ON THE PRESENCE OF CHOLIN AND NEURIN IN THE INTESTINAL CANAL DURING ITS COMPLETE OBSTRUCTION.

A RESEARCH ON AUTOINTOXICATION.

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In the normal processes of digestion the proteids and carbohydrates of our food are changed into more readily assimilated compounds, which are further altered before reaching the tissues; for example, the peptones,* which, if absorbed unaltered into the system would be very toxic, are changed into nutritive material in passing the intestinal wall. As a result of bacterial activity we may have these compounds broken up in a different manner, giving rise, either as immediate or terminal products of the decomposition of the proteid or carbohydrate molecule, to substances of more or less toxic character. Some of these substances, as phenol, the cresols, the dihydroxy-benzenes, indol and skatol, are known to occur as a result of the constant action of putrefactive bacteria in the large intestine. We may also have a large number of organic acids of the fatty series, as acetic, lactic, butyric, caproic, caprylic, etc., which have been shown to occur in various catarrhal conditions of the intes-

* According to E. Flquet the poisonous effects usually ascribed to peptones and albumoses are in reality due to ptomaines or other toxins which have not been removed by the ordinary processes of purification. Compt. rendus Acad. d. sc., 1897, p. 1371.
tinal tract, and whose irritating effects have been studied by Bókai.*

Gases, as hydrogen sulphide, methylmercaptan,† carbon dioxide, methane and ptomaines, as putrescin (tetramethylendiamin),‡ cadaverin (pentamethylendiamin),† ethyldiamin, are also more or less constantly present.

Opinions vary greatly on the toxicity of these substances, as the pharmacology of but one of them (phenol) has been carefully worked out.

For consideration in this respect they may be divided into three classes: (1) the fatty acids and the various gases, whose action in this connection is almost wholly irritative and need not be considered from the point of view of absorption, except perhaps in the case of infants; (2) substances of the aromatic series, which include phenol, the cresols, the dihydroxy-benzenes, indol and skatol, and (3) the diamines, including putrescin, cadaverin and ethyldiamin. Substances of the second group are all excreted in the urine as conjugate or ethereal sulphates, and it is by their estimation that we judge of the extent of the putrefactive processes, more especially in the large intestine. From the fact that their molecules are of fairly simple structure, and in the case of most of them completely oxidized, we may consider the amount excreted as a reasonable index of the quantity absorbed.

With phenol (carbolic acid) we are well acquainted; its use as an antiseptic has for years been general, as a poison it has been taken in large quantities, sometimes without fatal results. It has been administered in various diseases, more especially those in which intestinal antisepsis was sought, as typhoid fever, in amount so much larger than the quantity produced in the intestine and absorbed that a comparison would be ridiculous. Thus Brieger§ has found the amount excreted in 24 hours by a healthy individual to be about 15 milligrammes, while the textbooks on therapeutics set the maximum daily dose at 600 milligrammes. The cresols or methyl-hydroxy-benzenes are a later addition to the materia medica introduced by Laplace, and have undoubtedly many advantages over carbolic acid. It has been claimed that they are three times less toxic than phenol.|| I think from my own experiments that

* Bókai, Arch. f. exp. Pathol. u. Pharmakol., xxiii, 599; xxiv, 153.
† Neesk, Jahrb. u. Thier-Chemic, xx, 309.
‡ Udartszky, and Baumann, Zetlehr. f. physiol. Chem., xiii, 592; xv, 77.
 Werigo, Pflüger's Archiv, li, 362.
 García, ibid., xvii, 543.
 || Charteris, Lancet, 1894, i, 801.
this figure is too high, but I have found that, while their action on blood-pressure and respiration presents the general phenol picture, recovery is more prompt. The injection of 50 cc. of 0.5 per cent sol. of cresol in normal saline solution into the jugular vein of a dog weighing 4 kilos caused the blood-pressure to fall 41 mm.; it returned to the normal in 13 minutes. Paracresol, according to Baumann and Brieger, occurs in largest amount among the members of this series, all of which give on distillation a reaction with bromine, the ortho- and meta-cresols occurring in traces.

The dihydroxy-benzenes—resorcin, hydroquinon and pyrocatechin—are present in the urine only in traces. All three have been used as medicines, their daily doses being respectively, 0.1-0.6 grammes, 0.25-1.5 grammes, and 0.3-0.2 grammes.

