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Upon the intracellular constituents of the typhoid bacillus.

[From the Jenner Institute of Preventive Medicine, London.]

By Dr. **Allan Macfadyen** and **Sydney Rowland.**

With 2 Figures.

The following paper contains an account of the results that have bee obtained as regards the typhoid bacillus since the publication of our firs communication in the Centralblatt für Bakteriologie. Abt. I. Vol. XXX. 190 No. 20.

The investigations undertaken had, as was then stated, a speci object in view, viz: the study of certain of the intracellular factors i health and disease by obtaining directly the cell constituents and elim nating as far as possible excreted substances and those formed by th cell in a given environment. The ordinary laboratory methods could n be employed for this purpose, and it was necessary in the first instanc to devise the means of carrying out the research. The progress of th inquiry has therefore necessarily been slow, as many technical difficultie had to be overcome. The investigation has now been successfully ad vanced in various directions. The intracellular juices of healthy and morbi tissues, of leucocytes and of a number of micro-organisms have been ob tained and submitted to examination by the writers and their colleague The results, in so far as published, are referred to at the end of th paper. The experiments carried out with the typhoid organism and th results obtained were of the following nature.

I. Experiments with reference to an extracellular typhoid toxin.

The existence of a specific toxin produced by the typhoid bacillu has hitherto not been demonstrated although it has been assumed b analogy with other organisms, and by reasoning from the clinical cours of the disease. Such a poison must be either extracellular or intracellula The endeavours however, to demonstrate the production of an extra cellular toxin by the typhoid bacillus have not hitherto led to any definit results. That a toxin of this character does not exist in filtered culture of the organism is the common experience of bacteriologists. The typhoi organism when grown in the ordinary culture media does not produce an soluble products with marked poisonous properties. The absence fro such cultures of definite toxins might be due to the unsuitability of th soil used for growing the typhoid bacillus.

We considered it of importance to retest the question, since th detection of such a toxin would constitute a great advance in the unde standing and the treatment of the disease.

The first step in the search for the body in question consisted i substituting for the usual broth and peptone media, culture fluids ap proaching more nearly in constitution the natural body soils which clin cally support the life of the bacillus. A number of experiments extendin over a year were made in this direction. We endeavoured to cultiva the typhoid bacillus in fresh juices obtained from various organs of th

animal body, and representing the intracellular juices of the tissues. The spleen, lymphatic glands and intestinal mucous membrane were triturated and the juices expressed at a low temperature in order to prevent changes in the material during the grinding process. Such media would approximate more closely in constitution to the substances the typhoid organism might be expected to meet in the course of its stay in the body of a host in which it is producing toxic symptoms, and particularly if heat were avoided in their preparation. The fresh organs or tissues from the ox or the calf, as received from the slaughterhouse, were finely minced in a mincing machine and the resulting pulp disintegrated according to the methods employed by us in the preparation of Zymase, viz: with the aid of sand, the mass being kept cool during the process by an outer jacket of brine or carbonic acid (1).

The fresh juices thus obtained were passed through a Berkefeld filter to ensure sterility, and in each instance the intracellular juice was brought to the requisite degree of alkalinity by the addition of sodic carbonate. These juices were employed as a culture soil for the typhoid bacillus under the following conditions:

1) The organ juice per se.

2) The organ juice with an admixture of fresh human serum.

3) The organ juice after heating to 55° C for twenty minutes, and with or without the subsequent addition of fresh human serum.

The above conditions were applied to organ juices obtained from the fresh spleen of the ox, the lymphatic glands (mesenteric) of the calf and the intestinal epithelium of the ox and a few other animals. The media were inoculated with the typhoid bacillus alone and in a series of experiments, with the typhoid bacillus in conjunction with the bacillus coli communis. In every instance a parallel series of cultures was made under aerobic and anaerobic conditions. We were able to cultivate the typhoid bacillus under the above conditions and to determine in how far its toxicity was thereby affected. After an incubation at blood heat for four weeks the cultures were examined as to the presence of growth and freedom from contamination. The cultures were then passed through a Berkefeld filter to remove the organisms, and the filtrates injected into animals in small and large doses (e. g. 5 cub. c. and more). The experimental animals were guinea pigs, rabbits and monkeys. With the possible exception of one spleen juice, none of the fluids thus obtained exhibited any acute toxic power, either when used as culture soils for the typhoid organism or in conjunction with the colon bacillus. In the case of the rabbit and the monkey the fluids were practically innocuous. As regards the guinea pig no immediate toxic effect was observed. In a certain number of cases, however, the guinea pigs eventually died at the end of a period which averaged about six weeks. If one excludes the possibility of substances toxic to the guinea pig being naturally present in the organ juices (of which we possess a certain amount of experimental evidence), the result might be interpreted as being due to some slowly acting soluble toxin or toxins derived from the typhoid bacillus. We have not however as yet been able to observe any distinct effects on post mortem examination, and are at present unable to ascribe any definite significance to the result. It will be sufficient in the meanwhile to record the fact, and to omit the table of results as they are not essential to the present paper. Such toxins, if they exist, are quite different in properties to the intracellular toxin we are about to describe. The experiments were suf-

