14.16	0.004,0	+	5 hours.
14.78	0.006;0	+	3 hours.
13.50	0.008,0	+	2 hours.

FLUID GLYCERATE DIGITALIS.

Mouse weight.	Fluid glycerate, dose in c. c. per gram.	Result.	Time till death.
Grams.			
17.32	0.009	—	—
18.19	0.010	+	(?)
15.38	0.012	+	4 hours.
14.44	0.016	+	2 hours.
14.39	0.020	+	1 hour.

From these tables the least fatal dose of the fluid extract is seen to be 0.004 c. c. per gram mouse weight, that of the fluid glycerate 0.010 c. c. Thus the fluid extract assayed on mice approximately 2.5 times as strong as the fluid glycerate. Judging by the ratios in potency of the fluid extract and the fluid glycerate on the two species of animals it seems reasonable to say that the glycerin-water menstruum extracts only about 40 per cent of the active principles in crude digitalis leaves and is therefore not suited for making commercial preparations of this drug.

Digalen.—It has long been clearly recognized that digitalis medication is complicated by a number of undesirable secondary effects. Briefly, these are its accumulative action, its tendency to produce disorders of appetite and digestion, and, finally, its irritant action, which interferes with its use by the hypodermic method. These untoward effects are so marked at times as to prevent the use of the drug; they always make great caution necessary in the administration in order that dangerous toxic effects may not follow.

The use of digitalis is further complicated by the fact that its therapeutic effects often do not appear until about the second day after medication is started, a drawback which makes it practically no value in cases of acute heart failure. In certain cases the effect fails to appear even after several days, while in others the first indication that the drug has been absorbed is the appearance of symptoms of poisoning. These latter disagreeable features of the drug's action are usually ascribed to differences in the reaction of the patient,

but it seems equally or even more probable that they are often due to variations in the potency of the drug used, the preparation being in one case much weaker, in another much more active than the normal.

Many attempts have been made from time to time to secure a digitalis preparation without these various undesirable qualities. Unfortunately, however, these efforts have not resulted in much success, although a large number of special formulas, part of which have already been considered under the questions of variability and deterioration with age, have been used in preparing the crude drug for therapeutic use. In this connection, the so-called pure principles of digitalis have been suggested as offering a solution of the problem. These, however, although possessing the desirable effects of the crude drug are by no means free from undesirable secondary effects. Of these digitoxin, the glucoside which occurs in the greatest amount in digitalis leaves, is by far the most potent. The use of digitoxin in therapeutics is made practically impossible, however, by its excessively irritant action, its tendency to produce disorders of digestion, its marked accumulative action, and, less important, its insolubility in water. In 1904, however, Cloetta[1] announced that as a result of several years labor he had been able to isolate a digitoxin from digitalis leaves which was almost entirely devoid of these drawbacks. This new digitoxin (for sale under the trade name digalen) from a physical standpoint, differed from the crystalline product of Schmiedeberg and Kiliani in being amorphous and in being much more soluble in water. From the therapeutic standpoint, according to Cloetta, it differed even more widely, being easily absorbed, the digitalis effect appearing within twenty-four hours, being without accumulative or toxic action, and being so free from irritant qualities as to make it suitable for subcutaneous, intramuscular, or intravenous injection. Moreover, being a definite chemical substance, it afforded a preparation entirely devoid of the variability so often present, as has been pointed out, in the crude drug.

Aside from Cloetta's own investigation, the only work done regarding the identity of digalen with crystalline digitoxin was carried out by Kiliani,[2] who has done far more work on the chemistry of digitalis than any other investigator. He was unable to accept Cloetta's view, but rather pointed out that the formula given by Cloetta[3] for digalen does not correspond to that for the crystalline substance, the former having a formula of $C_{14}H_{23}O_5$ with a molecular weight of 287, the latter of $C_{34}H_{54}O_{11}$, a molecular weight of 638. Kiliani concludes that digalen is only a high percentage of digitalein.

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[1] Cloetta: München med. Wchnschr., 1904, **51**, 1466–1468.

[2] Kiliani: München med. Wchnschr., 1907, **54**, 886, 1112; Ber. d. deutsch. chem. Gesellsch., 1907, **40**, 2996.

[3] For Cloetta's reply to this criticism see München med. Wchnschr., 1907, **54**, 987.

Certain clinical results would seem to indicate that Cloetta and a large number of the clinicians who reported *invariably* good effects from digalen were partly in error (or based their conclusions on an inadequate number of cases) regarding the superiority of amorphous digitoxin over the older preparations of digitalis. A review of the literature (Reitter,[1] Vlach,[2] Frankel,[3] Mueller[4]) shows that digalen is not always devoid of cumulative action; that it possesses marked irritating properties, so irritant as to make subcutaneous injections practically impossible, causing edema and pain, lasting in some cases for two and three days and often causing great pain when given intramuscularly, or if given intravenously, causing in some cases edema and thrombosis (Kottmann,[5] Hochheim,[6] Liverato,[7] Veiel,[8] Teichmann[9]); that it causes disturbances of digestion apparently as often as the older galenicals, with loss of appetite, nausea, and vomiting (Eichhorst,[10] Veiel,[11] Teichmann,[12] Mueller[13]), and that its effects do not appear more rapidly than from corresponding doses (based on the manufacturer's claim that 0.3 mg. amorphous digitoxin (Cloetta) = 0.150 gm. folia digitalis) of the older preparations. Mueller,[5] in a series of 18 cases, observed effects from doses of 6 c. c. of digalen on the second day, namely, increased amounts of urine or a decrease in heart rate. From doses of 5 c. c. the effect appeared first on the second and third day, doses of from 3 to 4 c. c. on the third to fifth day, and from doses of 2.25 c. c. the action appeared on the fourth to the fifth day. From doses of 400 mg. folia digitalis (in terms of digalen 2.66 c. c.) the effect appeared on the second day; 300 of the leaves (equal to 2 c. c. digalen) produced effects on the third day, and Mueller remarks that these effects were not only more constant but were also more powerful than from digalen. To facilitate comparison, these results are summarized in the following table:

