

in doses of from 1/6 to 1/2 grain. With great restlessness and jactitation hyoscin hydrobromate is indicated. In the case of children the dose must be cautiously regulated.

Ergot, belladonna, cannabis indica, gelsemium, chloral, zinc oxid and the bromids are of inferior value. They are frequently, however, given in connection with opium or morphia. Inhalations of ether or chloroform may be cautiously employed to secure temporary relief from torturing pain, jactitation and spasm. Veratrum viride and aconite are mentioned only to condemn their use in this disease. The modern analgesics may be employed alone or in conjunction with opium and its derivatives in the management of the milder cases of the disease. Large doses are to be avoided.

R. Phenacetini ..... 3|  
Chart No. viii.  
Div. S. One powder q. tertia vel quarta hora.

R. Pactophenini ..... 2|  
Sacch. alb. .... 1|  
Chart No. vi.  
Div. S. One powder q. t. v. q. h.

R. Antipyrini ..... 3|  
Sacch. alb. .... 1|  
Chart No. viii.  
Div. S. One powder q. t. v. q. h.

If the headaches and pains increase cerebrospinal fluid may be withdrawn by lumbar puncture in order to diminish the intracranial pressure. Performed early in the course of the disease and repeated at intervals, it has been frequently followed by beneficial results.

Manges has reported 3 successful cases treated by the injection of lysol in 1 per cent. solution into the spinal canal after the removal of the exudate. This method was originally suggested by Carlos Franca at Lisbon in 1903. Wolff, of Hartford, Conn., basing the procedure on an antagonism which he found to exist between the *Diplococcus intracellularis* and the Klebs-Loeffler bacillus and the fact that the former were precipitated from active bouillon cultures on admixture with diphtheria antitoxin serum, was led to use the antitoxin in the treatment of this disease with satisfactory results, and Waitzfelder<sup>2</sup> treated 17 cases in the same manner, employing from 4,000 to 10,000 units, according to the age of the patient and the severity of the symptoms, at intervals of two or three days. Of his 17 cases, 5 recovered completely, 3 died and 9 were still under observation at the time of the publication of the report, 5 of the last "giving promise of speedy recovery." These observations are now being extensively repeated. In two cases of which I have knowledge the antitoxin treatment was employed without result, but these two cases were of fulminant type, one dying at the end of forty-eight hours and the other in a little more than twenty-four hours.

During a period of depression stimulants are to be freely given. Preparations of alcohol, ammonium carbonate and the spirit of chloroform are recommended. Cold effusion may be occasionally practiced.

During convalescence the bitter tonics and iron may be given. Cod liver oil and iodid of potassium are also employed. Delirium, spasm and irritability of the stomach too often in the severe cases render the administration of medicine and food impracticable. As recovery takes place the appetite is often excellent and an abundant, easily digested and nutritious diet of mixed solid food should be given.

## THE ACTION OF THE INTRACELLULAR POISON OF THE COLON BACILLUS.\*

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It is now generally conceded that the pathogenic bacteria bring about those changes in the body cells which form the basis of what we call disease by virtue of certain poisons which they produce. These poisons are supposed to be specific for each kind of bacteria and to them the name of toxin has been given. Certain bacteria possess a toxin which is not an integral part of the cell itself, and hence is not essential to its existence. As examples of such organisms may be mentioned the bacillus of tetanus and that of diphtheria.

With these bacteria the toxin is found free in the culture medium and there are differences in opinion concerning its origin, some regarding it as a cleavage product formed by the action of the bacillus either directly or through a chemical ferment on the constituents of the culture medium, while others think that the soluble toxin represents a group of the bacterial cell which easily drops off. On the other hand, the filtrates of cultures from such bacteria as the typhoid and the colon bacillus are non-toxic, or, at the most, possessed of but feeble toxicity. In the later instance the poison exists as an integral portion of the bacillus itself and can be set free only on the death of the latter. We therefore have two distinct classes of so-called specific bacterial poisons, the one being easily cast off from the living cell and hence called an extracellular toxin to distinguish it from the toxin which can not be split off from the bacterial cell without destroying the latter, the intracellular toxin.

That the colon bacillus, when introduced into the animal body, is one of the most deadly of all bacteria is a fact which is too well known to require further proof. There is no other germ which causes more anxiety to the abdominal surgeon, and the fatal results following typhoid perforation, strangulated hernia, and appendicitis when left to pursue their course uninterruptedly are in almost all instances due to its ravages.

The colon bacillus is a normal inhabitant of the intestinal canal, and so long as it remains within the canal it is practically outside of the body and can do no harm. When it obtains an entrance into the peritoneal cavity, however, it multiplies and at the same time is disintegrated by the protective agencies of the body. As it is broken up, its intracellular poison is set free and then, and not until then, is it capable of acting on the body of the host. It is not, therefore, from the growth of the bacterial cell in the peritoneal cavity that death directly results, but rather from its destruction. Primarily, of course, it is essential that the growth of the organism should have advanced to such a degree that enough bacterial cellular substance shall be present to furnish a sufficient quantity of the poison to cause a fatal issue in the body on its liberation. The necessity of employing a large amount of the living culture in order to obtain a fatal dose of poison from it has long been urged as one of the principal arguments against the intracellular poisons being true toxins. As compared with diphtheria and tetanus, this is, indeed, true, but the comparison is hardly a fair one. Thus, in the case of the diphtheria bacillus, the same germ may go on producing toxin without loss of life, for all one knows, indefinitely, whereas, in the case

of the colon bacillus, the bacterial cell must die and undergo disintegration before its toxin can be liberated, and then only a definite amount can be obtained from each bacillus.