ficient to lead us to the conclusion that in no case was an extracellula toxin developed comparable in any way to those obtained from pure cul tures of undoubtely extracellular toxin-producing organisms, e. g. th diphtheria bacillus, etc. It did not appear that this line of investigatio would be likely to lead to any practical results. The very large numbe of experiments made with the most natural soils obtainable had not bee successful in demonstrating the presence in cultures of the typhoid bacillu of any definite toxin of likely value for immunising purposes.

II. Experiments with reference to an intracellular typhoid toxin.

The experiments having failed to establish the presence of any definit extracellular toxin, it became necessary to search within the typhoid or ganism itself for the missing toxin. The research was thus directed no to the products of the typhoid bacillus, but to the organism itself an its intracellular constituents. For this purpose the endeavour was mad to obtain the fresh unmodified cell plasma of the organism and the metho originally employed was as follows: The virulent typhoid bacilli wer grown on the surface of nutrient agar in flat rectangular bottles, eac giving a surface of 200 sq. cm; one hundred such culture bottles wer required in order to yield a growth sufficient for trituration by the metho that was in the first instance adopted. After cultivation for about 36 hour at blood heat, the bottles on being washed out with salt solution, yielde about one litre of a thick emulsion of the bacilli. The bacilli were sepa rated from the emulsion by means of a high speed centrifuge, and wer at the same time thoroughly washed free of possible excretory products b repeated additions of physiological salt solution. The washed and separate bacilli were then mixed with fine silver sand and triturated in a cold jacketed metal cylinder by means of small vanes revolving at a hig velocity. The intercollision of sand particles and bacilli resulted in th rupture of the bacterial cells, and the process usually occupied from thre to four hours. The resultant mass was filtered through Kieselguhr wit the aid of a hydraulic press. The filtrate represented a rich watery solution or suspension of the intracellular constituents of the typhoi bacillus in so far as these were capable of passage through the Kiesel guhr. There remained at the end of the pressing a hard cake of Kiesel guhr, which was found to contain a considerable amount of retained al buminous and other organic substances. Repeated extractions of this cake made with glycerine and with a solution of carbonate of soda, demonstrate that the Kieselguhr cake contained physiologically active constituents o the typhoid organism. There had undoubtedly been held back intracellula elements of possible importance to the experiments we desired to carr out. The entire operation lasted about six hours and the average yiel of juice from the first pressing was about 8 ccm. An account has alread been given of the experiments made with such juices upon guinea pig and rabbits with a view to testing their toxicity and immunising propertie against the bacillus typhosus. It was found that the fluid, injected i doses of 1, 0.5 and 0.2 ccm completely protected the experimental animal against one to ten lethal doses of virulent typhoid bacilli, and the pro tection following one such injection lasted about four weeks. The result were identical whether a first, second or third pressing of the juice throug the Kieselguhr was employed. The juices preserved their immunising pro perties as regards the typhoid bacillus for a considerable period of time

s at the end of four months they were still found to be active in this espect. The cellplasma on subcutaneous inoculation was very quickly bsorbed without evidence of local irritation. The quick absorption of he cell juices by the tissues and the absence of local irritation we regard s a point of considerable practical importance. If the full immunising ffect as regards the typhoid bacillus per se is to be attained by the in-ection of the plasma obtained from its cell substance, such a method of rocedure would undoubtedly present considerable advantages over the ther methods that have hitherto been employed with the same end in iew, e. g. the use of heated cultures and the intact bodies of the bacilli s vaccines etc. The ideal method of procedure would be to obtain an nmunising substance directly from the bacterial cells, of nonirritating roperties, capable of rapid and complete absorption by the tissues, and reed from all the superfluous material present in the ordinary culture ıedia.