TABLE 1.—*Time of onset of the therapeutic effects of digalen and folia digitalis with corresponding doses (doses of folia digitalis based on the manufacturer's claim that 1 c. c. digalen=150 mg. of the leaves).*

Doses in c. c. per day	6	5	4	3	2.66	2.25	2
Day effects from digalen appeared	2	2.3	3–5	3–5	.....	4–5	.....
Day effects from folia digitalis appeared	.....	.....	.....	.....	2	.....	3

[1] Reitter: Wien. med. Wchnschr., 1905, **55**, 2245.
[2] Vlach: Prag. med. Wchnschr., 1906, **31**, 43.
[3] Frankel: Arch. f. exper. Path. u. Pharmakol., 1907, **57**, 123.
[4] Mueller: München. med. Wchnschr., 1909, **56**, 904.
[5] Kottmann: Ztschr. f. klin. med., 1905, **56**, 128.
[6] Hochheim: Zentralbl. f. inn. med., 1905, **20**, 545.
[7] Liverato: Cron. d. clin. med. di Genova, 1905, **11**, 276.
[8] Veiel: München. med. Wchnschr., 1906, **53**, 2140.
[9] Teichmann: Therap. d. Gegenw., 1907, **48**, 199.
[10] Eichhorst: Deutsch. med. Wchnschr., 1905, **31**, 49.
[11] Veiel: München. med. Wchnschr., 1906, **53**, 2140.
[12] Teichmann: Therap. d. Gegenw., 1907, **48**, 199.
[13] Mueller: München. med. Wchnschr., 1909, **56**, 904.

Walti [1] and Teichmann [2] also were unable to observe effects in the cases reported by them until the second or third day, and Frankel,[3] from observations of the pulse and amplitude, reports that a digitalis action is manifest after 0.3 gm. doses [4] of digitalis leaves as follows:

Patient.	Effects.	Patient.	Effects.
	Hours.		*Hours.*
1	22	5	25
2	20	6	23
3	17	7	23
4	24	8	24

Regarding the claim that digalen is an efficient remedy in acute heart failure, Von Ketly [5] reported that the effects did not appear more quickly when given subcutaneously than by the mouth. Kottmann [6] reported, however, that if digalen were given *intravenously* in doses of 1–5 c. c. (amounts which are necessary to produce a distinct digitalis action), the action appeared in from two to five minutes, and that 5 c. c. was an absolutely maximum dose. The danger of this method of exhibition makes such a use very questionable, however, as Teichmann [7] observed edema and thrombosis and, in one case after a dose of 4 c. c., general tonic-clonic convulsions.

It seems rather strange that digalen has been accorded so much attention at the hands of the clinician in view of the contradictory reports concerning its chemical identity and its action and that so few experiments have been carried out on animals. A few investigations have been carried out, however, and these have tended to show that digalen had a true digitalis action—a result entirely in accord with Kiliani's report, however, that digalen was an impure digitalein—the latter glucoside also having the same effect as digitoxin on the heart, excepting that it is manifested to a lesser degree. It seems doubly strange on account of Kiliani's denial of the chemical identity of this new digitoxin with the older crystalline substance that comparative studies were not made along the lines used in the biologic standardization of the digitalis series. Neave,[8] apparently the only investigator to make such a study, reported from his experiments on frogs and rabbits that, although digalen has the characteristic action, it is very much less active than a like amount of crystallized digitoxin, 1.2 mg. amorphous digitoxin (Cloetta) causing a rise in blood pressure of only 14 per cent—a slowing in rate of 2

[1] Walti: Deutsch. Aerzte Ztg., 1904, p. 461.
[2] Teichmann: Therap. d. Gegenw., 1907, **48**, 199.
[3] Frankel, A.: Arch. f. exper. Path. u. Pharm., 1907, **57**, 131.
[4] It is to be noted that this amount corresponds to 2 c. c. digalen, which produces its first effect about the fourth to fifth day, according to Mueller.
[5] Von Ketly: Therap. Monatsh., 1906, ,**20**, 272.
[6] Kottmann: Corr.-blat. f. Schweiz.-Aerzte., 1907, **38**, 306.
[7] Teichmann; Therap. d. Gegenw., 1907, **48**, 199.
[8] Neave: Scot. Med. and Surg. Jour., 1907, **20**, 390.

per cent as compared with a rise in pressure of 21.9 per cent, a slowing in rate of 14.7 per cent from 0.5 mg. crystalline digitoxin.

Owing to the claims of the advertising literature which ignores these various reports, and in view of the statement of Kottmann and others that digalen equaled crystalline digitoxin in activity, as it should if chemically identical with it, a series of experiments were undertaken to determine biologically the relation of its activity to that of digitalein and crystalline digitoxin. Three methods were used in determining the relative potency of these substances. The first method to be employed was the one hour frog heart method already described. Five different samples of digalen [1] were examined according to this method. These were all obtained in the open market, but one had been in stock at this laboratory for about two years and two others for about three years; the remaining two (samples 4 and 5) were obtained during the summer.[2] The appearance of the various samples was very much the same, although the older preparations appeared somewhat more tinged with yellow. One sample, however, that of 1905, had evidently undergone some marked chemical change since it contained a considerable amount of a dark brown granular substance,[3] a precipitate not present in any of the other samples.

These various samples were tested for their effect on the frog's heart in the manner described above, and gave the results shown in Table 2.

TABLE 2.—*Determination of the systolic stoppage of the heart of Rana pipiens in one hour. Drug injected into abdominal lymph sac. The doses are given in milligrams per gram of body weight.*

DIGALEN No. 1.

Number of frogs used.	Dose.	Result.
	Mg.	
1	0.020	—
1	0.030	—
1	0.035	—
1	0.035	—
1	0.040	[9] +

[1] Through the courtesy of the manufacturer the dates of manufacture for the several samples were obtained, viz., No. 1, 1905; No. 2, 1906; No. 3, 1907; Nos. 4 and 5, 1908.