It is not the purpose of this paper, however, to prove absolutely that the intracellular poison of the colon bacillus is a true toxin but to show that such a poison can be obtained and to compare its action on the animal body with that of the living organism. Whether or not this poison is a true toxin will be discussed when we take up the subject of immunity in a later paper. The fact that a potent intracellular poison can be obtained from the colon bacillus is of especial interest, since several authors have recently denied the existence of the so-called endotoxins, affirming that the colon and typhoid germs cause disease in the same manner as does the diphtheria bacillus, by the production of soluble poisons within the body of their host. These authors explain the absence of such poisons from artificial cultures on the ground that the medium is not suitable for their production, and that consequently the soluble toxins are elaborated solely within the animal body. It is needless to state that such a theory rests almost entirely on negative evidence and if it can be conclusively shown that the colon bacillus, when grown on artificial media, does produce a powerful poison which causes death in much the same manner as does the living germ, the endotoxic explanation would seem to be much more plausible. In discussing the action of the intracellular poison of the colon bacillus, it will be found convenient to take up the action of (1) fatal doses of living cultures, (2) fatal doses of the dead germ substance in which the poison still exists in the bacterial cell, which has, however, been deprived of life; (3) doses of the poison obtained by chemically splitting up the bacterial cell by boiling with a 2 per cent. solution of sodium hydrate in alcohol. In this case the poison is obtained in a comparatively free form.

#### THE ACTION OF THE LIVING GERM.

When a guinea-pig is inoculated with a fatal dose of the living colon germ, practically no symptoms whatever are noticeable for a period varying from five to seven hours, according to the size of the dose given. This may be considered as the period of incubation and is roughly proportional to the amount of living germ injected. We have always worked with a bacillus 1 c.c. of a 12-hour or older, bouillon culture of which has invariably proved fatal for guinea-pigs within 24 hours. If 1 c.c. of such a culture is given, no effects will be seen for a period of from 10 to 12 hours. If, on the other hand, 2 c.c. of the same culture be injected, the animal will begin to manifest symptoms of illness in from 8 to 10 hours, and if still larger doses are given the symptoms will become apparent in a still shorter time. This period of incubation undoubtedly represents the time taken for the bacillus to multiply and to be destroyed to such an extent that sufficient poison may be liberated through its disintegration to produce noticeable toxic effects in the animal. This period of incubation is, therefore, in reality the crisis of the disease and the outcome depends solely on whether all germs have been destroyed before a lethal dose of the poison has been set free or not. It is during this period that individual resistance and acquired immunity are important factors acting by causing increased bacteriolysis and the destruction of all germs before a fatal dose of poison has been set free. During this time the

temperature of the animal may rise to a greater or less extent or may remain stationary; the animal remains active, eats; its coat is not roughened and it appears in all respects as well as a normal animal. At the end of this period, however, the appearance changes. The animal becomes less active. it remains in one corner of its cage; its coat becomes roughened; it hangs its head and apparently enters into a state of stupor. At the same time the rectal temperature begins to fall abruptly, as can be seen from a study of Chart 1.

Indeed, this fall of body temperature is often the first marked symptom and, when occurring to a marked degree, it is invariably a bad omen. The body temperature will often fall from 101 F. to 94 F. or even lower within from 2 to 4 hours, and this fall is progressive and continuous until the animal's death, immediately preceding which a temperature as low as 87 F. or 86 F. is not uncommon. At the same time the animal shows signs of the most marked peritoneal inflammation, as is evidenced by rigidity and spasm of the abdominal muscles on pressure. At autopsy, the only gross lesion present is a marked hemorrhagic peritonitis with a large amount of bloody fluid containing intact red corpuscles and leucocytes in the peritoneal cavity.

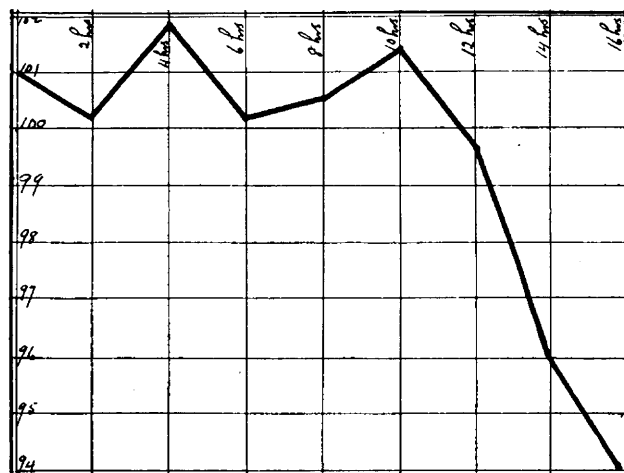


Chart 1.—Temperature curve of guinea-pig after inoculation with 1 c.c., 16 hr. bouillon culture of the colon bacillus. Death occurred 20 hours after inoculation.