In this respect the methods we were employing appeared to furnish ıe hope of obtaining an active and at the same time a purer material ıan had hitherto been found possible. The appearance of the agglutiation reaction in the blood of the treated animals afforded evidence that e were dealing with intracellular juices which possessed active physio-ogical properties. This reaction appeared very quickly and persisted for considerable period of time, and was still present when the specific rotective substances had disappeared from or ceased to be active in the lood. In the case of the rabbit an agglutination of the typhoid bacillus ccurred nine months subsequent to the injection of the typhoid cell juice ubcutaneously. On intravenous injection we have succeeded in obtaining ıe agglutination reaction within seventeen hours, and at times in two ours, after inoculation with a dilution of 1 in 100 of rabbits blood. One ıjection of the cell juice was sufficient to develop antibacterial properties ı the blood of the treated animals. At the end of a month the serum as actively bacteriolytic. A complete destruction of the typhoid bacilli y the serum in doses of $^1/_{10}$, $^1/_{20}$ and $^1/_{50}$ ccm, occured within two ours. The agglutinative and bacteriolytic action was obtained with the lood serum of treated rabbits and monkeys.

The experiments at this stage had demonstrated that the typhoid cell lasma, obtained by the above methods, possessed active physiological pro-erties, and that on injection they afforded a certain protection against irulent typhoid organisms in virtue of specific bacteriolytic properties eveloped in the blood of the treated animals. At the same time the ield of active cell plasma by the above mentioned triturating process did ot prove to be of a quantitative character. A considerable amount of ıe cell constituents was retained in the Kieselguhr sponge. The method ppeared in this respect to be capable of improvement and particularly ith reference to the minute cells that we were dealing with. A method hich would eliminate the sand and Kieselguhr, as employed by other bservers (Buchner, Hahn etc.) and by ourselves, and would at the ame time produce a rapid trituration of the organisms was, we found y experience, essential. The method, if it could be successfully devised, ould yield the entire intracellular constituents of the micro-organisms in uestion for the purpose of experiment. We had likewise noted the tole-ance exhibited by the treated animals, and particularly by the guinea pig,) the injection of large quantities of the expressed cell plasma of the yphoid bacillus. Whilst the immunising properties of the cell juices, as

regards the typhoid organism, had been demonstrated, acute and definit toxic effects had proved remarkable by their absence.

These various observations led us to endeavour to improve the method employed, and to relinquish the procedure on lines analogous to those Buchner, Hahn and other investigators in the study of expressed ce juices. The results, on the injection of such expressed cell juices int animals, were purely of an antibacterial character, an active toxin in th cell plasma and consequently antitoxic properties in the blood of th treated animals had not been demonstrated. This constituted a seriou gap in the experiments, if we assume that an intoxication of the syste in the case of enteric fever is a grave and perhaps the cardinal facto to be considered in the treatment of the disease.

The filtering action of the Kieselguhr used in the filter pressing ap peared to be the most likely reason for lack of success in this direction The disintegration of the organisms was therefore attempted without th admixture of any foreign material which would render a subsequen filtration through Kieselguhr necessary.

III. Experiments with cold grinding methods.

The mechanical method of disintegration that appeared to be mos likely to lead to successful results in the case of bacteria was their tri turation whilst in a frozen and brittle condition. It had already bee demonstrated (2) that an exposure to the temperature of liquid air (abou —190° C) did not injure or destroy the vitality of bacteria, and that micro organisms might be kept for as long a period as six months at this lo temperature without any deleterious effect.

This important point being determined, it appeared probable that th brittleness of the cells at this low temperature would favour their mechanica disintegration without any admixture of sand or other foreign substance The most convenient agent for the production of the necessary cold wa liquid air. Liquid air possessed two practical advantages: — it could b more conveniently handled than other substances that might possibly hav given the necessary conditions of cold at higher temperatures than — 190° C and it furnished a fluid freezing bath in which the material t be ground could be directly immersed. These properties have proved o great practical value in the course of the experiments. A further ad vantage was that at such a temperature the ordinary chemical processe would cease, changes due to heat would be eliminated, and the process i successful would furnish a quantitative yield of unaltered cell plasma.

The experiments were successful and the feasibility of disintegratin micro-organisms per se, without any admixture of triturating substance was demonstrated. The complete disintegration of the typhoid bacillu was accomplished at the temperature of liquid air in a period of abou two hours without the addition of sand or other foreign substance.

The method has likewise been successfully applied to a number o bacteria, to other types of vegetable cells, and to animal organs an tissues, and their intracellular juices obtained for experimental purposes

The method entirely obviates the use of any accessory grinding o filtering substances and fulfils the conditions we desired to obtain for th study of intracellular constituents.