[2] Both samples 4 and 5 were obtained at a local pharmacy in August, 1909, at which time the experiments which follow were carried out and *are such preparations as would have been dispensed on a physician's prescription*, no statement having been made regarding the use to which the drug was to be put. On this account final comparisons of value have been made on the basis of the value of sample 5, in order to be entirely fair to the manufacturers. However, it seems unwarranted from the evidence as given in the summary to Table No. 2 to claim *absolute* uniformity and stability, as preparations made the same year varied very considerably in activity.

[3] I am indebted to Mr. M. I. Wilbert for this sample and for the statement that when the precipitate first formed it was nearly white and that it had become dark on standing. It is also to be noted that in no instance had these samples been opened, so that this change came about as a result of natural causes and was not a deterioration due to opening the bottle, etc.

[9] (?) Diastole.

TABLE 2.—*Determination of the systolic stoppage of the heart of Rana pipiens in one hour. Drug injected into abdominal lymph sac. The doses are given in milligrams per gram of body weight*—Continued.

DIGALEN No. 2.

1	0.020	−
1	0.025	−
1	0.030	−
1	0.030	+
1	0.035	+

DIGALEN No. 3.

1	0.020	−
1	0.025	−
1	0.030	−
1	0.035	+
1	0.040	+

DIGALEN No. 4.

1	0.020	−
1	0.020	−
1	0.025	−
1	0.025	+
1	0.027	−
1	0.030	+

DIGALEN No. 5.

1	0.010	−
1	0.015	−
1	0.020	[1] −
1	0.022	+
1	0.025	+
1	0.030	+

SUMMARY.

	Minimum lethal dose.
	Mg.
Digalen No. 1	0.040 (?)
Digalen No. 2	0.030
Digalen No. 3	0.035
Digalen No. 4	0.027
Digalen No. 5	0.022

[1] (?) Diastole.

From this summary it will be noted that the several samples of digalen varied in value from about 0.02 to over 0.04, the older preparations generally testing weaker than those of more recent manufacture, thus indicating some deterioration with age, despite the claim that digalen is a stable preparation of digitalis.

In order to compare the value of these samples of digalen with digitoxin and digitalein, experiments were carried out on frogs, using the same experimental process as nearly as possible. The results, using Merck's crystalline digitoxin and Merck's digitalein, are shown in Table 3.

TABLE 3.—*Determination of the systolic stoppage of the heart of Rana pipiens in one hour. Drug injected into the abdominal lymph sac. The doses are given in milligrams per gram of body weight.*

DIGITOXIN IN 25 PER CENT ALCOHOL.

Number of animals used.	Dose.	Result.
	Mg.	
1	0.006	−
1	0.007	−
1	0.008	+
1	0.008	+
1	0.009	+

DIGITALEIN 7 PER CENT ALCOHOL, 25 PER CENT GLYCERIN.

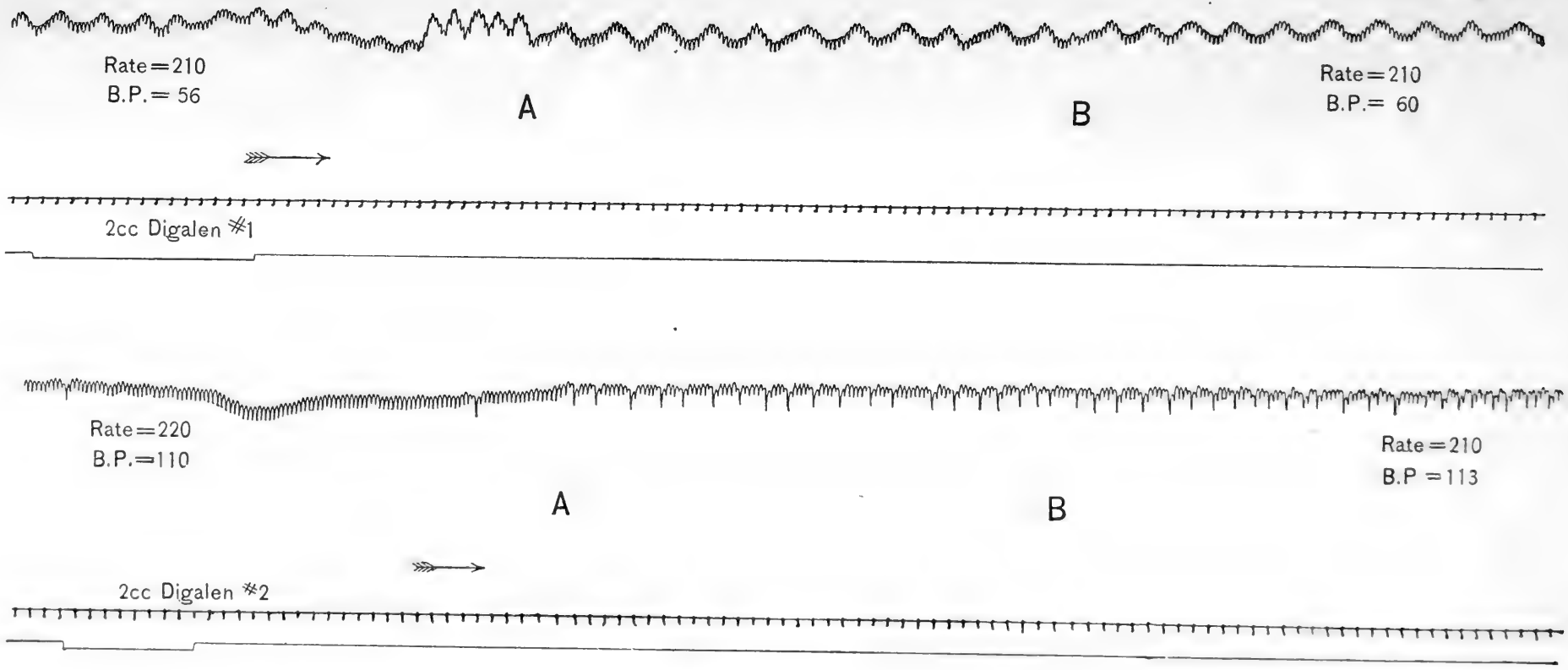
1	0.025	−
1	0.026	−
1	0.026	+
1	0.027	−
1	0.028	+
1	0.028	+
1	0.029	+

The results of these experiments on frogs would indicate that digalen, basing conclusions on the assay of the *best testing samples*, occupied a place between crystalline digitoxin and digitalein, the comparative values, according to the above tests, being crystalline digitoxin 0.008, digalen 0.022, and digitalein 0.028.