The parietal and visceral peritoneum are studded with minute punctiform hemorrhages. Hemorrhage is an especially prominent feature in the great omentum and is present to a less marked degree in the mesentery.

#### THE ACTION OF THE DEAD GERM.

The dead germ substance used in the following work was obtained by growing a large amount of the colon germ on tanks filled with agar for a period of two weeks at room temperature. At the end of this time the growth was removed from the tanks and extracted with absolute alcohol and ether. The crude bacterial substance thus obtained was reduced to a fine powder by pulverization in an agate mortar and was then ready for use.

It is interesting to note that the person who did the pulverizing was often quite seriously poisoned during the process unless he took the precaution of wearing a mask which hindered the inhalation of the powder. The symptoms of such poisoning were exceedingly interesting. The first thing noticed was a marked irritation of the nasal mucous membrane and a huskiness of the voice, due no doubt to the mechanical irritation

of the inhaled powder. This was followed by a feeling of depression and malaise, and chilly sensations. Occasionally a decided chill would be experienced. It is unfortunate that no accurate observations of temperatures were taken in these cases. Nausea and even vomiting were occasionally noted. After a period of discomfort varying from 6 to 10 hours, during which the patient often complained of dull pain in the various joints, recovery would rapidly and completely take place.

On examining the powder obtained in this manner microscopically we found that it consisted of colon bacilli which still retained their morphologic characteristics and could still be stained by anilin dyes. On the other hand, cultures made from this powder have, of course, never given a growth. In other words, the bacillus has not been broken up by this treatment, but simply has been deprived of life and of the power of reproduction. It is worthy of note that neither by the action of alcohol, ether, physiologic salt solution, distilled water nor any simple solvent have we been able to extract a poison from the colon bacillus. Nor, again, can a poison be split off by the action of heat even when the germ substance is heated to 184 degrees C. in a sealed tube for 30 minutes. It is only when we make use of agents which will chemically break up the colon bacillus that we are enabled to obtain a poison apart from the rest of the germ substance. The powdered germ substance is not soluble, but can be held in suspension in normal salt solution and, since it can be boiled without appreciably affecting its toxicity, suspensions were always heated to 100 degrees for 15 minutes before injection in order to insure sterility.

This coarsely powdered germ substance killed guinea-pigs when injected intraperitoneally in doses of 1 to 40,000, body weight, and invariably proved fatal within 12 hours, usually causing death at the end of from 6 to 8 hours. Smaller doses did not produce a fatal result. On the injection of a fatal dose of the germ substance intraperitoneally, we noticed that the most marked change was in the length of the period of incubation. Thus, whereas in the case of the living germ from 8 to 12 hours passed before noticeable symptoms appeared, in that of the dead germ substance the animal almost invariably showed symptoms of illness at the end of 4 hours. In regard to the character of these symptoms it may be stated that they are similar in all respects to those induced by the living germ. The temperature remains the same or may rise slightly during the first two hours. At the end of the 4 hours it has begun to fall and there is a decided drop from then on until the time of death, provided the dose given is a fatal one. If a non-fatal dose has been injected intraperitoneally the temperature, as will still be seen from reference to the accompanying chart, has reached a minimum at the end of from 6 to 8 hours and has returned to normal again in from 12 to 20 hours.

Moreover, as a general rule, it may be stated that the fall in temperature in non-fatal cases seems to be directly proportional to the amount of bacterial substance injected. That this should be the case seems to be only natural when we consider the fact that in this instance we have largely done away with that factor which is known as the individual resistance of the animal. As has been previously mentioned in the case of the living bacillus, the individual resistance plays an important part in determining the amount of poison which will ultimately be set free in the body. For example, whereas 1 c.c. of a 12-hour culture of our colon bacillus invari-

ably proved fatal, .25 c.c. never did. The explanation of this is to be found in the fact that all animals were able to cause disintegration of all bacilli injected before a fatal dose of poison was set free. If now .5 c.c. be given some would recover, while others would die. In this case we would speak of the former as possessing a greater individual resistance than the latter. This simply means that, in the first instance the animal has possessed a sufficient quantity of bactericidal substance directly available to cause disintegration of all bacilli before the latter have multiplied to a sufficient extent to furnish enough poison to kill the animal on its liberation. On the other hand, those animals which succumbed did not possess quite enough of the bactericidal substance, or at least did not possess it in a form available for immediate use. When, however, the dead bacterial substance is given the dose of poison which the animal receives is a certain definite amount and is not capable of subsequent increase.