Possibly the substance which is attracting more attention than any of these at the present time is indol, which appears in the urine as indican, or indoxyl potassium sulphate. The amount of indican excreted is for an average man about 12 milligrammes per day; this is equivalent, if all is excreted that is absorbed, to an absorption of 6 milligrammes of indol in 24 hours. Experiments on the toxicity of indol and on its fate in the organism have been made by a number of investigators. Jaffé,* Nencki † and also Baumann ‡ administered considerable quantities of indol to dogs both by subcutaneous injection and by feeding, and although the object of these experiments was to determine the amount of indol that was converted into indican, it was at the same time observed that indol is not a toxic substance. Jaffé found no toxic symptoms following the subcutaneous injection of considerable quantities of indol prepared according to Baeyer's synthesis, and Nencki noted that a dog showed no signs of intoxication after receiving 1 gramme of indol by mouth, but with a dose of 3 grammes during 24 hours developed diarrhoea. Experiments more directly relating to the toxic influence of this substance were next undertaken by Christiani,§ who found that a fowl gave no signs of poisoning when it received 0.07 gramme of indol mixed with bread crumb, but that frogs reacted with decided symptoms in about an hour after the subcutaneous administration of from 1.2-2.4 milligrammes in solutions of 1:1000. The average fatal dose for frogs was 12 mmg. in 1 per cent solution subcutaneously administered. The symptoms were in general like those following the administration of phenol and need
not be given in detail. In recent years Rovighi * and Herter † have published more extended researches on the toxicity of indol. Rovighi finds that for rabbits the lethal dose of indol or skatol lies between 1.5 and 2 grammes administered in the course of 24 hours by subcutaneous injection, and that these two products of intestinal putrefaction have a similar physiological action. As summarized in Malý’s *Jahresbericht*, the symptoms of poisoning are: torpor, somnolence, widespread paresis, weak action of the heart, reduction of temperature and retention of urine and feces. The autopsy in cases of acute poisoning showed the portal vessels and the supra-hepatic veins to be highly congested, while in cases of chronic poisoning, especially after the administration of indol, the bile ducts were surrounded with infiltrating small cells, which also filled up the intercellular spaces. The kidneys were congested.

Herter’s experiments relate to acute indol poisoning in rabbits and dogs, to chronic indol poisoning in rabbits, and to the effects on man of moderate doses taken by the stomach. As in the experiments of Rovighi, it was found that in acute poisoning with considerable quantities, say 70 cc. of a 0.1 per cent solution of indol injected slowly into the femoral vein of a dog weighing 15 lbs., the symptoms were cardiac and respiratory depression, general prostration, irregular clonic spasms, increased reflex excitability and marked contraction of the pupils. The cause of death appeared to be cardiac rather than respiratory failure. Observations on the temperature and on arterial pressure were not made. Of great interest are Herter’s experiments on chronic indol poisoning in rabbits. The daily injection of such small quantities of indol as 10 cc. of a 0.1 per cent solution led to death in the course of 13-22 days. Diminished activity, loss of appetite, profound disturbance of nutrition with marked loss of body weight are the points especially emphasized in this connection. A small ring-tailed monkey was found to be far less susceptible than rabbits, for the monkey received 4 cc. of a 0.1 per cent solution daily for two months without any apparent effect. Highly interesting, too, are Herter’s contributions to the study of indol poisoning in man. Three healthy men, varying in age from 25 to 32 years, were induced to take indol during periods of from 6 to 13 days in daily quantities varying in the several subjects from 0.025 to 2 grammes. One of these men, a vigorous medical student, aged 25 years and weighing 160 lbs., consumed no less than 6.8 grammes in divided doses in 6 days, taking on one day

* Abstract in Malý’s *Jahresbericht*, Thier-Chemie, xxvi, 456.
as much as 2 grammes. The first day, after a dose of 1 gramme, no symptoms whatever were noted. Further administration with slightly increased doses led to disturbances of sleep and headache but no distinctly toxic symptoms. Without going further into the details of Herter’s work, which is of especial value when the clinical significance of indol absorption is to be considered, I will only state that I agree with his conclusion that indol does not ordinarily exert highly toxic effects even when absorbed in unusually large amounts.

My own experiments on indol and skatol relate merely to their effect on arterial pressure. The indol used in my experiments was made according to Nencki’s synthesis, acting with dichlor-ether on anilin. I believe that pure indol is more easily secured in this way than from putrefying fibrin. I have found that when injected in doses of 0.1 gramme into the jugular vein of the dog it produces no effect on arterial pressure. In frogs, as pointed out by Christiani,* it produces convulsions similar to those caused by phenol. What has been said of indol holds also for skatol, which has been fed to a dog weighing 55 kilos at the rate of 30 grammes in 21 days without any serious effect.† In my experiments no change of arterial pressure was produced by jugular injections of 0.1 gramme. In fact, I am satisfied that 20 times as much of either of these substances as are excreted daily by a man of 70 kilos weight may be injected at one time into the jugular vein of a dog of 4 kilos without producing an appreciable effect on the circulation or respiration. Indol, however, is much the more important of the two, as skatol, though formed in larger quantities, is absorbed only in traces.‡

When we consider, therefore, the amounts in which any of these substances could probably be formed under the most favorable circumstances, and compare these with the quantities which have been administered empirically or experimentally, we cannot but feel that to account for the symptoms in acute cases of intoxication something more active is necessary.