As these results indicated that digalen was far from being as active as crystalline digitoxin, it was thought to be worth while to carry out comparative tests using other methods of comparison in order to avoid any possibility of error.

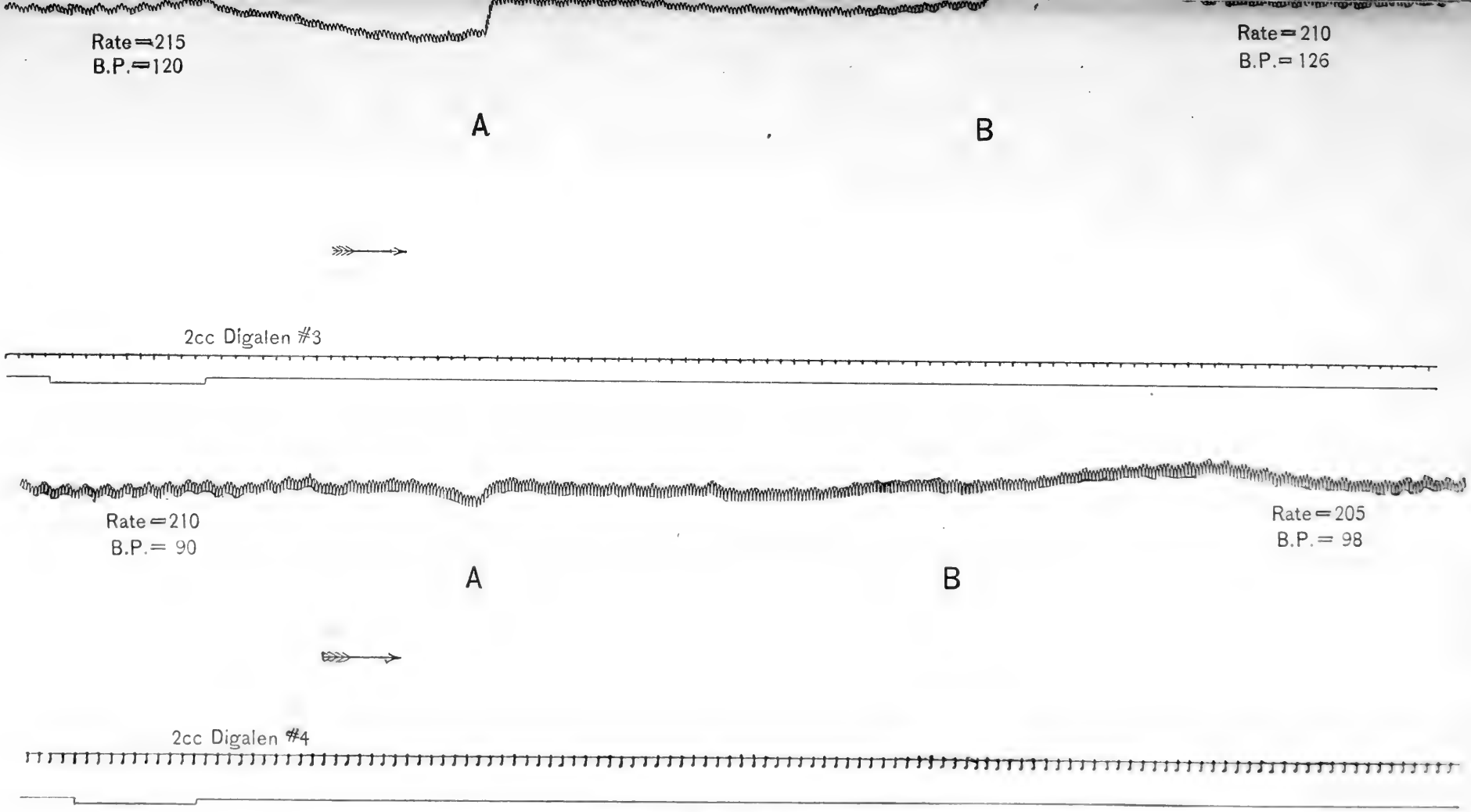
In line with this, experiments were carried out to determine the comparative effect of the several samples of digalen on the blood pressure. For this purpose cats of about the same weight (3,000 to 3,600 grams) were used and were anesthetized for the experiments by ethyl carbamate (urethane) and chloral given by the stomach. The blood pressures were taken from the carotid artery and the injections of the drug made into the external jugular vein. In working with digitalis it is ordinarily advisable, on account of its accumulative action, to take no reading except the first as determinative, but in this series, after an interval of an hour, further injections were made and the readings incorporated in the results to make up the averages. The following tracings from these experiments illustrate the effect of the different samples of digalen in doses intravenously of 2 c. c. each. Tracings from blood-pressure experiments using digitoxin and digitalein are also given for the sake of comparison.

