First. The bowel can not return to its normal position within the cavity of the abdomen, which, by reason of the perforation, is the site of the most intense inflammation of the adjacent peritoneum and intestines. In the old method, when the bowel is returned, it will come to rest at this point and bathe the sutures in pus, with the consequence that the sutures are more liable to pull out.

Second. Acute obstruction is not so liable to follow for the same reason.

Third. The contents of the bowel will flow outside of the abdomen and not inside in case the sutures in the bowel tear out.

Fourth. An artificial anus can easily be made should partial or complete obstruction develop.

The toilet of the peritoneal cavity is important. The constant repetition of the procedure carried out in every reported case was principally to impress this fact. Every part of the greater peritoneal cavity from the pelvis to the diaphragm should be thoroughly flushed out with warm normal salt solution. The flushing should be continued until the fluid coming out is clear.

There is always fluid in the abdominal cavity following a perforation. Sometimes it is almost amber colored, like ascitic fluid. At other times it is flocculent, dirty and bloody, and may contain bands of organized lymph and fecal matter. To leave this in the abdominal cavity is a serious mistake. It is only a pabulum for micro-organisms when present. The fluid can not be removed by sponges at all thoroughly, and flushing is the only procedure to follow. After flushing, dry the abdominal cavity by means of sponges or by means of a suction syringe, or both.

When this is done the cavity will be in as nearly a normal condition as it is possible to make it under the circumstances.

The insertion of a large glass drainage tube to the bottom of the pelvis is for the purpose of removing any fluid collecting there.

The patient being placed in Fowler's position, the fluid can not go elsewhere than in the pelvis. As a rule, there is but little fluid to remove after all of the salt solution is out.

The drainage tube or gauze is removed as soon as possible, depending on the amount of fluid and the nature of the organisms present at the time of operation.

Fecal fistulas are not pleasant nor to be desired by either the patient or the surgeon, but do not give much trouble, and usually close spontaneously.

I am deeply indebted to Dr. A. Letevec³ for the interest shown in making many microscopic examinations and cultures of the abdominal fluid. This procedure is very necessary. It aids in the after-treatment, and it also aids in making a more certain prognosis.

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Medicine as a science and as an art ranks higher than it has ever done. Physicians and surgeons win victories over disease and death of which our predecessors never dreamed. We have progressed and the future holds for us even better things. Let us have done with the prevailing pessimism; it is an infection which has been sown into our ranks from the ranks of unbelievers. We know better things of medicine than they and we must not let the unbelief of an outside few deter us in our onward and upward march.—*Western Medical Review*.

THE EXTRACTION OF THE INTRACELLULAR TOXIN OF THE COLON BACILLUS.

WITH A PRELIMINARY STATEMENT CONCERNING ITS PHYSICAL AND CHEMICAL PROPERTIES.

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INTRODUCTION.

The large tanks for the growth of bacteria in mass in use for some years in the hygienic laboratory of the University of Michigan permit the collection of bacterial cell substance in large amount and thus afford opportunity for the study of cellular chemistry not obtainable in any other way. Already several researches along this line have been carried on in this laboratory, and recently Dr. Vaughan has published his theory of the composition of bacterial cells and the principles of immunity developed from these studies. It is the purpose of this paper to detail some of the work which has been done recently in the extraction of bacterial intracellular toxins, with special reference to the toxin of the colon bacillus, and to give some preliminary statements concerning its physical and chemical properties. The method for the extraction of the toxin has been followed repeatedly and with several species of bacteria, so that now it can be given as reliable in results, and it is believed suited for general application.

EXTRACTION OF TOXIN.

The details of the method for extracting the bacterial intracellular toxins may be stated as follows: The organism to be studied is grown on 3 per cent. agar on the large tanks for from ten to fourteen days at room temperature. When the tanks are kept at 38 degrees centigrade, a shorter time may suffice to give the best yield. When the growth has ripened, the layer of bacterial substance is detached from the agar with glass rakes and drawn into flasks by means of suction pumps. Should the growth be too dry to be removed in this way, sufficient 95 per cent. alcohol is added to permit the suspended germ substance to pass into the flask. The flasks are emptied into large precipitating jars and the germ substance washed several times with 95 per cent. alcohol by decantation. It is then covered with absolute alcohol, and is allowed to stand for several hours with frequent stirring, when it is collected on a filter and all the alcohol is allowed to drain off thoroughly. Prolonged contact with absolute alcohol hardens the cell substance and renders the subsequent extraction of the toxin difficult. The cellular substance is now placed in large Soxhlets and extracted with ether, after which it is air dried, pulverized and weighed. The process thus far is preparatory. As obtained in this manner the dry germ powder kills guinea-pigs in doses of 1 to 40,000 body weight within twelve hours.