The third class of substances comprises putrescin, cadaverin and ethylidendiamin, all belonging to the diamins. Udránzsky and Baumann§ have fed both putrescin and cadaverin to dogs in large doses without effect. Grawitz|| has shown that they are both capable in 2.5 per cent solution of producing severe inflammation and necrosis,

*a Loc. cit.
† Mester, Zeitschr. f. physiol. Chem., xii, 130; Brieger, ibid., iv, 414.
‡ Brieger, ibid., iv, 241.
§ Ibid., xv, 77.
|| Virchow’s Archiv, cx, 1.
while Behring* has found cadaverin, taken in large doses, poisonous to mice, guinea-pigs and rabbits. The substance found by Kulneff † in a case of gastroptosis is probably ethyldendiamin. It is more poisonous to mice and guinea-pigs than to frogs. In the former it causes lachrymation and salivation followed by violent dyspncea, lasting until death, which follows in 24 hours or more. So of these substances it may be said that the first two are not extremely toxic and the chemical position of the last is still uncertain.

Of the various toxins which are known to be formed by the action of bacteria, we have not definite knowledge enough to speak until their principles are more completely isolated so that they can be studied as individuals. Of these, however, many are albumoses or of proteid nature and are destroyed according to Nencki by various digestive juices.‡

As the first three classes of these substances differ from what we commonly have in mind when we speak of poisons, so do the symptoms which they are supposed to produce in the so-called autointoxications differ from the toxic picture we see in a case of ileus or acute intestinal obstruction.

We know that the chief symptoms of ileus, such as pain, vomiting, cold clammy sweat, pallid and shrunken features, with possibly sub-normal temperature and ultimate complete muscular relaxation, all of which often result in death within one or two days, can be simulated by poisons formed by bacterial activity, and that, too, within a comparatively few hours as, for instance, by the tyrotoxin of Vaughan.§ Lépine and Molière || have occasionally observed in cases of intestinal occlusion symptoms like those seen in atropin poisoning, namely, dilated pupils and marked redness of the skin, and these authors surmise that death in these instances may be in some degree due to autointoxication from absorption of ptomaines from the intestine.

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†Berl. klin. Wochenschr., 1891, p. 1071.
‡Ransom (Deutsche med. Wochenschr., 1898, p. 117), however, finds that tetanus toxin passes in large part unchanged through the alimentary canal, its harmlessness when administered by the stomach being due to incapacity of the stomach and intestine to absorb it. Behring (ibid., p. 662) considers that other proteid-like bacterial toxins behave in the same way.
§Zeitschr. f. physiol. Chem., x, 146.
||Cited from Eichhorst, Darmstenose, Real-Encyclop. d. gesamm. Heilk., iii edit., v, 430.
It is not my purpose to offer a chemical theory in explanation of any of these various symptoms that arise in the course of an acute and complete obstruction of the intestinal canal at different points in its course. It is my object rather to present a chemical study of the intestinal contents in cases of complete obstruction of the small intestine in order to learn whether other or more powerful poisons than the putrefactive products already isolated can be found under such circumstances. Such poisons if present must exert their action and play their part, be it great or small, in the symptomatology of ileus; certainly the substances so far observed in the intestinal canal are not sufficiently toxic to account for any of the symptoms observed in intestinal obstruction. On the other hand, "shock" and similar expressions are far from giving a rational explanation of the condition described.

When we consider the chemical and physical conditions which exist in a case of this kind we find, first, a closure of the bowel, it may be by hernia, volvulus, intussusception or pressure, but the effect is to convert so much of the digestive tract as may be above the constricted portion into a closed thermostatic tube containing culture materials in the shape of proteids, carbohydrates, etc., kept at body temperature and infected by a varied bacterial flora, air being excluded. In this respect, the conditions are similar to an experiment conducted in the laboratory, where the same materials are used and inoculated with intestinal bacteria, but with this striking difference, that in the former case the tube is composed of animal membrane through which many of the products may pass by absorption, to be taken up later by the portal system and if unchanged in their passage through the intestinal wall (as pointed out before in the case of peptone), perhaps to be oxidized or otherwise changed by the liver cells before reaching the tissues. So that for a chemical theory not only would poisons have to be formed, but in order to produce alarming effects they must be of such a composition that they are not destroyed by the liver, or they must be produced in such quantities that the liver is unable to destroy them as fast as they are absorbed.