Digalen seemed to cause some stimulation of the central nervous system in addition to its action on the circulation antagonizing, in several experiments, the effect of the anæsthetic which was sufficiently



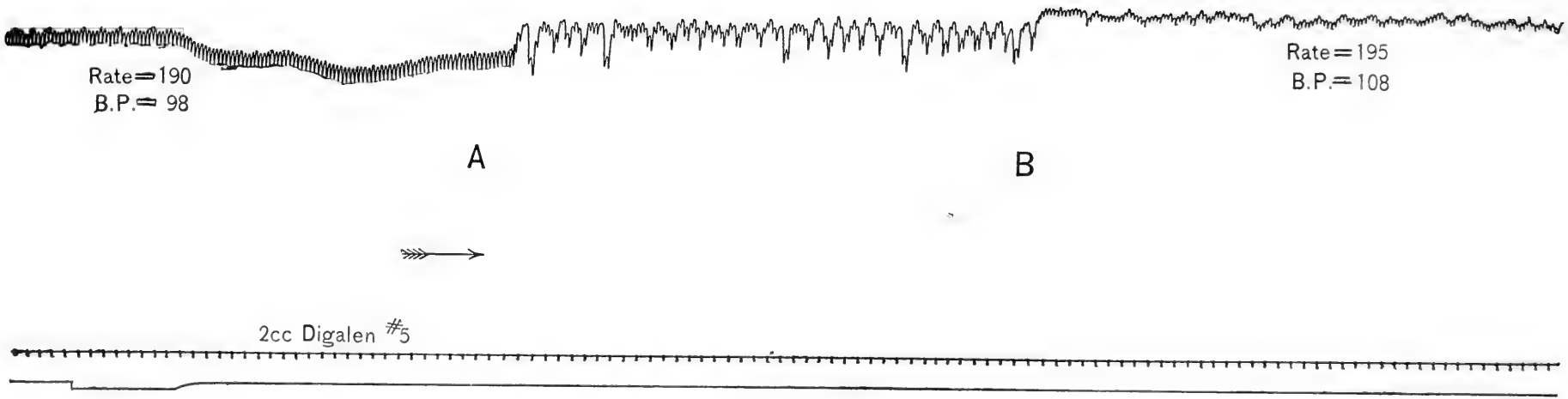
TRACING I.

Upper curve: Blood pressure curve from cat; 2 cc. Digalen No. 1; one minute out at A; one minute out at B.
Lower curve: Blood pressure curve from cat; 2 cc. Digalen No. 2; base line raised; one minute out at A; one minute out at B.



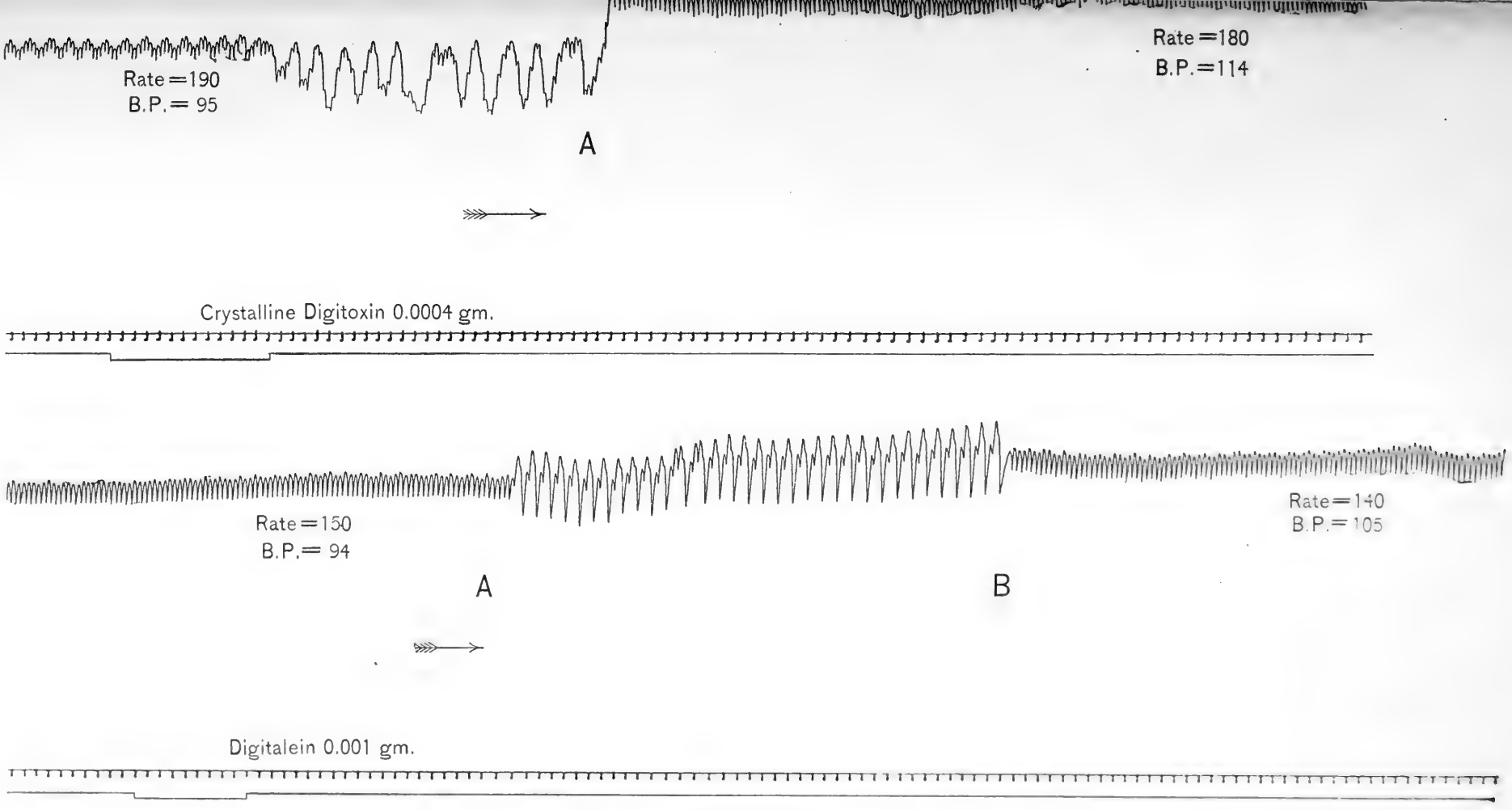
TRACING II.