The germ substance, thus purified, is boiled for one hour on the water-bath, under a reflux condenser, with from fifteen to twenty-five times its weight of absolute alcohol, in which 2 per cent. by weight of pure sodium hydrate had been previously dissolved. It is essential that the alcohol used in this extraction should be absolute, and that every trace of water in the germ substance should be removed before the extraction of the toxin is undertaken. If this point be neglected the toxin will be obtained in a sticky, gummy mass which does not give satisfactory results. By this process the germ

substance is divided into two distinct parts. From the golden brown alcoholic solution there is obtained a powder representing about one-third of the weight of the original dry germ powder, which is very toxic, killing guinea-pigs in small doses in about thirty minutes, while the residue insoluble in alcohol represents about two-thirds of the weight of the germ substance and is not poisonous. The germ substance does not yield all its toxin, as a rule, until the extraction with sodium hydrate in alcohol has been repeated at least three times. Boiling the germ substance thus with sodium hydrate in alcohol splits the cells along certain definite lines of cleavage, for when the extraction has been repeated several times, boiling for one hour at each extraction, it will be found that all the toxin is dissolved in the alcohol, that no more can be split off, and that the portion insoluble in the alcohol is now non-toxic. After these portions have been separated by filtration, the filtrate, which contains the toxin dissolved in absolute alcohol, and is alkaline in reaction, is neutralized with hydrochloric acid. This throws down practically all the base and acid as sodium chlorid, which is removed by filtration. There is thus obtained a solution of the toxic part of the germ cell in absolute alcohol. This is evaporated in vacuo at a temperature of 40 C., redissolved in absolute alcohol to get rid of remaining traces of sodium chlorid and again evaporated in vacuo at 40 C. The toxic part of the cell substance obtained in this way, provided there is no water present, forms a brown scale on the dish which may be pulverized to a brownish powder that is toxic in very small doses.

It is believed that this poisonous substance is an integral part of the bacterial cell split off from it by the alkali, and that it is a synthetic product formed by the germ during its growth. In order to demonstrate this point the colon germ was grown for seven days in flasks containing Fraenkel's modification of Uchinsky's medium of the following composition:

Water	10,000 parts.
Sodium chlorid	50 parts.
Asparagin	34 parts.
Ammonium lactate	63 parts.
Di-sodium hydrogen phosphate	20 parts.

After a week's development the contents of the flasks were poured into from two to three volumes of 95 per cent. alcohol. The precipitated substance was filtered out and put into absolute alcohol. Filtered from this it was extracted in Soxhlet with ether, then dried and powdered. From this germ substance the process for the extraction of a poisonous body with 2 per cent. sodium hydrate in absolute alcohol was carried out as given above. On concentration of the alcoholic solution there was obtained a poisonous body just as from the extraction of germ substance grown on agar. The same dose kills guinea-pigs in thirty minutes, with characteristic symptoms of colon toxin. Also, the toxin obtained from germ grown on Fraenkel's modification of Uchinsky's medium gives all the proteid color tests hereafter described as being obtained from colon toxin from agar-grown cultures. This demonstrates the fact that the toxic body is an integral part of the germ split off from the cell by alkali; and in consideration of the apparent proteid nature of the toxic part, set forth in detail subsequently, it is evident that the bacterial cell must synthetically produce this proteid body during its growth from the chemical compounds of the medium.

PROPERTIES OF THE CRUDE SOLUBLE TOXIN.

The poison split off from the cell substance by the

method above given is designated as the crude soluble toxin; "crude" because it is undoubtedly a mixture of a number of substances, and "soluble" in contradistinction to the germ substance from which it has been obtained and which is insoluble.

The brownish toxic powder from the colon bacillus is very hygroscopic and dissolves freely in water, forming an opalescent solution, acid in reaction, and pale yellow to golden brown in color, according to its strength. The opalescence is removed by filtration through a hardened filter, but the toxicity of the solution is not thereby diminished, showing that a small but non-toxic part of the powder is insoluble in water, but the larger part is so freely soluble that for practical purposes the opalescence may be disregarded. On neutralizing the opalescent solution with sodium hydrate it becomes clear; on neutralizing with sodium bicarbonate, a brownish non-toxic precipitate is formed, small in amount, and when this is removed by filtration the solution obtained is clear and highly toxic. In absolute alcohol the solution is perfectly clear. After long standing in an absolute alcohol solution, there is a small part that settles out and becomes insoluble; also, after repeated evaporation to dryness and resolution in absolute alcohol, a small part each time becomes insoluble. The powder is insoluble in ether, chloroform and petroleum ether. Each of these will remove a very small amount of fatty substance, which is non-toxic, but they do not dissolve an appreciable amount of the poison. The powder is soluble in strong mineral acids, remaining clear on being boiled and on diluting with water. A few drops of mineral acid, however, added to a water solution, causes a precipitate. This would seem to indicate that the acidity of the water solution of the toxin is caused by the presence of some organic acid.