Accompanying the fall in temperature there is apparent lassitude, stupor and roughening of the coat. In cases in which many times the fatal dose has been given, the animals occasionally die within from 4 to 6 hours with convulsions, a feature which can now and then be observed after the injection of large quantities of the living bacillus. At autopsy we find a picture similar in all respects to that following inoculation with

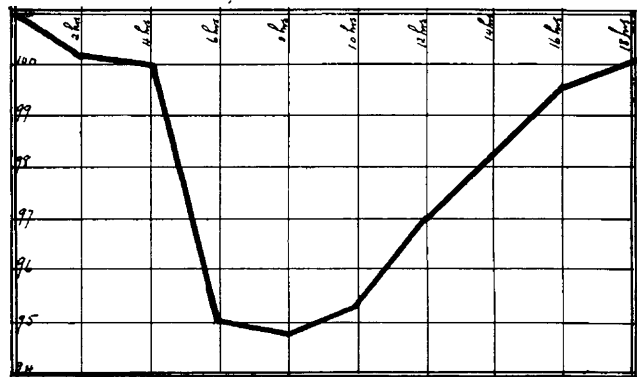


Chart 2.—Temperature curve of guinea-pig after intraperitoneal injection of non-fatal dose of crude bacterial cell substance.

the living colon bacillus. There is a marked hemorrhagic peritonitis, the peritoneal cavity containing bloody fluid, together with unabsorbed bacterial cell substance, and the omentum and mesentery showing numerous punctiform hemorrhages. It is needless to state that in all cases cultures were made from the peritoneal cavity and heart's blood immediately after death, and these proved to be sterile. From this we see that practically the sole difference between the effects following inoculation with the living bacillus and the injection of the dead bacterial substance is a shortening of the period of incubation due, no doubt, to the fact that the intracellular poison is liberated much more rapidly and in greater concentration in the second case. As will be seen later, it is not so much the absolute quantity of the poison which is injected that determines the result, as the amount which is active at a given time.

#### THE ACTION OF THE SOLUBLE POISON.

On heating the crude bacterial substance used in the above experiments with a 2 per cent. solution of sodium hydrate in absolute alcohol, precipitating with hydrochloric acid to remove the excess of sodium and evap-

orating the filtrate *in vacuo*, a residue is obtained which can be reduced to a powder which is freely soluble in water. The resulting solution is acid in reaction, and consequently is rendered neutral or slightly alkaline with sodium bicarbonate before it is injected into animals in order to avoid possible irritative effects from the acid. It may be stated here that all the poison contained in the colon bacillus is present in this portion, the residue after extraction with the sodium in alcohol being possessed of no toxic properties whatever. The poison is not obtained in a pure state and, as has been seen in the paper by Dr. Wheeler, it probably makes up but a small portion of the powder, there being more or less sodium chlorid certainly, and other constituents of the colon bacillus itself probably, present. Although we are certain that the poison is not pure, for reasons which will appear later, we are inclined to believe that it exists in an uncombined or very loosely combined state.

When doses of this powder are given intraperitoneally in amounts varying from 8 to 60 milligrams, according as to whether we have been careful to remove most of the common salt or not, a fatal result follows in guinea-pigs in from 30 to 60 minutes. Within 15 minutes after injection the temperature begins to fall and sometimes within half an hour has reached 94 F. or even lower. At first, after an interval of from 5 to 10 minutes immediately following the injection the animal appears restless, runs about the cage and shows a great tendency to scratch itself, this undoubtedly being due to itching sensations in the skin caused by irritation of the peripheral nerves. The animal then begins to show evidence of lack of co-ordination, which is rapidly followed by partial paralysis, which is especially marked in the hind extremities. This stage lasts for from 5 to 10 minutes, during the later part of which the animal usually lies quietly on one side. From this state the animal passes into what one might term the convulsive stage. These convulsions are usually clonic in nature and, as a rule, at first involve only the neck muscles, the head being momentarily drawn backward on the back. At first these convulsions are but slight in degree and are separated by considerable intervals of time. Soon, however, they become much more frequent and of much greater severity. Gradually they become more and more general in their extent until all the muscles of the body become involved in violent clonic convulsions. This stage when present invariably presages a fatal outcome. During a convulsion, or occasionally in the interval of calm, respiration ceases. The heart, however, continues to beat, at first with perfect regularity and no acceleration; indeed, the rate seems to be somewhat slower than normal. Gradually the beat becomes more and more feeble, the rate and regularity being preserved to the end. It is usually only after an interval of from 3 to 4 minutes after the cessation of respiration that the heart ceases to beat. As has been previously stated, a fatal issue, if it occurs at all, always results within one hour after injection and usually within from 30 to 40 minutes. This is to a large extent independent of whether the dose is the minimum lethal one or two or three times that amount. It is certainly entirely independent of the size of the pig. Death, of course, results at slightly different times with different batches of the poison, but even in this case the interval of time between injection and a fatal issue does not vary to any great extent. A dose which has proved to be the minimum

fatal dose for one pig will almost surely prove to be the same for another. In other words, we have done away practically entirely with the period of incubation, and the poison acts so rapidly that individual resistance plays no part; hence, the animal acts almost with the exactitude of a chemical compound into which for all practical purposes it has been converted. The period of incubation has ceased to exist since the poison is no longer contained within either the dead or the living bacillus, but is present in a free and uncombined form, capable of uniting immediately with those body cells for which it may possess a special affinity.