For the purpose of this research lecithin was chosen, a substance which is a constituent of all food materials and is widely distributed
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in nature. The products formed by its decomposition are not only in some instances of extreme toxicity, but also capable of positive detection and identification. It has been found as a constant accompaniment of cell life, animal and vegetable, but chiefly in brain and nerve tissue, yolk of eggs and the germinating sprouts of plants, to a lesser degree in milk, muscles, etc.

Chemistry and Fate of Lecithin in the Economy.—It has been known for a long time that there are different lecithins according to the fatty-acid radicle contained, but more recently Lippman* found two lecithins in beet residue, one of which gave cholin on decomposition and the other betain; he has therefore suggested that we may have different lecithins depending on the interchangeability of the basic radicle, as we have different lecithins according to the acid radicles present.

We know that these complex molecules split up into different compounds with different arrangement of their component radicles according to the agents employed, but as a result of chemical action and putrefactive processes it has been abundantly shown that lecithin breaks up into glycerophosphoric acid, fatty acids and basic bodies.

As regards the decomposition and fate in the economy of the different radicles composing the lecithin molecule, Bókai considers it analogous to the fats, and states that lecithin is decomposed during the digestive processes into glycerophosphoric acid, fatty acids and cholin, and that these products are severally absorbed. According to this view it might be dangerous to consume a great deal of food rich in lecithin (eggs for instance) as cholin is certainly not a harmless substance. Bókai† subjected lecithin to the action of the pancreatic ferments and found that it was split up as above, but he mentions also that bacterial agency was not excluded. From the more recent experiments of P. v. Walther,$ it seems fair to assume that some lecithin may be absorbed without decomposition, as he always found it present in the chyle of the dog to the extent of from 0.03-0.096 per cent. Hasebroek§ has shown in putrefactive experiments, practically anaerobic as he used slime from the river Ill as the source of bacteria, that under these conditions cholin is broken up into methylamin, carbon dioxide and methane.

† Zeitsehr. f. physiol. Chem., i, 162.
In the case of the lecithin under consideration, which is much the more common form in foods, the basic body contained in its molecule is almost wholly cholin* or trimethyl-oxyethyl-ammonium hydroxide. The composition of this base is

\[
\begin{align*}
N \left\{ \begin{array}{l}
(CH_3)_3 \\
CH_2 - CH_2 - OH \\
\text{OH}
\end{array} \right.
\end{align*}
\]

It is readily oxidized to a highly poisonous compound isomeric with muscarin and, on losing a molecule of water, a process which may easily occur in the intestine, it yields the almost equally poisonous compound neurin. Neurin is trimethylvinyl-ammonium hydroxide and has the composition,

\[
\begin{align*}
N \left\{ \begin{array}{l}
(CH_3)_3 \\
CH = CH_2 \\
\text{OH}
\end{array} \right.
\end{align*}
\]

The intimate relation between cholin and neurin is further shown by the fact that, as proved by E. Schmidt, neurin can be changed back to the oxyethyl compound, cholin. It has further been shown by Schmidt† that cholin chloride is decomposed almost entirely by putrefactive action, at 20-30° C., at the end of 14 days yielding large quantities of trimethylamin and a small quantity of a base whose platin chloride is similar in crystallization and solubility to the neurin salt, and also agrees with neurin in its physiological action. When decomposition was carried on for ten days at 30-33° C. neither cholin nor neurin were present, nor could the presence of trimethylamin be determined with certainty. There can be but little doubt that the crystals isolated by Schmidt from solutions of neutral cholin chloride, which had been infected with hay infusions, consisted in reality of the chloride of neurin, and we must therefore regard the conversion of the relatively non-toxic cholin into the highly poisonous neurin as being within the power of perhaps numerous varieties of bacteria. Hasebroek, as mentioned in connection with lecithin, found on treating cholin solutions with sewage from the Ill, that it was entirely decomposed, yielding methane and carbon dioxide. The solution on treatment with alkali gave an odor of methylamin, and Brieger found that a cholin solution, after the action on it of putrefactive bacteria, gave trimethylamin on treatment with alkali.

It will be seen that strikingly different results have occurred from putre-

† _Archiv d. Pharmacie_, cccxix, 481.
active experiments on cholin. This, however, is to be expected as the flora in many cases is entirely different, but all observers agree that in the examination of the products of putrefaction whenever cholin is present neurin is also present, although it may be in traces only.

In reference to the toxicity of these substances it has been shown that cholin, previously considered non-toxic, is fairly active, since Gahtgens* has proved that 0.59 gramme produced almost instantaneous death in a cat. It has been further shown that cholin chloride produces the same muscarin-like symptoms as neurin although the latter are much more intense. Briggs † found that 0.005 gramme of neurin chloride would produce the same symptoms in a rabbit as 0.1 gramme of cholin chloride. He further found that the fatal dose of cholin per kilogramme of rabbit was 0.5 gramme or ten times that of neurin. Boehm ‡ considers the curara-like, paralyzing action of cholin to be like that of artificial muscarin, but the latter is 500 times more toxic.