Although the toxic powder is so readily soluble in absolute alcohol, the tests point to the presence of an albuminous or proteid body. Practically all the proteid color reactions are obtained, with the exception of Molisch's test. It is interesting, too, that the part separating out from an alcoholic solution on long standing gives none of the proteid color reactions, indicating that the proteid part is permanently soluble in absolute alcohol. The Millon's reaction shows most perfectly, even in dilute solution, as does also the biuret test. Heated with strong nitric acid a clear pale yellow solution is formed, which, when ammonia to excess is added, turns to a deep orange color, thus giving the xanthoproteic test. The reaction of Adamkiewicz is positive, for when the substance is heated with acetic acid and sulphuric acid is added, a beautiful violet color develops. On boiling with concentrated hydrochloric acid, to which a drop of concentrated sulphuric acid has been added, the powder passes into solution and a violet color results, thus giving Lieberman's test. The reaction of Adamkiewicz is supposed to be referable to the presence in the proteid molecule of aromatic groups, together with a carbohydrate radicle, while Lieberman's reaction is conceded to depend on the formation of furfural, and, although the toxic substance gives both these reactions perfectly, it does not respond to Molisch's test, which is given by the formation of furfural on the splitting up of a carbohydrate group with sulphuric acid. Neither does the powder reduce Fehling solution, either directly or after boiling with dilute mineral acid.

The ordinary test for sulphur in proteids, that of heating with excess of sodium hydrate in the presence

of a small amount of the acetate of lead—is not given by the portion of the germ split off by alkali in absolute alcohol. If, however, a portion of the substance in a test tube is fused with metallic sodium, and the cooled mass treated with water, a few drops of a freshly prepared solution of sodium nitroprussiate added to part of the clear filtrate, give a beautiful violet color, indicating the presence of sulphur. Also, if the other part of the clear filtrate be treated with a lead acetate solution and acidified with acetic acid, lead sulphid is precipitated. If the solution is acidified before lead acetate is added a faint but unmistakable odor of hydrogen sulphid is detected. It is known that sulphur may exist in the albuminous molecule in at least two forms, one part being readily split off on heating with dilute alkalies as hydrogen sulphid, the other being obtained only when the destruction of the proteid molecule is carried much farther. Since the nitroprussiate reaction is very delicate, no conclusion as to the amount of sulphur can be drawn from this test, and although a good precipitate of lead sulphid is formed, the amount of sulphur is probably not large, since Leach, in this laboratory, failed entirely to find sulphur present in the ash of the colon bacillus. It may be of interest to note parenthetically that both the germ substance and the residue which remains after extraction of the toxin, respond to the nitroprussiate test for sulphur, and also give the lead sulphid precipitate in the clear acidified filtrate from the fused mass.

A solution of the toxic substance is not coagulated by heat in acid, neutral or alkaline solution, though, as already stated, a few drops of a mineral acid causes the appearance of a considerable precipitate which is not soluble on heating or on the further addition of acid. This precipitate is produced regardless of whether or not the opalescence of the water solution is first removed.

Among the metallic salts, copper sulphate produces no precipitate and ferric chlorid only on heating. Silver nitrate naturally precipitates any trace of chlorids present, but after the addition of an excess of ammonia there still remains a small precipitate. Potassium ferrocyanid gives a precipitate, also potassium bismuth iodid in acid solution. Lead acetate, mercuric chlorid, and platinum chlorid all produce heavy precipitates. These latter precipitates have been examined more in detail and will be referred to again.

From a water solution of the powder, bodies giving proteid reactions may be salted out by the addition of ammonium sulphate or sodium chlorid to saturation, but on the removal of the neutral salt from the filtrates, these filtrates still respond to the proteid color tests. In the case of the salting out with ammonium sulphate, the precipitate shows little physiologic effect, whereas the residue from the filtrate, after removal of the ammonium sulphate, is still toxic and still gives the proteid color tests.

NO EVIDENCE OF ALKALOIDAL BODY IN CRUDE SOLUBLE TOXIN.