Blood pressure curves from cat; 2 cc. Digalen Nos. 3 and 4; one minute out at A; one minute out at B.



TRACING III.

Blood pressure curve from cat; 2 cc. Digalen No. 5; one minute out at A; one minute out at B.



TRACING IV.

Upper curve: Blood pressure curve from cat; 0.0004 gram crystalline digitoxin; two and one-half minutes out at A.
Lower curve: Blood pressure curve from cat; 0.001 gram digitalein; A, one minute out; B, one minute out.

deep before injecting the drug to prevent spontaneous movement from the operative procedure. This action is illustrated by the following protocol:

10.30. 2 c. c. digalen.
10.35. Struggling.
10.36. 2. c. c. anæsthetic per jugular; 30 c. c. having been given for complete operative anæsthesia.
10.48. Struggling.
10.52. 1 c. c. anæsthetic, intravenous.
11.01. 2 c. c. digalen.
11.03. Struggling.
11.19. Struggling.
11.20. 2 c. c. anæsthetic, intravenous.
11.32. Struggling.
11.34. 1 c. c. anæsthetic, intravenous.
11.48. 2 c. c. digalen.
12.05. Struggling.
12.07. 2 c. c. digalen.
12.15. 2 c. c.-digalen.
12.24. 4 c. c. digalen, total 14 c. c.; (amorphous digitoxin 4.2 mg.) animal killed.

The results of this series of blood-pressure experiments are summarized in Table 4.

TABLE 4.—*Summary of experiments made on the blood pressure of cats.*

	Average decrease in rate.	Average increase in blood pressure.
	Per cent.	*Per cent.*
Digalen No. 1 (two readings)	2.0	7.1
Digalen No. 2 (two readings)	7.7	3.6
Digalen No. 3 (two readings)	6.9	5.0
Digalen No. 4 (four readings)	5.0	7.7
Digalen No. 5 (four readings)	6.2	10.2

The results tabulated in this summary agree in a general way with those obtained in the experiments on the frog's heart, viz, the oldest preparations give the smallest increase in pressure. Exceptions are to be noted, however, in the case of sample 1, which gives a relatively marked increase in blood pressure, but failed to stop the frog's heart in systole. Also sample 2, which assayed on the frog's heart somewhat higher than sample 3, has somewhat the lesser effect on the blood pressure.

Blood-pressure experiments were also carried out with digitoxin (Merck) and digitalein (Merck) in order to compare their effect with that obtained from the use of digalen.

The digitoxin was dissolved in 25 per cent alcohol, the digitalein in 25 per cent glycerin and 7 per cent alcohol, the latter solvent being the same as that used in the manufacture of digalen.

The digitoxin solution was injected into the external jugular vein of cats and the blood pressure taken from the carotid. The following table gives the rise in blood pressure and the change in rate:

Time.	Notes.	Rate.	Blood pressure.	Per cent rise in pressure.
12.03	Normal	205	112	
12.04	0.2 mg. digitoxin			
12.04[2]		180	96	
12.07		190	134	19.7
1.06	Normal [1]	180	102	
1.07	0.2 mg. digitoxin			
1.07[3]		180	100	
1.11		170	118	15.7
10.25	Normal	190	84	
10.26	0.4 mg. digitoxin			
10.26[3]		160	80	
10.30[3]		175	110	30.9
11.14	Normal [1]	190	94	
11.15	0.4 mg. digitoxin			
11.15[2]		180	85	
11.19		180	114	21.2

[1] Second injection after one hour, using the same cat.

Digitalein in solution was also tested in the same manner for its effect on the blood pressure. Three different experiments were carried out using 1-milligram doses at each injection, two determinative readings being made on each animal. The average rise in blood pressure was 9 per cent; the heart rate was slowed on the average 10 per cent.

In contrast to the apparent stimulating action of digalen neither digitoxin nor digitalein in the series of experiments as given above caused any symptoms of central stimulation aside from the action on the circulation.

Basing comparisons on the effect of these drugs on the blood pressure, it will be noted that digalen (the best testing sample), in doses of 0.6 mg. calculated as amorphous digitoxin, Cloetta, increased the pressure 10.2 per cent; that digitoxin in an average dose of 0.3 mg. gave an average rise of 21.9 per cent; and that digitalein, in a dose of 1 mg., gave an average rise of 9 per cent.

One may conclude, therefore, that digalen is considerably less active than crystalline digitoxin [1] and that it is of about the same or

[1] Miller, Jour. Am. Med. Ass., 1908, 51, 1745, tested four bottles of Digalen and was unable to get any effect on frogs or on dogs. In quantitative tests Hatcher, Am. Jour. of Phar., 1910, 83, 360, found Digalen to be about one-fourth the strength of crystalline digitoxin.

of slightly greater potency than corresponding amounts of digitalein. This conclusion is also borne out by the following series of experiments.

White mice of the same lot were weighed, placed in separate jars and injected beneath the loose skin of the back with digalen, digitoxin (Merck) and digitalein (Merck). The doses were calculated in terms of grams of body weight. Increasing amounts were injected until the minimum lethal dose was obtained. The results of these experiments are given in Table 5.

TABLE 5.—*Determination of the minimum lethal dose for white mice, subcutaneous injection. Dose in milligrams per gram of body weight.*

DIGITOXIN IN 25 PER CENT ALCOHOL.

Number of mice used.	Dose.	Result.
1	0.008	−
1	0.011	−
1	0.014	+
1	0.016	+
1	0.017	+

DIGALEN SAMPLE No. 5.

1	0.030	−
2	0.040	−
1	0.050	+
1	0.060	+

DIGITALEIN 7 PER CENT ALCOHOL, 25 PER CENT GLYCERIN.

1	0.015	−
1	0.025	−
1	0.040	−
1	0.055	−
1	0.065	+
1	0.070	+

From this table it is apparent that the minimum lethal dose for digalen again is considerably larger than that for digitoxin (for mice about four times). Digitalein, as in other methods of testing, assayed weaker than digalen, but, according to this method, appeared comparatively to be very little weaker.