If therefore we take it for granted that putrefaction takes place in the intestinal canal during obstruction, the toxicity of the substances formed will depend upon the material present and the character of the intestinal flora. It may be that at the time of obstruction the canal is comparatively free from those bacteria which would give rise to toxic substances, or, on the other hand, it may be highly infected.

If the lecithin contained in the food is decomposed in such a way as to give rise to cholin and possibly to neurin we may demonstrate their presence. On the other hand, failure to demonstrate the presence of cholin would not prove positively that the decomposition does not go on in this way; since, as in all experiments on the digestive tract, the substances formed are either further modified or are absorbed so rapidly that it is almost impossible at any one time to obtain a sufficient quantity for positive identification. This difficulty is naturally greatly increased when two or three days are allowed to elapse between feeding and the removal of the intestinal content. The intestinal content is then very small and contains so much bile that it is very difficult to handle. Out of six experiments on dogs in

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† Ueber Ptomaine, i, 39.
‡ Arch. f. exp. Pathol. u. Pharmacol., xix, 87.
It was my intention, in these experiments, to determine whether the lecithin content of the food could give rise to cholin and possibly neurin by decomposition in the intestine in cases of obstruction. The dogs used were therefore fed for two or three days before the operation of closing the intestine was performed on yolk of eggs, which is very rich in lecithin.

The following protocol from my notebook will serve to illustrate the entire series of four experiments:

Expt. 3. Friday, March 29, 2 P. M., anæsthetized dog, male, weight 55 lbs. Placed ligature around intestine just above ileo-caecal valve. Animal had been fed for three days previously on yolk of eggs. Saturday, March 30, 6 P. M. Animal quiet, does not seem very sick, drinks well but does not eat. Urine of sp. gr. 103.2, acid in reaction, no albumin; strong indican reaction. Sunday, March 31. Dog drinks but does not eat, appears much the same, urine 274 cc., sp. gr. 102.8, reaction acid, no albumin. Indican reaction strong. Monday, April 1. Dog seemed better, but about 2 P. M. managed to tear open the incision in the abdominal wall, and in consequence a loop of the intestine escaped. Dog was killed with chloroform and an autopsy made. Urine for this day up to this time, 160 cc., sp. gr. 103.1, reaction acid, strong indican reaction. It may be said that in no case was there any marked anuria, as the dogs drank freely and did not vomit. As far as the indican reaction was concerned it was strong, but not much more so than I have seen in apparently healthy dogs.

It must be remembered that the indican reactions as usually made cannot be considered quantitative, as the color is produced by oxidation of the indoxyl which cannot be regulated to give quantitative results, as the same agent at the same time produces indigo red and indigo white. I consider Baumann's the best test, namely, equal volumes of urine and strong hydrochloric acid with a few drops of ferric chloride, as there is less chance of over-oxidation by this method.

Autopsy.—Evidences of peritonitis; some excess of peritoneal fluid containing flakes of fibrin, intense venous congestion. This was found to be due to perforation at point of ligature. There were slight adhesions between neighboring intestinal loops. Renal cortex much engorged, papilae pale, capsule non-adherent. Liver hyperæmic, consistence nor-
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Gall bladder distended, contents green; adjacent tissues stained yellow. Spleen hyperemic, veins on surface distended. Heart, veins on surface distended, otherwise normal. Lungs, highly pigmented; some calcareous nodules; otherwise normal. Stomach contents small, reaction acid, whole internal surface hyperemic, pyloric third stained yellow. Two small ulcerations in cardiac portion about middle of inferior curvature.

Intestinal content small, reaction acid, intensely bile-stained and whole surface hyperemic. The acid character of the contents continued to within 15 inches of ligature and this lower portion of the ileum was very dark and had apparently lost all tone. It was filled with faecal material, bright green in color. The most of the mucous surface of the ileum was highly congested and in the lower portion it was easily separable.

In all the experimental cases, except the foregoing, in which there was perforation at point of ligature, the tendency, with ordinary aseptic precautions, is toward recovery. Plastic processes connect the intestinal walls around ligature, necrosis occurs at the point of ligature and a passage is usually established in 5-7 days. This was a source of disappointment in the earlier experiments as, in waiting for the full effects of obstruction in order to obtain as much material as possible, the experiments failed because of the escape of material through newly formed passages, re-establishing the continuity of the intestine. In those animals which were chloroformed in from 70-80 hours after ligature, there was no abnormal appearance of the internal organs, with the exception of the kidneys, in which there was much engorgement of the capsular veins and intense hyperemia of the cortex, though the papillae remained pale.

Microscopic Examination.—Kidneys showed infiltration of Bowman’s capsule, cloudy swelling of epithelium of convoluted tubules, some necrosis of the epithelium and tube casts.