Phosphotungstic, phosphomolybdic and picric acids all give abundant precipitates. Since these reagents are also used in the precipitation of alkaloidal bodies, the precipitates with phosphomolybdic and phosphotungstic acids were further examined, the possibility suggesting itself that the toxic body might be alkaloidal in nature and that the proteid part might be entirely separate from the poison. A sample was precipitated with phosphomolybdic acid in acid solution, the pre-

cipitate removed, washed and dissolved in ammoniacal water. This solution was then shaken with amyl alcohol. The alcohol was not colored and the residue obtained on concentration was so slight as to be practically nothing. Another sample was precipitated with phosphotungstic acid, the solution being acid in reaction. The precipitate was allowed to settle, removed by filtration, washed with acidulated water, decomposed with a saturated solution of barium hydrate and the remaining insoluble part filtered out. So far as possible the barium was removed from the filtrate with carbon dioxide, alternating with concentration and further addition of carbon dioxide. The solution was then allowed to concentrate to dryness, when the residue was dissolved in absolute alcohol, leaving barium salts behind. On concentrating the slightly opalescent solution, more barium salts came down during the process and were filtered out. The dry residue was taken up in water and ammonium carbonate used to precipitate the barium that still remained. After removing the barium carbonate, by evaporating on the water bath, both carbon dioxide and ammonia were expelled, the solution again becoming acid. Dryness being reached, absolute alcohol was once more used, leaving undissolved a small amount of inorganic material. In this way the final residue after evaporation of the alcohol was practically freed from inorganic impurities. Sulphuric acid no longer gave a barium precipitate in water solution. The amount obtained by this method was very small and an exceedingly small part of the original toxic powder used. Since the substance obtained in this way still gives good Millon's, biuret and xanthoproteic proteid reactions, it is fair to say that it is not alkaloidal. The very small amount obtained by this method was given a guinea-pig intra-abdominally and, though the animal was sick, it did not die. Either phosphotungstic acid does not precipitate the toxic body or else the amount obtained was less than a fatal dose.

Should the toxic powder contain an alkaloidal body existing as a salt in the acid solution, the possibility of extracting the base with ether or chloroform after the solution had been made alkaline with ammonia, is apparent. This was tried with negative results. To a water solution of the toxin, acid in reaction, ammonia was added, drop by drop, to a slightly alkaline reaction, the mixture shaken with ether, the ether separated and evaporated. The residue left was non-toxic. The ammoniacal water solution was next shaken with chloroform, the slightly colored chloroform drawn off and evaporated at low temperature, leaving a small amount of a dark, thick semi-liquid, which was non-toxic either as it was or after faintly acidifying with hydrochloric acid. The water solution remaining being still toxic, it is evident that the toxic part is not an alkaloidal body capable of being extracted directly.

Potassium bismuth iodid in acid solution of the crude soluble toxin produces an abundant precipitate, apparently more or less soluble in excess. This precipitate is soluble in ammoniacal water. On shaking with amyl alcohol so small an amount of substance is extracted as to be practically nothing.

Lead acetate produces a heavy precipitate which settles readily. The Millon test is given by the filtrate in this case and not by the precipitate. After removing the lead from both parts by means of hydrogen sulphid, both solutions were concentrated to a thick syrup. The residue from the filtrate still gave the Millon test, while that from the precipitate did not.

The residue from the filtrate was toxic for guinea-pigs, that from the precipitate was not, showing that lead acetate does not precipitate the toxic part. A determination of nitrogen by the Kjeldahl method in the toxic residue from the filtrate showed 9.90 per cent.

A saturated water solution of mercuric chlorid produces in a water solution of the poisonous powder, acid in reaction, an abundant rather curdy or fibrous-looking precipitate of a brownish color, but here again the proteid reactions are given by the filtrate and not by the precipitate and again after the mercury has been removed by hydrogen sulphid, the residue from the filtrate is toxic, that from the precipitate is not.

Platinum chlorid gives a good precipitate in both water and alcoholic solution of the crude soluble toxin. The alcoholic precipitate is perhaps the more abundant. It has been found that from 10 to 15 per cent. of the crude poison is thus precipitated. The precipitate is insoluble in water, hot or cold, acid, alkaline or neutral in reaction, in alcohol, ether, chloroform or acetone, so, naturally, all attempts to crystallize it failed. It gives the Millon test perfectly, also the biuret and xanthoproteic reactions. These tests are not given by the filtrate or by the residue from it after removal of the platinum by hydrogen sulphid. The precipitate after removal of the platinum is very toxic, having twice the toxicity of the crude poison.