With regard to the general action of digalen on both frogs and mammals, it appears to possess considerably more stimulant action on the central nervous system than other digitalis preparations. In almost every case in the frog experiments the early symptoms of absorption were convulsive movements. At times these were clonic, but more often tonic in character, the legs being extended and remaining in hyperextension for some time. The effect of digalen on mice was much the same, except that the convulsions were usually

clonic in character. In the blood-pressure experiments no convulsions were so clearly present, the animal being anæsthetized, but, as has been noted, struggling was often a sequel of the injection of even small amounts of digalen and, to prevent this, further small doses of ethyl carbamate were necessary. It is of interest in this connection to remember that Teichmann[1] reported similar symptoms of excessive central nervous system stimulation in a patient who was given 4 c. c. digalen intravenously.

CONCLUSIONS.

Digalen is not a uniformly stable preparation, as is shown by the gross appearance of sample 1 and by biologic tests of the five different samples. Biologic tests also indicate that digalen is relatively much less potent than corresponding amounts of crystalline digitoxin, but that it is of about the same activity as digitalein.

The experience of clinicians vary, some reporting good results from the use of digalen, but many reports indicate that it is much less effective than is claimed and that the secondary action of the digitalis group appears equally often after its use as with the older and cheaper galenicals. Its use in cases of acute heart failure, whether by intramuscular or intravenous injection, seems to open serious objection (on account of the pain and danger of thrombosis), and it would apparently be better practice in such cases to use either an *assayed* strophanthin[2] to be given intramuscularly or intravenously or very small amounts of one of the preparations of the suprarenal gland by intravenous injection.

Digipuratum.—Another of the more recent digitalis preparations, the use of which is claimed to produce few or none of the undesirable secondary effects of digitalis medication, is digipuratum (extract of digitalis depuratum, Knoll). According to the statement of the letters patent, this preparation is made by treating the alcoholic extract according to a method originated by Gottlieb[3] with ether. As a result the extract is freed from about 85 per cent of inert and undesirable material, including the irritating saponin-like body digitonin. The remaining 15 per cent is said to represent the full activity, less about 5 per cent, of the original extract and to contain both digitoxin and digitalin. The digitalis extract having been thus purified, the resulting yellowish solution is mixed with such amounts of milk-sugar that when tested biologically on frogs it has the same activity as a like amount of strongly active digitalis leaves—that is, 1 gram digipuratum equals in activity 1 gram powdered digitalis leaves.

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[1] Teichmann: Therap. d. Gegenw., 1907, **48**, 199.

[2] The importance of an assayed strophanthin has recently been pointed out by Hatcher, J. A. M. A., 1910, **54**, 1050.

[3] Hoepffner: München. med. Wchnschr., 1908, **55**, 1774.

Digipuratum is further said to be insoluble in cold water and in acids, but to be easily soluble in weak alkalies. Theoretically, therefore, it is said to have no action on the stomach and to be absorbed only after reaching the small intestine. Clinically also Mueller asserts that symptoms on the part of the stomach do not often develop;[1] but too few cases have been reported as yet to establish this claim of lessened toxic secondary effects when compared with the older preparations. Such questions must be left largely to the clinician, and the experiments which follow and the conclusions drawn from them were carried out merely to determine the drug's activity according to the biologic method of assay used at this laboratory.

In order to prepare the powdered digipuratum for injection, 1 gm. of the powder was taken and to this 100 mg. sodium bicarbonate was added; this mixture of alkali and digipuratum powder was then shaken from time to time for 24 hours with 8 c. c. 25 per cent alcohol. The insoluble material was then allowed to settle and the resulting clear portion was decanted and set aside. The insoluble residue was further treated with small amounts of 25 per cent alcohol until a total of 20 c. c. alcohol had been used in this shaking out process. The later decantations were evaporated over a water bath at 80° until the total extractive when added to the first decantation equaled 10 c. c., or a solution conforming to the U. S. P. tincture of digitalis.

Digipuratum is also marketed in tablet form, each tablet representing 1½ grains (100 mg.) of the powder. Ten such tablets were treated in the manner above described, so that a resulting alkaline 25 per cent alcoholic solution of 10 c. c. was obtained.

An assay of the powder was undertaken to determine the amount of powdered digipuratum in 10 per cent solution necessary to produce systolic stoppage of the heart of *Rana pipiens* at the end of one hour. Doses were calculated in cubic centimeters per gram of frog body weight. The experiments gave the results shown in Table 6.

TABLE 6.—*Effect of powdered digipuratum on the frog.*

Weight of frog.	Dose per gram weight.	Result.
49	0.004	−
48	0.005	−
45	0.006	+
53	0.007	+
55	0.008	+
49	0.010	+
60	0.012	+

[1] Mueller: München. med. Wchnschr., 1908, **55**, 2651.

An assay of the tablets of digipuratum was made to determine the amount of digipuratum tablets in 10 per cent solution necessary to produce systolic stoppage of the heart of *Rana pipiens* at the end of one hour. Doses were calculated in cubic centimeters per gram of frog body weight. The experiment gave the results shown in Table 7.

TABLE 7.—*Effect of digipuratum tablets on the frog.*

Weight of frog.	Dose per gram weight.	Result.
42	0.004	−
47	0.005	−
58	0.006	+
57	0.007	+
56	0.008	+
43	0.010	+

These results may be summarized as follows:

Preparation.	Dose to produce systole.
Digipuratum powder.....	0.006 c. c.=0.0006 gm.
Digipuratum tablets.....	0.006 c. c.=0.0006 gm.

These results indicate that the two samples are of the same strength, a result which accords with the manufacturers' claim that digipuratum is an assayed product. They also show that digipuratum in 10 per cent solution (corresponding to the official tincture in strength) is of about the same activity as strongly active preparations of the official tincture when assayed by the same method.[1] However, when compared with a series of assays [2] of official fluid extracts, diluted to 10 per cent strength, digipuratum in 10 per cent solution was more active.