At autopsy no special gross lesions can be made out. The peritoneum is smooth and shiny throughout and there is not the slightest evidence of either hemorrhage or even marked congestion in the omentum or mesentery. This is a very important feature and in marked contrast to the hemorrhagic peritonitis found after injection of either the living or the dead colon bacillus. We are inclined to believe that it is the distinguishing feature between the injection of the poison in a comparatively free and in a combined state. At one time we attempted to obtain the poison by a simpler method, omitting the extraction of the crude substance with ether. The result was that on evaporation of the alcoholic filtrate we obtained a sticky residue which it was utterly impossible to pulverize or to weigh. We were compelled, therefore, to content ourselves with evaporating it to a sticky mass, which was then immediately dissolved in water. The solution of the poison thus prepared was very toxic but, as a rule, took from one to two hours or even longer to bring about a fatal result. The animals showed the roughening of the coat and the stupor characteristic of the living and dead bacillus, but not as a rule seen in the case of the powdered poison. Furthermore, the majority of these animals showed during life unmistakable signs of peritoneal inflammation. They died in convulsions. At autopsy an intense hemorrhagic peritonitis was present, which was particularly prominent in the omentum and mesentery, and hemorrhage was often present in the capsules of the liver and the spleen. From the fact that death was slower in these cases and that the symptoms were more like those seen after inoculation with the living bacillus, we are inclined to believe that in this instance the poison, although split off from the bacillus itself, still exists in combination with some other cell group and that it is essential that this combination be broken up before the poison can be set free and can act on the body cell.

Another interesting fact in this connection is furnished by the action of the poison in solutions which have been rendered strongly alkaline by the addition of sodium bicarbonate. As has been previously stated, the aqueous solutions of the poison are slightly acid in reaction and in order to avoid the irritative effects which might follow their injection into the peritoneal cavity, they were neutralized or rendered slightly alkaline by the addition of sodium bicarbonate. At first no attempt was made to secure perfect neutralization, with the result that sometimes we were making use of neutral, while again slightly or decidedly alkaline solutions were employed. It was soon noticed, however, that the results obtained in the three cases were very different. Thus, whereas in the neutral or faintly alkaline solution the injection of 60 mgs. of the powder invariably killed, in the case of a stronger alkaline solution the same amount did not cause a fatal result, although

the animals were very ill. From this fact it became evident that some change had taken place in the poison on standing in alkaline solution. In order to study this change more in detail, experiments were conducted with solutions of different degrees of alkalinity, with the results found in the following tables:

TABLE 1.

RESULTS WITH SOLUTION OF POISON BARELY NEUTRALIZED WITH SODIUM BICARBONATE AND PLACED IN INCUBATOR.

No. of Animal.	Dose of Poison.	Solution Kept in Incubator.	Weight of Pig.	Result of Injection.	Time of Death After Injection.
1.....	60 mgs.	Fresh.	325 gms.	+	30 minutes.
2.....	60 mgs.	2 hours.	330 gms.	+	20 minutes.
3.....	60 mgs.	20 hours.	370 gms.	+	20 minutes.
4.....	60 mgs.	2 days.	370 gms.	+	15 minutes.
5.....	60 mgs.	4 days.	320 gms.	+	20 minutes.
6.....	60 mgs.	6 days.	350 gms.	+	45 minutes.
7.....	60 mgs.	8 days.	350 gms.	+	20 minutes.

TABLE 2.

RESULTS WITH SOLUTION OF POISON RENDERED DECIDEDLY ALKALINE WITH SODIUM BICARBONATE AND PLACED IN INCUBATOR.

No. of Animal.	Dose of Poison.	Solution Kept in Incubator.	Weight of Pig.	Result of Injection.	Time of Death After Injection.
1.....	60 mgs. (barely neut.)	Fresh.	350 gms.	+	30 minutes.
2.....	60 mgs. (decidedly alk.)	Fresh.	370 gms.	Very sick for 2 hrs. [sick.]	Recovered.
3.....	60 mgs.	2 hours.	325 gms.	Not very Sick.	Recovered.
4.....	80 mgs.	4 hours.	310 gms.	+	Recovered. More than 5 hours.
5.....	120 mgs.	24 hours.	280 gms.	+	7 hours.
6.....	160 mgs.	3 days.	350 gms.	+	

TABLE 3.

RESULTS WITH SOLUTION OF POISON RENDERED DECIDEDLY ALKALINE WITH SODIUM BICARBONATE AND KEPT AT ROOM TEMPERATURE.

No. of Animal.	Dose of Poison.	Time at Room Temperature.	Weight of Pig.	Result of Injection.	Time of Death After Injection.
1.....	60 mgs.	Fresh.	350 gms.	+	35 minutes.
2.....	60 mgs.	12 hours.	265 gms.	Sick.	Recovered.
3.....	90 mgs.	2 days.	280 gms.	Sick.	Recovered.
4.....	120 mgs.	2 days.	460 gms.	+	20 minutes.
5.....	120 mgs.	7 days.	405 gms.	Sick for 5 hours.	Recovered.
6.....	160 mgs.	7 days.	440 gms.	Sick for several hours.	Recovered.