As has already been stated, that part of the germ split off by alkali in alcoholic solution and designated as the "crude soluble toxin" is undoubtedly a mixture of substances. At present it is not known whether the toxic part itself is a single chemical compound or not. There is no evidence that it is an alkaloidal body, and it is interesting and it may be significant as well, that wherever the toxic part is found the proteid color reactions are given also. Another apparently constant factor is the acidity of the poisonous body. In places where, by precipitations, washings and purifications, there is no chance for any trace of inorganic acid used to remain, this acidity of the toxic part still remains, e. g., when the toxin has been precipitated by platinum chlorid in alcoholic solution, the precipitate carefully washed with alcohol, the platinum removed by hydrogen sulphid and the clear resulting solution concentrated to dryness on the water bath, the very toxic residue remaining is still acid in reaction.

TOXIN BROKEN UP WITH CONCENTRATED HYDROCHLORIC ACID.

Assuming the compound nature of the toxic substance and the possibility of splitting off therefrom a less complex toxic group, the effect of breaking up the crude poison by prolonged heating with concentrated hydrochloric acid was tried. Samples of the toxin were heated on a boiling water bath for five hours with from 20 to 30 volumes of concentrated hydrochloric acid. Practically everything was dissolved, the solution being very dark brown, almost black. This solution, after filtering, was neutralized with sodium carbonate and evaporated to dryness on the water bath, when the residue was thoroughly extracted with absolute alcohol, forming a pale yellow solution. This, on the addition of a saturated alcoholic solution of mercuric chlorid, gave a heavy white precipitate which, after thoroughly washing with alcohol, was dissolved so far as possible in boiling water, the precipitate being difficultly soluble in a large amount of hot water and leaving an insoluble part. The clear solution was concentrated on a water bath and from it were obtained sheaf-like ag-

gregations of rather long, narrow, fragile-looking prisms. These were filtered out, put into water, and the mercury removed by hydrogen sulphid. On evaporation there remained a small amount of a brownish residue, acid in reaction again and very toxic, the symptoms produced resembling those given by the crude soluble toxin. It is known also that this residue dissolved in alcohol gives a precipitate with alcoholic platinum chlorid. Work is now in progress in the preparation of both the mercury and platinum compounds in quantities sufficient for their study and identification.

CONCLUSION.

Briefly, then, there is split off from the germ substance of the colon bacillus by treatment with a 2 per cent. solution of sodium hydrate in absolute alcohol a crude soluble toxin, leaving undissolved a residue which is almost completely soluble in water but which is not poisonous. That a definite cleavage takes place is shown by the fact that the germ substance is split so decidedly into two distinct and different parts, a point being reached where no more toxin can be split off, thus leaving an inert residue. The toxin is obtained whether the germ be grown on agar or on Fraenkel's modification of Uschinsky's medium, demonstrating that it is an integral part of the cell. The use of the modified Uschinsky's medium shows also that the organism is able to build the toxin synthetically from chemical compounds.

This poisonous substance, freely soluble in water, but more readily and perfectly in absolute alcohol, gives all the ordinary proteid color reactions, but does not yield a reducing carbohydrate. There is no evidence that it is alkaloidal in nature, the toxic part being precipitated directly by platinum chlorid alone, with the probable exception of phosphotungstic acid, and in both these cases the toxic body obtained still gives the proteid reactions. It may be broken up by concentrated acid, and a toxic crystallizable body precipitated by the use of mercuric chlorid.

The cell substance of typhoid and anthrax bacilli, as well as that from the colon bacillus, is broken up by dilute alkali in alcohol at 78 degrees, into portions A and B, the former being in solution in the alcohol while the latter forms an insoluble residue. Both A and B are soluble in water and aqueous solutions of A are poisonous, while similar solutions of B are inert or relatively so. Portion A from the colon bacillus, at least (the others not having been so thoroughly studied as yet), can be divided into two portions, *a* and *a'*, by precipitation with platinum chlorid. Portion *a* constitutes from 10 to 15 per cent. of A and is poisonous, while *a'* is inert. The proteid color reactions follow the poisonous portion in these separations. By breaking up portion A with concentrated hydrochloric acid, a crystalline toxic body is obtained.

The relation of these poisonous, alcohol-soluble proteid derivatives to similar substances obtainable from other proteids, also their relation to the alcohol-soluble proteins obtained by Osborne from seeds, are matters for further investigation.

A detailed study of the action of the poisonous portion on animals will be presented in a paper by a co-worker in this laboratory.

Case Reports.—Every physician sees instructive cases not duplicated in the experience of others. If he does not report them, remarks the editor of the *Medical Council*, their lessons are lost to medical science.