Chemical Treatment of the Intestinal Contents.—As it was my intention to ascertain by the presence or absence of cholin, whether there had been decomposition of lecithin during the obstruction of the intestine, it was first necessary to choose a method that would totally obviate, if possible, the chemical decomposition of the lecithin in the analytical processes employed. The most suggestive work in this connection is that of Marino Zuco,* who claims that, by the methods of the toxicologists, it is possible to obtain cholin from fresh tissues, blood, etc., and that the

cholin thus found originates from the splitting up of the lecithins under the influence of the acids and alkalis.

The intestinal contents of the animal described in Experiment 3 were removed with the aid of as little water as possible. Together with the water added the intestinal contents amounted to 280 cc. and had an acid reaction. The whole was treated with four times its volume of absolute alcohol and left with occasional shakings for 48 hours. It was then filtered and being still acid was evaporated in a large dish on the water-bath, the temperature of the fluid not going above 70° C. at any time; absolute alcohol was occasionally added to carry off the balance of water at the same low temperature. When the material had been reduced to a thin syrup it was mixed with a large quantity of powdered glass, evaporated to dryness in vacuo at 45°-50° C. and then placed in a Soxhlet extracter and thoroughly extracted with ether. This removes all of the lecithin, cholesterin and fats, a great deal of coloring matter, extractives, etc. It is of course understood that the method of treatment was governed by the substance sought. If no cholin was present my question could not be answered in the affirmative; on the other hand, the varying statements in reference to the ease or difficulty with which lecithin is decomposed made it imperative that the possibility of its decomposition should be avoided. Marino Zuco is the chief authority for the statement that lecithin is easily decomposed by analytical methods, and the method devised by him includes digesting on the water-bath for 24 hours at 70°. It is apparent therefore that much less injury must result from evaporating the fluid at the same temperature in one-eighth of the time. Further, I find that drying at first and extracting with ether in Soxhlet's apparatus much facilitates succeeding operations.

Schulze and Steiger * claim that in the examinations of certain seed contents made by previous investigators, all the lecithin was not extracted by ether, and they make these deductions from the fact that after shaking the finely ground seeds in a flask with a quantity of ether, allowing it to stand for some hours and then repeating the process two or three times, they were still able to obtain lecithin. This, however, is quite different from 36 hours' extraction in Soxhlet's apparatus, as in my experiment. After extracting with ether for this length of time one may rest assured that every trace of lecithin has been removed.

After the substance had been extracted with ether as described, it was removed, dried, and extracted with absolute alcohol, acidified with hydrochloric acid. Of the more common putrefactive bases only the chlorides

of cholin and neurin are soluble in absolute alcohol and also the chlorides of some of the amines. The alcoholic extracts were united and evaporated to a small bulk and were then treated with an alcoholic solution of platinum chloride, the precipitate was thoroughly washed on a filter with alcohol and ether, and was then dissolved off with cold water, in which it proved to be almost entirely soluble. This solution of the platinum chloride double salt was then decomposed with hydrogen sulphide, was boiled and filtered, and a portion of the filtrate was neutralized and tested with the following alkaloidal reagents:

<table>
<thead>
<tr>
<th>Reagents</th>
<th>Reaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phosphomolybdic acid</td>
<td>abundant yellow caseous ppt.</td>
</tr>
<tr>
<td>Phosphotungstic acid</td>
<td>white ppt. crystalline</td>
</tr>
<tr>
<td>Potassium bismuth iodide</td>
<td>dark brown pulverulent ppt. somewhat sol. in excess.</td>
</tr>
<tr>
<td>Potassium cadmium iodide</td>
<td>white ppt. sol. in excess.</td>
</tr>
<tr>
<td>Potassium mercuric iodide</td>
<td>yellow crystalline ppt.</td>
</tr>
<tr>
<td>Potassium iodide and iodine</td>
<td>dark brown ppt.</td>
</tr>
<tr>
<td>Bromine water</td>
<td>reddish ppt.</td>
</tr>
<tr>
<td>Mercuric chloride</td>
<td>ppt. white, gradually becoming crystalline.</td>
</tr>
<tr>
<td>Gold chloride</td>
<td>yellow granular ppt. sol. on heating.</td>
</tr>
<tr>
<td>Platinum chloride</td>
<td>slight cloudiness.</td>
</tr>
<tr>
<td>Tannic acid</td>
<td>white finely flocculent ppt.</td>
</tr>
<tr>
<td>Picric acid</td>
<td>no precipitate.</td>
</tr>
</tbody>
</table>

The balance of the filtrate was now evaporated down and precipitated with gold chloride and filtered, the gold salt decomposed with H₂S, and the solution boiled and filtered. The filtrate gave the following alkaloidal tests:

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<td>white ppt. gradually becoming crystalline.</td>
</tr>
<tr>
<td>Gold chloride</td>
<td>yellow granular ppt.</td>
</tr>
</tbody>
</table>

On the basis of the above reactions the solution was considered to contain only pure cholin chloride, and it was therefore evaporated down, taken up in absolute alcohol and precipitated with alcoholic platinum chloride. The yellow precipitate of double salts was filtered off, washed with alcohol and ether, dried in vacuo at 100° and analyzed.
0.1457 gramme of this salt gave 0.0463 gramme platinum or 31.77 per cent. For cholin: Theory requires 31.64 per cent platinum. Found 31.77 per cent. platinum.