From the above tables it will be seen that the degree of alkalinity of the solution, and especially the length of time that the poison has stood in alkaline solution are very important factors in determining its toxicity. Thus in Table 1, in which the solution was barely neutralized, the poison seems to have retained its full potency after eight days in the incubator, whereas, in the case of the strongly alkaline solution, the potency has decreased markedly within from 24 to 48 hours. Again, there are great differences to be seen depending on whether the strongly alkaline solution has been kept at room temperature or at that of the incubator, the decrease in toxicity being much less rapid in the first instance.

A more detailed result of the effects on animals than it was possible to give in the above tables is not without interest. For example, in Table 2, No. 2, which received 60 milligrams immediately after the solution had been rendered decidedly alkaline, was very sick indeed, whereas No. 3, which received the same amount after two hours in the incubator, was only slightly affected. In the case of Nos. 5 and 6 the effects observed corresponded more closely to those obtained with the crude bacterial cell substance. It is unfortunate that the time of death was not ascertained in the case of No. 5. No. 6 did not succumb until seven hours after the injection.

On autopsy there was considerable fluid in the peritoneal cavity and the vessels of the mesentery were markedly congested. The omentum was particularly injected and a few minute hemorrhages could be made out. The most plausible explanation of the above facts is found in the theory that the poison has not been destroyed in the alkaline solution but rather has entered into chemical combination with the alkali and that we are again dealing with it in a combined instead of in a free state. The fact that the same amount will not cause a fatal result is thus readily explained, since the outcome depends largely on the rapidity with which the poison acts. If it is present in a state of combination which must be broken up before it can exert its deleterious action on the body, and if this combination is only slowly decomposed, the nerve cells, for which it apparently has a special affinity, are not subjected to an overwhelming dose at one time, as in the case of the intraperitoneal injection of the free poison.

The results obtained in animals Nos. 5 and 6, Table 3, are very interesting. In these instances there were two distinct illnesses, the first becoming manifest within from 20 to 30 minutes after the injection and corresponding in all respects to that following a non-fatal dose of what we have for convenience termed the free poison. The animals were decidedly in better condition at the end of an hour; however, they then began to show symptoms similar to those noticed after the injection of the crude cell substance, i. e., roughening of the coat, stupor and slight convulsive movements. Recovery from this state did not occur until after the lapse of from 5 to 6 hours. It is evident that here the first signs of illness were due to some of the poison which had not as yet combined with the alkali and hence still existed in the free state, whereas the later symptoms were due to the effects of the slow liberation of the same poison from its combination. In this connection it is interesting to note that the combination between the poison and the alkali which apparently takes place in decidedly alkaline solutions is not an immediate one but occurs gradually and reaches a maximum only after the lapse of a considerable interval of time. That the rapidity with which this combination is effected depends largely on temperature is shown by the fact that it occurs much more rapidly in a solution kept in the incubator than in one which is allowed to stand at room temperature.

The results which follow the injection of a fatal dose of the soluble poison intraperitoneally have already been described. When a non-fatal dose has been injected the symptoms first noticed are similar in all respects to those following a fatal dose. The animal becomes restless, shows signs of irritation of the peripheral nerves, inco-ordination and partial paralysis. The convulsive stage is not present, as a rule, and when it is noticed is evidenced solely by slight movements separated by considerable intervals of time. We have never seen a case showing marked generalized convulsions which recovered. Recovery is apparently rapid and complete and within two hours after injection the animal which has been desperately ill appears as well as any untreated animal. The maximum effect is obtained within from 45 to 60 minutes in every instance. The study of the changes in temperature in these animals is particularly interesting. Within 15 minutes the rectal temperature has begun to fall and has reached a minimum within one hour.

It remains stationary for a short time and then be-

gins to rise again and at the end of three hours after the injection has usually returned to normal or above.

On injecting the soluble poison subcutaneously, we find that the animals are able to withstand a much larger dose than when the poison is given intraperitoneally. Thus, in the case of a poison, 60 mgs. of which invariably killed when given intraperitoneally, it was found that 120 mgs. could be given subcutaneously without causing a fatal result. However, the injection of a solution containing 180 mgs. invariably caused death, the fatal issue occurring in about the same length of time as in the case of animals treated intraperitoneally. Thus, a dose of 180 mgs. always proved fatal in from one-half to three-quarters of an hour. The symptoms are practically identical with those following the intraperitoneal injection with the exception of the fact that the various stages are much more sharply defined. For example, the stage of peripheral irritation is much more marked. The animal soon after