These conditions were as follows:

1) That no chemical or heat changes should take place during th process of disintegration,

2) That the disintegration should be accomplished without the addition of any triturating substance, the necessary subsequent removal of which might vitiate the composition of the resulting mass.

3) That the process should furnish a quantitative yield of the unmodified cell plasma.

In this communication we will confine ourselves to the results obtained with the typhoid bacillus. (Schluß folgt.)

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(Schluß.)

IV. Apparatus and methods.

It will be advisable in the first instance to give a full description of he methods that have been specially devised and employed for obtaining irectly the intracellular juices of the typhoid bacillus and other orga- isms. The general principle consists in freezing the micro-organisms to n extreme degree of brittleness by means of liquid air, and disintegrating he cells per se in a mechanically operated mill.

In the case of the expressed juices obtained by the sand and Kiesel- uhr method, about 100 agar culture bottles were required to furnish an dequate growth of the orga- isms for grinding purposes. n the present method, ten uch agar cultures are suffi- ient for a single grind of the nicro-organism in question. This in itself is a great saving n time and material.

The virulent typhoid orga- isms are grown on the surface f ten agar bottles at blood eat for 24 to 30 hours. The growth is then washed off with alt solution and the resultant mulsion of bacilli is spun in high speed centrifuge. The rocess is repeated several imes with freshly added salt olution, in order to cleanse he organisms from any extra- neous matter. The spun out acteria are next reduced to he consistency of a pasty mass y a rapid drying on the sur- face of a Chamberland filter through which air is being sucked.

The average yield of washed bacteria, when freed as far as possible from ad- herent water, was about 0.15 g per culture plate. This repre- sented quantitatively 1.5 ccm

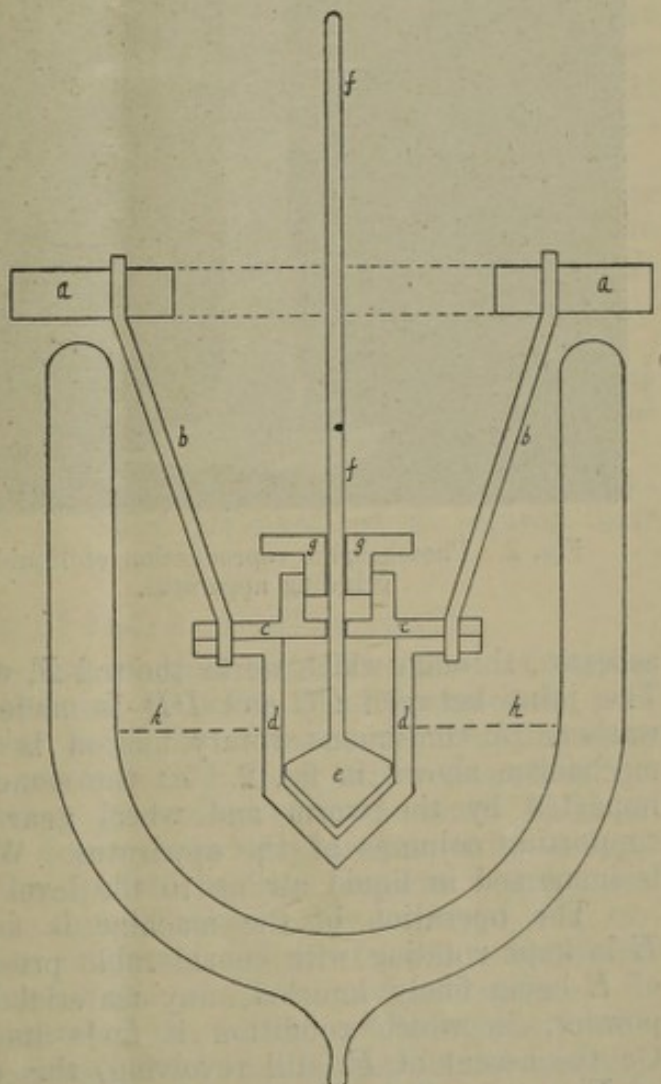

Fig. 1. Diagrammatic vertical section of liquid air grinding apparatus.