The presence of cholin in the intestinal contents of the animal experimented upon is therefore proven.

On the Presence of Neurin in the Intestinal Contents Examined.—From the fact that there was a precipitate with potassium cadmium iodide, tannic acid and also a slight precipitate with platinum chloride, I considered there was neurin as well as cholin present in my final solutions. Gulewitsch,* in one of the most complete chemical studies of cholin which has yet been published, draws attention particularly to the fact that Brieger and others, working with such solutions and using tannic acid to distinguish qualitatively between cholin and neurin, fell into an error in using this reagent. Cholin chloride, in acid solution, will not give a precipitate with tannic acid, but in neutral solution invariably does so. I have stated previously that neurin has been considered invariably to accompany cholin. It is possible that, using only qualitative tests, an error may have occurred when experimenters did not note whether the solution of chlorides was neutral or acid.

It has already been stated that when the platinum salt of cholin was dissolved on the filter by the free use of cold water a small quantity of a platinum double salt remained undissolved. This salt was, however, found to dissolve in hot water and when the solution had cooled, small, yellow octahedral crystals were deposited which resembled crystals of the corresponding salt of neurin as described by Gulewitsch t in his recent paper on neurin and its compounds. Now, these octahedra could not consist of the platinum salt of one of the amines or diamines, for, the former were excluded by testing the original solution from which the cholin and neurin were precipitated by the chlorides of platinum and gold, and the latter were excluded by the fact that their platinum double salts differ in crystalline character from the octahedral crystals here described. On warming gently the original solution with a slight excess of alkali or of a solution of sodium bicarbonate it was not possible to detect the odor of an amine. Unfortunately, this platinum salt, soluble only in hot water, was not obtained in sufficient amount to warrant decomposing it and performing pharmacological tests with it. Nevertheless, I consider the presence of neurin in the intestinal contents, under the experimental conditions set forth in this paper, as almost a certainty.

† Zeitschr. f. physiol. Chem., xxvi, 175.
The following is a tabular statement of the reactions for cholin obtained with the intestinal contents of the dogs used in Experiments 1, 2 and 4, after these contents had been subjected to the chemical treatment already described. In none of these experiments was enough of a salt of cholin obtained to warrant an analysis. The reactions are stated very briefly, but coincide entirely in appearance and character with those given under Exp. 3, and prove that cholin was present in the intestines of all of these animals though in less amount than in those of the animal used in Exp. 3:

<table>
<thead>
<tr>
<th>REAGENT</th>
<th>EXP. 1</th>
<th>EXP. 2</th>
<th>EXP. 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phosphomolybdic acid</td>
<td>ppt.</td>
<td>ppt.</td>
<td>ppt.</td>
</tr>
<tr>
<td>Phosphotungstic acid</td>
<td>ppt.</td>
<td>ppt.</td>
<td>ppt.</td>
</tr>
<tr>
<td>Pot. bismuth iodide</td>
<td>ppt. brown</td>
<td>ppt. brown</td>
<td>ppt. brown</td>
</tr>
<tr>
<td>Pot. cadmium iodide</td>
<td>ppt. white</td>
<td>ppt. white</td>
<td>ppt. white</td>
</tr>
<tr>
<td>Pot. mercuric iodide</td>
<td>ppt. yellow</td>
<td>ppt. yellow</td>
<td>ppt. yellow</td>
</tr>
<tr>
<td>Pot. lodine and iodine</td>
<td>ppt. brown</td>
<td>ppt. brown</td>
<td>ppt. brown</td>
</tr>
<tr>
<td>Bromine</td>
<td>ppt. brown</td>
<td>ppt. brown</td>
<td>ppt. brown</td>
</tr>
<tr>
<td>Mercury chloride</td>
<td>ppt.</td>
<td>ppt.</td>
<td>ppt.</td>
</tr>
<tr>
<td>Gold chloride</td>
<td>ppt.</td>
<td>ppt.</td>
<td>ppt.</td>
</tr>
<tr>
<td>Platinum chloride</td>
<td>ppt. yellow</td>
<td>slight cloudiness</td>
<td>slight cloudiness</td>
</tr>
<tr>
<td>Tannic acid</td>
<td>slight cloudiness</td>
<td>slight cloudiness</td>
<td>slight cloudiness</td>
</tr>
<tr>
<td>Picric Acid</td>
<td>slight cloudiness</td>
<td>slight cloudiness</td>
<td>slight cloudiness</td>
</tr>
</tbody>
</table>

The absence of the precipitates with tannic acid in Exps. 1 and 2 were due most probably to the fact that the solution was acid, whereas in 3 and 4 it was neutralized before tests were made.