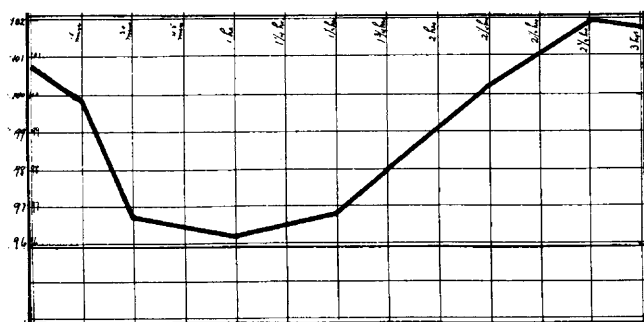


Chart 3.—Temperature curve of guinea-pig treated with 45 mgs. of the soluble poison intraperitoneally.

injection becomes very restless, runs around his cage and scratches his body. This itching seems, however, to be general from the outset and is not, apparently, more pronounced in the immediate neighborhood of the site of injection. If the animal has been injected under the skin of the abdomen, his attention is not necessarily first attracted to this spot, but he may begin by scratching his nose or one of the extremities. Another peculiar symptom, which is probably due to peripheral irritation and which is seldom seen in cases of intraperitoneal injection, is the tendency which the animals show to dig furiously in the shavings in the bottom of their cages. This feature is quite characteristic and is seldom absent in pigs which have been treated subcutaneously. The later stages are similar in all respects to those seen following the intraperitoneal injection. The animal shows symptoms of inco-ordination, lies on one side and finally develops convulsions, with failure of respiration, the heart continuing to beat regularly for some time after the complete cessation of respiration. Here also the symptoms are accompanied by a decided fall in the body temperature.

The results following the intravenous injection of the soluble poison are given in the following table:

TABLE 4.

No. of Animal.	Amount of Poison Injected Intravenously.	Time of Cessation of Respiration After Injection.	Time of Cessation of Heart Beat After Injection.
1.....	10 mgs.	4 minutes.	7 minutes.
2.....	10 mgs.	Recovered.	
3.....	10 mgs.	3 minutes.	6 minutes.
4.....	10 mgs.	Recovered.	
5.....	15 mgs.	4 minutes.	6 minutes.
6.....	15 mgs.	3 minutes.	5 minutes.
7.....	15 mgs.	4 minutes.	7 minutes.
8.....	15 mgs.	4 minutes.	6 minutes.
9.....	20 mgs.	3 minutes.	5 minutes.
10.....	20 mgs.	4 minutes.	6 minutes.
11.....	20 mgs.	3 minutes.	6 minutes.
12.....	20 mgs.	3 minutes.	7 minutes.

From the above table it will be seen that in all cases respiration ceased within four minutes after injection. Indeed, the respiratory embarrassment becomes pronounced immediately following the injection. The animal struggles for breath and there is violent retraction of the sternum. No convulsions are seen following the intravenous injection, this being probably due to the inhibitory influence of the anesthetic which has been used during the preparation of the animal for the operation. The failure of respiration in the absence of convulsions would seem to be conclusive evidence that the cessation of this function is due not to mechanical interference during a convulsive attack but to a direct paralysis of the respiratory center itself. Furthermore, the fact that the heart continues to beat in a perfectly normal manner for from two to four minutes after respiration has entirely ceased would tend to show that the immediate cause of death is asphyxia brought about by paralysis of respiration through the action of the poison.

This action of the heart after the cessation of respiration is exceedingly interesting, and is entirely analogous to that mentioned as following the intraperitoneal injection. The rate of the beat is decidedly lessened and at first the individual beats are stronger. They gradually become more and more feeble, however, until finally the heart stops in diastole, the rate after the preliminary slowing remaining unchanged until the end. It is worthy of note that in the case of intravenous injections the fall of temperature, which is so marked a feature after the intraperitoneal and subcutaneous injections of the poison, does not occur. The explanation of this fact is doubtless to be found in the very short interval of time which elapses between the injection and a fatal outcome. As regards the size of the lethal dose when given intravenously, we see that 10 milligrams often, and 15 milligrams invariably, proved fatal.

For purposes of comparison, we have always made use of the poison obtained from the same extraction in our intravenous, subcutaneous and intraperitoneal injections and have therefore been able to ascertain with a fair degree of accuracy the differences in dose required to bring about a fatal result in the three cases. Thus, whereas 60 mgs. represents the fatal dose when given intraperitoneally, it requires between 120 and 180 mgs. subcutaneously and only from 10 to 15 mgs. of the same poison to cause death when given intravenously. These differences are undoubtedly due to the rapidity of absorption in the various cases, and a fatal issue depends entirely on whether sufficient poison reaches the respiratory center at one time to cause cessation of respiration or not.

It has now been shown that a very powerful intracellular poison can be obtained from the colon bacillus. As has been previously stated, the results given in the foregoing experiments are those obtained with the poison from one extraction only. It must be understood that the poison is not in a pure state and when it is stated that 60 mgs. causes death when injected intraperitoneally we refer simply to the powder obtained from a given extraction. We have been able to procure powders which kill in doses of 15 and even as low as 8 milligrams when given intraperitoneally. This difference is in large amount due to the presence of sodium chlorid, since no attempt has been made to remove this salt in the case of the less toxic powders by redissolving in absolute alcohol.