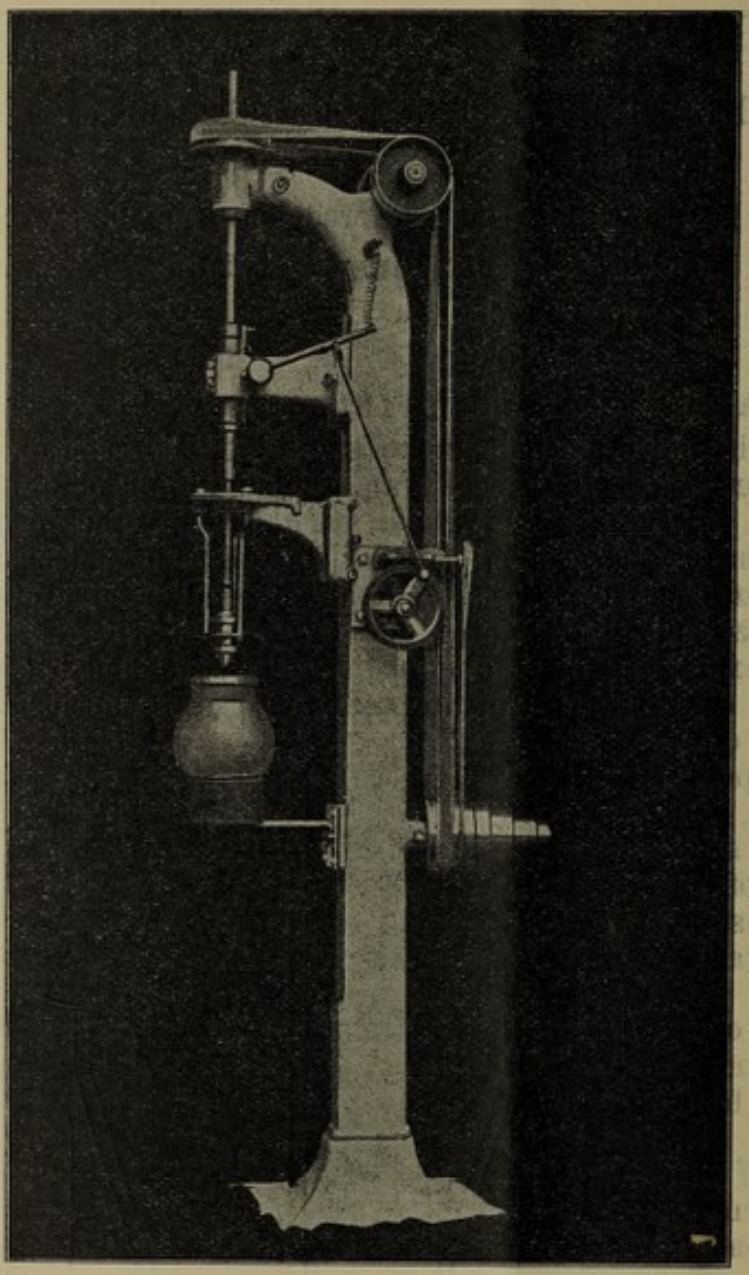

Fig. 2. Photographic reproduction of liquid air grinding apparatus.

of the 10 per cent s
solution of the c
juice prepared aft
the disintegration
the cells.

The pasty ma
of washed organis
is removed from t
Chamberland filt
and is introduced in
the apparatus sho
in diagrammatic se
tion in fig. 1. Th
apparatus is construc
ed as follows: T
horizontal plate
(also seen in the phot
graphic reproducti
fig. 2) supports by t
rods *BB* the plate *C*
This plate, circular
plan forms the cov
of the conical recep
acle *DD* on which
free to revolve th
doubly coned plung
E. The vessel *D* ca
be removed from t
covering plate *C*,
which it is attache
by bolts, for the pu
pose of introducing th
material to be disint
grated, as in this i
stance the typho
bacilli. The plate *C*
is furnished with
gland *GG*, close
packed with ignite
asbestos, through which works the rod *F*, which rigidly supports the cone *E*
The joint between *CC* and *DD* is made by means of an annular pap
washer. A continuous rotary motion is imparted to *F* by means of th
mechanism shown in fig. 2. At the same time a reciprocating motion
imparted by the worm and wheel gear seen at the side of the ma
supporting columns of the apparatus. When in operation, the apparatu
is immersed in liquid air up to the level *HH*.

The operation of the machine is as follows. On the descent of *E*
E is kept rotating with considerable pressure against *D*, and the surfac
of *E* being finely knurled, any material between *E* and *D* is reduced
powder, in which condition it finds its way into the upper cone of *E*
On the ascent of *F* (still revolving) this coarse powder falls to the botto
of *D*, and is again forcibly rubbed between the lower cone of *E* and th
bottom of *D*, on the next descent of *F*. This sequence of operations

ntinued until no entire micro-organisms are found on microscopical xamination, a result which can be accomplished in from $1^1/_2$ to 2 hours, hen dealing with 0.5 to 1 g of pasty organisms. It must be remembered iat as the above sequence of operations will take place at about — 0° C., the contents of *D* are in the condition of a dry powder.