Experiments were also carried out to determine the action of digipuratum on the blood pressure. For this purpose cats weighing between 2,200 and 2,700 grams were used, being anaesthetized for the experiments with ethyl carbemate (urethane) and chloral given by the stomach or with chloroform and magnesium chloride. The blood pressure was taken from the carotid, using the ordinary mercury manometer, and the drug was injected into the external jugular vein.

The effect produced by a 1 c. c. dose of a 10 per cent solution of digipuratum is shown in the following tracing:

[1] Edmunds, Charles Wallis: The Standardization of Cardiac Remedies. The Journal A. M. A., May 25, 1907, **48**, 1744. The doses recorded in this paper are based on the amounts necessary to produce systolic stoppage of the heart of 20-gram frogs and should be divided therefore by 20 to permit of comparison. Also see pp. 31 and 32.

[2] Edmunds and Hale: Bull. 48, Hyg. Lab., U. S. P. H. and M. H. S., Washington.

Smaller doses were also used and the following protocol illustrates the marked action on the blood pressure from ½ c. c. injections of a 10 per cent digipuratum solution:

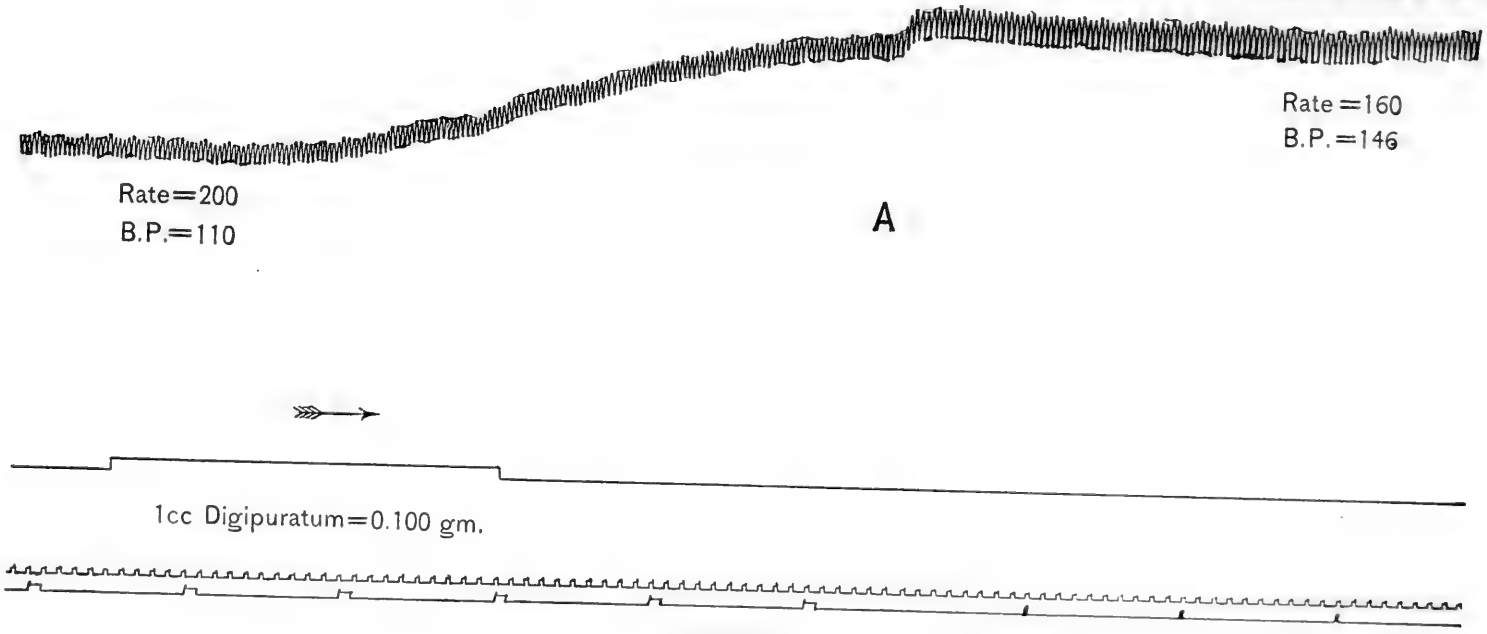
November 6, 1909. Cat, 2,280 gms. Anaesthesia urethane 0.050 + chloral 0.02 gms. per 100 gms. cat + ether. Artificial erspiration.

Time.	Notes.	B. P. increase.	Rate.	B. P.
		Per cent.		
11.13	Normal		210	137
11.13 [1]	½ c. c. digipuratum			
11.14			200	142
11.18		8.0	180	148
11.23			190	138
11.42	Normal		190	104
11.42 [2]	½ c. c. digipuratum			
11.44			190	112
11.48		25.0	210	130
11.52			210	112
12.17 [2]	Normal		190	58
12.17 [3]	½ c. c. digipuratum			
12.18 [1]		27.5	200	74
12.19			200	64
12.21			200	48
12.32	½ c. c. digipuratum			
12.35		Dead.		

Total injection, 2.0 c. c.

These biologic tests may, therefore, be said to show that digipuratum is of the same strength whether purchased in the tablet or in the powdered form, and that it is of about the same activity as the strongest official digitalis preparation on the market.

To what extent the use of digipuratum is justified on the grounds of lessened secondary effects is outside the province of this paper to discuss. On general grounds, however, it may be said that its use rather than a physiologically standardized official preparation seems to offer no special advantages and, except in those cases in which secondary toxic effects are produced by them, that the official preparations would be equally efficient and are about 1,500 per cent cheaper. Whether digipuratum also produces secondary toxic effects as often as the official galenicals do is a matter which must be decided by the clinician.



TRACING V.

Blood pressure curve from cat; 1 cc. Digipuratum=0.100 gram; A, two minutes out.

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.

70066°—Bull. 74—11——5

*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.

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