There are several facts which lead us to believe



that this poison is the one which causes the symptoms of illness and death in animals infected with the colon germ. Most of these facts have already been brought out, but it may not be out of place to briefly recapitulate at this point. As has been previously seen, the results obtained with the living germ, the dead bacterial substance and the soluble poison can best be explained on the ground that the toxic body in each case is the same. The differences in action are not differences in symptoms but simply in the rapidity with which these symptoms become manifest. While it is undoubtedly true that in animals dying with the minimum fatal dose of the living germ, the convulsive stage is not present or is only slightly marked, it is rarely absent in cases where from three to four times the fatal amount has been given. The sole difference between the living germ and the soluble poison which would appear to demand an explanation is the lack of evidence of a peritonitis in the latter case. This, we think, is best explained by the fact that in the case of the soluble poison the toxic substance exists in an uncombined form, which, of course, is not true in the case of either the living or the dead bacterial cell. The uncombined poison is rapidly absorbed from the peritoneal cavity and hence the irritative effects which would result from its retention in this place are absent.

As has been stated, one of the first signs of the action of the poison is a lowering of the body temperature. This hypothermia is usually present to a marked degree and is noticeable before any visible symptoms occur. It is therefore, the best index which we have as to the exact time at which the poison begins to exert its effect. In the same manner, the rise of temperature after the development of hypothermia is the first indication of recovery. Moreover, if in an animal with a subnormal temperature a rise occurs, it is an infallible sign of ultimate recovery, no matter how grave the general condition may appear to be at the time. We have laid great stress, therefore, on the changes in body temperature as furnishing the most delicate test of the action of the poison. It may be here stated that the body temperature of guinea-pigs is ordinarily fairly constant within certain narrow limits, and they are much more satisfactory animals to work with in this respect than are rabbits. Moreover, their temperature does not seem to be materially altered by the injections of sterile salt solution or of such inert substances as a suspension of pumice stone into the peritoneal cavity. As has been seen in the case of the living germ, it is only after the lapse of several hours that a fall in temperature occurs. This would indicate that it is not until this time that sufficient poison is liberated to cause noticeable toxic effects in the animal. That it takes an appreciable time for the poison to be liberated from the bodies of the bacilli is well illustrated in the instance of the dead bacterial substance. Here it is only a question of dissolution of the bacilli and the setting free of the contained poison, and yet it will be noticed that an interval of at least two hours and usually longer elapses before there is any noticeable fall in temperature. The maximum effect in this case is reached between four and six hours, and if the dose has been a non-fatal one, recovery begins at the end of from eight to ten hours, as is indicated by the upward trend of the temperature curve at this point. In the case of the soluble poison, the toxic effect begins at once. Within fifteen minutes the temperature has begun to drop and within an hour has reached a minimum.

Recovery then begins and within three hours the effect of the poison has worn off, as is best evidenced by the return of the body temperature to normal or above at this point.

As has been previously mentioned, it is not the purpose of this paper to prove that this poison is a true toxin; such proof can be established only by the demonstration of the production of an antitoxin. It is our intention to treat of this subject in a later paper. Again, it is not claimed as yet that the intracellular poison of the colon bacillus is specific in the sense that similar poisons can not be obtained from other sources. A comparison between the poison of the colon bacillus and certain poisons obtained from other proteid substances will form the basis of a subsequent paper.

Finally, as has been shown in the paper of Dr. Wheeler, this poison can be obtained from the colon germ when the latter is grown on a medium which contains no proteids. This would seem to indicate conclusively that the colon germ is able to build up its poison synthetically, since the latter is unquestionably a proteid body.

#### CONCLUSIONS.

The conclusions which we have drawn from the above data may be summarized as follows:

1. The colon bacillus produces a powerful poison when grown on artificial media.
2. This poison is intracellular in character, and is contained within both the living and the dead bacterial cell.
3. The poison can be separated from the other constituents of the bacterial cell only by means which chemically break up the latter.
4. The peritonitis which occurs after intraperitoneal inoculation with the colon bacillus is due to the presence of the poison in a combined and not in a free state.
5. The intracellular poison of the colon bacillus causes a marked fall in the body temperature.
6. The poison of the colon bacillus apparently causes death by paralysis of respiration.
7. The intracellular poison is an essential group of the bacillus, and can be built up synthetically on proteid-free media.
8. This intracellular poison is the poison which causes death in animals inoculated with cultures of the living colon bacillus.

#### THE DIAGNOSIS OF SYPHILOMA OF KIDNEYS.\*

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It will not be possible to consider all the syphilitic diseases of the kidney in the few minutes allotted to this subject. There can be no question that syphilis as an etiologic factor must be seriously taken into the consideration of the inflammatory kidney diseases. That syphilitic nephritis exists, however, is doubted by some of the best authorities, it being classed with nephritis, and syphilis is considered only as an etiologic factor. I do not wish to insist that a syphilitic treatment should be instituted in such cases; in fact, authorities differ; some advise strongly against the use of mercury in these conditions. It certainly

\* Read before a joint meeting of Chicago Urological and Chicago Medical societies.