As a result of this process, there is obtained a pasty mass (when iawed), consisting of the entire substance of the organisms used. Moreover, is substance, at the moment of thawing, is chemically identical with the ving protoplasm of the cell, the whole disintegration having occurred nder conditions which preclude the possibility of any chemical change iking place.

This pasty mass is mixed with salt solution (0.75 p. c.), rubbed up in n agate mortar, and the opaque suspension thus obtained is centrifugal- ed until free from suspended matter, and an opalescent solution of the tracellular constituents of the organism results.

The centrifugalisation of such minutely divided material is occas- onally a matter of difficulty, more especially if, by chance any unground rganisms are found in the suspension. In those cases, in which a sterile iice is required the centrifugalisation must be very vigorous, and for this urpose it has been found that the best results are given by a very simple rm of centrifuge, consisting of a horizontally mounted steel disc (36 in- ies in diameter) on the periphery of which are mounted strong glass ibes in steel cases hung on trunnions, the disc making from 3,000 re- olutions per minute. The glass tubes to prevent fracture under the great rain are floated in mercury, contained in the covering steel tubes. With ich a disc, sterile juices have been obtained.

The centrifugalised celljuice was as a rule equivalent to a 10 per nt solution of the intracellular constituents obtained. It represented the tracellular constituents of the typhoid organisms soluble in physiological lt solution, after a complete disintegration of the cells had been accom- lished.

It is this material which was used in the experiments we are about describe and it consisted of a bacterial celljuice practically identical ith that contained within the living cell.

These juices likewise had the merit of possessing a constant con- itution, and thus were capable of being standardised as regards their hysiological action.

V. Demonstration of intracellular toxin.

The first experiments were carried out with the view of testing hether the typhoid celljuices obtained by cold grinding methods pos- essed any toxic properties. It was found that if such a disintegrated mass e freed from whole bacilli (if present) and from other suspended in- oluble particles by centrifugalisation, an opalescent fluid results which on ioculation into animals in small doses, invariably proves toxic or fatal. t was therefore concluded that the typhoid bacillus contains within itself n intracellular toxin. The toxin thus obtained and employed for ex- eriment is a ten per cent solution in normal saline of such intracellular onstituents of the typhoid bacillus as are soluble in such a medium.

The standard adopted in estimating the toxicity of the typhoid cell- iice was the amount found to prove fatal on intraperitoneal injection ito the guinea pig. The intracellular fluid under these circumstances in- ariably proved toxic in a short period of time. The toxicity of the juices

varied pari passu with the virulence of the living bacilli for the guin pig. The greater the virulence of the organism employed, the great was the toxicity of the intracellular constituents obtained from it, a vice versa. For example, the toxicity of the plasma obtained from organism of which 0.1 ccm of a broth culture killed in a few hours w greater than in the case of an organism of which larger quantities of broth culture failed to produce death in the same period of time.

In the case of a broth culture of the typhoid organism of such degree of virulence that $^1/_{10}$ of a cube on intraperitoneal injectio produced death infive to ten hours, the toxicity of the celljuice obtain from the same organism would be on an average as follows. On intr peritoneal injection, 1 ccm of such a toxin killed in 3 hours; 0.5 ccm 3 to 4 hours; 0.2 and 0.1 ccm in 3 to 5 hours; 0.05 ccm in abo 12 hours and 0.02 ccm in about 40 hours.

We have likewise obtained a juice of which 0.02 ccm has killed six hours.

The best result so far obtained as regards acute toxicity, was in t case of a 10 per cent toxin of which 0.003 ccm killed within 24 hours.

No organisms were found in the blood or peritoneal cavity on postmorte examination. The effects were therefore produced by "devitalised" co stituents of the typhoid bacillus. The peritoneal cavity contained a co siderable amount of exudation; haemorrhages were present in the stomac the small intestine was acutely congested and the suprarenal capsules we injected.

Similar results to the above were obtained in a very large numb of repetition experiments and justified the conclusion that the typho bacillus contains an intracellular soluble toxin of considerable power.

The toxin on subcutaneous injection into the guinea pig produced toxin oedema at the seat of inoculation, and death has occurred after t injection of 0.5 ccm and 0.2 ccm of the toxin in about seven days.

One of the effects of a sublethal dose of the toxic juice was the ea and constant appearance in the blood of marked agglutinating properti and sometimes this occurred a few hours after e. g., an intravenous i jection. The constant presence of this reaction served to demonstrate t specificity of the celljuices with which we were dealing.