On the Occurrence of a Ptomaine accompanying the Cholin and Neurin.
—In Experiment 1, in which the dog died during the night and was examined the following morning, a ptomaine was found which possessed the following characteristics. Its hydrochloride is very soluble in alcohol and water and crystallizes in fine needles. It gave all the reactions of cholin, but both the platinum and gold salts were quite insoluble in cold water and difficultly soluble in hot water, the gold salt being more easily soluble than the platinum one. The platinum salt, which had been dissolved in hot water and filtered clear into a watch glass, on cooling gave a deposit, which, under the microscope, had the appearance of small, bright yellow spheres which reacted towards light like crystals. An iridescent scum formed on the surface of the water from gradual decomposition of the salt. The gold salt was dissolved in hot water and acidulated with hydrochloric acid, and, as it appeared to be reducing, the liquid solution was quickly filtered and cooled. On cooling it showed a fine yellow granular deposit of spheroidal crystals. On examination the following morning the deposit was quite
dark and a beautiful mirror had been formed on the sides and bottom of the crystallizing dish as well as on the surface of the liquid. Both the platinum and gold salt, therefore, are easily reduced compounds. It is also to be noted that the free base has a penetrating, sweetish odor, and that it is easily oxidized to a brown resin when its solutions are left exposed to the air.

The amount of this ptomaine at my disposal was insufficient for establishing its identity. It agrees in some of its properties, though not in others, with a ptomaine, C₅H₁₇N, obtained by Gautier and Étard * from putrefying mackerel and from the decomposing flesh of the ox and horse. It resembles, perhaps, more closely a base, C₁₉H₁₇N, which has been isolated from sea-polyps in an advanced stage of putrefaction by de Coninck.† The hydrochloride of de Coninck's base forms fine, deliquescent needles, changes to a brown resin when exposed to the air, and both the platino- and auro-chloride are decomposed by boiling water.

In the intestinal contents of animal No. 4 apparently the same base was present, for, on shaking out the ether from the Soxhlet apparatus with acidulated water a few milligrams of a gold salt were obtained which resembled the gold salt already described, and on heating, burnt with a smoky, oily flame which gave off a disagreeable odor.

**SUMMARY.**

My experiments lead me to believe that complete occlusion of the small intestine at its lower end will give rise to the occurrence of cholin, neurin and perhaps other bases, provided the food taken contains any considerable quantity of lecithin. It is not improbable that still other poisons are formed by bacterial action from other constituents of the food in cases of intestinal obstruction. While cholin would have to be absorbed in relatively large amounts to exert a marked toxic action in human beings it is otherwise with neurin, which is many times more intense in its action and must be classed with the exceedingly active poisons. It has been shown both by the experiments of Schmidt and Weiss and also by those recorded in this paper that the poisonous neurin may be formed from cholin by bacteria. In its physiological action neurin agrees closely with muscarin; especially to be noted here is the paralytic action on the heart and its power to

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* Vaughan and Novy, Ptomaines and Leucoaines, 3rd edit., 316.
† Ibid., 318.
increase the intestinal movements to such an extent that continual evacuations occur. Whether the ptomaine which was found by me is poisonous I cannot yet say. It must be considered proved, however, that highly toxic substances may arise in the intestinal canal during its complete occlusion. The method of treating cases of intestinal obstruction, before surgical means are resorted to, namely, washing out the stomach and as much of the gut as possible often reduces the violent peristalsis and this is due, perhaps, to the removal of substances out of which irritating and toxic products are formed by bacteria.

In conclusion, I would remark that our knowledge of the fate of lecithin in the digestive canal under normal conditions is very deficient. The assumption that it is saponified by the fat-splitting enzyme of the pancreatic juice, thus yielding cholin, glycerophosphoric acid and fatty acids, rests on the work of Bókai * in 1877 and, as that investigator himself admits, without excluding bacterial action. This omission throws grave doubts on the results. If the assumption of Bókai be correct, caution must be observed in the use of some foods that have been considered most nutritious and healthful; for instance, the ingestion of a meal made up largely of eggs would hardly be without danger because of the poisonous action of the large quantity of cholin liberated from the lecithin and the probability of the formation of the highly poisonous neurin.

It is my purpose in the near future to examine this question with the help of modern methods.