The heat relationships of the toxins derived from the typhoid bacill and other organisms are being investigated, and the results will be p blished in due course.

VI. Immunising properties of the typhoid celljuices.

It remained to test the typhoid celljuices for immunising and oth properties. The preliminary experiments in this direction were made up the rabbit and the monkey. The monkey was selected as an animal mo likely to furnish data of possible application to man. For this purpo the typhoid celljuice was administered subcutaneously to the monkey. T injections did not produce any general symptoms beyond a transient ri in temperature, whilst the material was quickly absorbed after each i jection without any traceable effect.

In this manner doses of 0.5 to 1 ccm of the material were inject at intervals. An immediate result was the agglutination of the typho bacillus by the serum of the treated monkeys, whereas no such effect w produced by the serum of monkeys which had not been treated.

This furnished useful evidence that the animals were under the i

uence of celljuices derived from the typhoid organism. The injections ere repeated at intervals of 3—4 days, and after a lapse of 4—6 weeks he animals were bled.

The serum obtained was then tested for immunising properties. The est objects were 1) a virulent culture of the typhoid bacillus and 2) the ntra-cellular toxic juice of the same organism. A varying amount of the irulent bacilli and of their toxic celljuice was mixed with a varying uantity of the serum. The respective mixtures were then injected into he peritoneal cavity of the guinea pig.

The broth cultures of the typhoid organism used in the experiments ere per se lethal in doses of 0.1 ccm in 5—10 hours. The typhoid elljuices were fatal in doses of 0.2 and 0.1 ccm in 3—5 hours and in oses of 0.05 ccm in about 12 hours. The serum was thus tested for) specific antibacterial and 2) specific antitoxic properties.

The experiments showed that the serum of the monkey, after in-ection of the typhoid celljuices, possessed antibacterial and antitoxic roperties, inasmuch as the serum protected the experimental animals gainst the bacilli, and also against an intracellular toxin obtained from hem.

A simultaneous injection of 1) serum with the bacilli, and 2) serum ith the toxic celljuice produced no lethal or toxic effects. The control nimals on the other hand invariably succumbed.

It was further investigated whether the serum possessed preventive nd curative properties.

The serum from treated monkeys was injected into guinea pigs, one njection being made in each instance, and the same animals received at n interval of 12—24 hours lethal doses of the typhoid bacillus and of ts toxic intracellular juice respectively. The treated animals survived the est, whilst the control animals succumbed. It was therefore concluded hat the serum had protective properties.

A third series of guinea pigs received lethal doses of the typhoid acillus and of its toxic celljuice respectively. The serum was then in-ected at various intervals into individual animals. It was found that the ives of the animals could be saved by one injection of the serum, from fatal infection or intoxication, even when half of the lethal period had lapsed in each instance. The serum therefore, possessed curative pro-erties. From the experiments made upon the monkey it would appear) That by the injection of the intracellular juices of the typhoid organism nto the monkey, it is possible to obtain a serum with both antibacterial nd antitoxic properties; 2) That such a serum possesses curative and reventive properties as regards the typhoid bacillus and an intracellular oxin present in the same organism. It is believed that this has furnished or the first time proof that in the case of one species of pathogenic or-anism, the intracellular juices of the organism when injected into a sui-able animal, give rise to the production of a serum which is both actericidal to the organism itself and antitoxic as regards a toxin con-ained in its substance. How far such properties of the celljuice are hared by other pathogenic organisms is being made the subject of further nquiry.

In the case of the rabbits treated with the typhoid celljuice, anti-acterial and antitoxic properties were likewise found to be developed in heir blood. The experiments which have been made with the goat are confirmatory of the above results, its serum likewise possessed antibacterial

and antitoxic properties as regards the typhoid bacillus and the solubl toxin derived from it. At the present moment the experiments are bein conducted on the horse.

The in vitro experiments that have been made with the variou serums obtained have confirmed the results obtained in the experimenta animals.

In was important to determine, whether in addition to being anti toxic, the serum obtained from the experimental animals was likely unde further treatment to possess an enhanced antitoxic value, and whether a in the case of diphtheria, any "overproduction" of antitoxin could be de monstrated. We have found that the serum of an animal immunised b repeated injections of celljuice in doses of 0.2 ccm, can completely neu tralise thetoxic effect of one hundred times the amount of a typhoi celljuice that is capable of producing death on intraperitoneal injectio into the guinea pig in six hours.

We conclude, therefore, that as the antitoxic value can be raised b repeated injections, there is every reason to hope that it can be sti further raised by longer treatment.

To those familiar with Ehrlich's elaborate standardising method with regard to the diphtheria toxin, the absence of similar data from thi paper will no doubt be noted. It will, however, be equally obvious tha a method of standardising and testing which has after much experimen by many observers, reached a high empirical standard of efficiency canno be applied to a toxin of an entirely different nature.

We are engaged in the consideration of the best method of stan dardisation to be adopted with special reference to the typhoid toxin an intracellular toxins in general, and the lengthy experiments involved hav not yet been completed. We must therefore, content ourselves with givin the protocoll of a typical experiment.

Experiments with serum from monkey B.

Injections intraperitoneal.

0.2 ccm toxin killed in 4½ hours.
0.25 „ broth cult. killed in 10 hours.

A. Injection of serum followed by injection of typhoid culture an toxin.

At 5 p. m. injection of serum made.

Guinea Pig 1	Guinea Pig 2	Guinea Pig 3	Guinea Pig 4
0.5 ccm	0.25 ccm	1 ccm	1 ccm

the following day at noon

0.25 typ. cult.	0.25 typ. cult.	0.2 toxin	0.1 toxin

B. Simultaneous injection of serum and broth culture of typhoi bacillus.

Guinea Pig 5	Guinea Pig 6	Guinea Pig 7	Guinea Pig 8
0.5 serum	0.25 serum	0.1 serum	0.05 serum
0.25 broth cult.	0.25 broth cult.	0.25 broth cult.	0.25 broth cult.

C. Simultaneous injection of serum and toxin.

Guinea Pig 9	Guinea Pig 10	Guinea Pig 11	Guinea Pig 12
1.0 ccm serum	0.5 ccm serum	0.2 ccm serum	0.1 ccm serum
0.2 „ toxin	0.2 „ toxin	0.2 „ toxin	0.2 „ toxin

D. Injection of typhoid bacillus followed by injection of serum.

	Guinea Pig 13	Guinea Pig 14	Guinea Pig 15
At 12.50	0.25 ccm typ. broth cult.	0.25 ccm typ. broth cult.	0.25 ccm typ. broth cul
	At 1.30	At 2.30	At 3.30
	0.25 ccm serum	0.4 ccm serum	0.5 ccm serum

	Guinea Pig 16	Guinea Pig 17
At 12.50	0.25 ccm typ. broth cult.	0.25 ccm typ. broth cult.
	At 4.30	At 5.30
	0.4 ccm serum	0.5 ccm serum

Injection of toxin followed by injection of serum.

Guinea Pig 18	Guinea Pig 19	Guinea Pig 20	Guinea Pig 21	Guinea Pig 22
At 1 p. m.	At 1 p. m.	At 1 p. m.	At 1 p. m.	At 1 p. m.
0.2 ccm toxin	0.2 ccm toxin	0.2 ccm toxin	0.2 ccm toxin	0.2 ccm toxin
At 1.30 p. m.	At 2 p. m.	At 2.30 p. m.	At 3 p. m.	At 3.30 p. m.
0.2 ccm serum	0.5 ccm serum	0.7 ccm serum	1 ccm serum	1 ccm serum

All the animals survived the above test with the exception of No. 2 ich died after two days, and No. 21 which survived $4^1/_2$ hours.

VII. General conclusions.

Experiments are at present being conducted on the horse.

It remains to be seen in how far the results already obtained are able of being utilised outside the laboratory in clinical directions.

It appears to us that the results detailed above possess considerable oretical interest. The experiments have furnished a demonstration of fact that it is possible to prepare a serum in the case of a given or-ism which is bactericidal to the organism in question and antitoxic as gards a toxin contained within its substance. Further, the experiments the demonstration of the presence of a specific intracellular toxin, may, is possible, serve to explain the most striking feature in the course of hoid fever — the intoxication.

As regards the practical methods of preparing bacteriolytic serums, immunisation of the animals by means of disintegrated cells offers many vantages in practice, through the absence of serious local reaction and rapidity of absorption of the inoculated material.

There appears also, the possibility of obtaining bacterial vaccines of ater purity and capable of more accurate standardisation in the case Enteric Fever, of Plague and other diseases, the symptoms of which y depend upon the presence of intracellular toxins in their exciting or-nisms. This matter is one that is engaging our careful attention.

In conclusion we have to express our appreciation of the valuable vice and help afforded by Professor James Dewar. F. R. S. in the rse of these and other investigations.

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