VACCINES AND FEVER.

BY

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(From the Lister Institute: The Constance Trotter Researches.)

In the great majority of infective diseases accompanied by marked systemic disturbance the most prominent clinical feature is that form of intoxication which is expressed in fever. The relationship of fever to general infection is so constant that whenever continued fever occurs we may be almost certain that an infective process is at work. Continued fever is in fact an index both of the continued activity of micro-organisms pathogenic to the individual infected and of the absence of effective protection against such activity. On the other hand, the absence of fever does not necessarily indicate the absence of infection. On the contrary, it is a familiar experience that in severe infections leading to a fatal issue there may be no fever on account of grave impairment of the reactive functions of the infected body. This impairment may exist prior to infection or may be the expression of extreme intoxication after infection has occurred. This observation can be verified with great ease. We have therefore learnt to recognize that whilst the continuance of fever is an excellent indicator of the continuance of infection and of the absence of effective immunity thereto, it is also an index that the power of reaction—whatever that may prove exactly to mean—is not yet lost. Now the power of reaction to infection in terms of fever is in some unknown way intimately associated with the complex problems presented by immunity. And we have some reason to believe that the mere presence of continued fever is a sure sign that the machinery of immunization has been set in motion and is still running. The solution therefore of the mystery that surrounds the nature and source of the substances that are the immediate cause of the fever of infective disease can hardly fail to throw light on many of those problems in immunity production which are at present totally obscure.

Hence determination of the essential cause of the fever of infective disease—from the pathological standpoint as opposed to that of physiology—is a matter of immediate concern to all students of immunity. And, as I shall show, this particularly applies to those who administer dead bacterial vaccines, whether for purposes of prophylaxis or of treatment, as well as to those who make use of bacterial emulsions in test tube reactions.

In all immunity problems, whether of the test tube or the living body, the keystone on which all inquiry rests has long been recognized to be the antigen, or substance that provokes the manifestation of antibodies. Similarly in the pathological problems presented by fever inquiry must always start from the pyrogen, or substance that provokes the manifestation of fever.

The antigens, then, and pyrogens of infective disease are substances so vital to the production of immunity and of fever that precise knowledge of their true source, as well as of their chemico-physical nature, must always be a necessary preliminary to an intelligible conception of how they act.

It is widely believed, largely in consequence of Pfeiffer's early work, that the main source of supply of both antigen and pyrogen in infective disease is dissolved bacterial protoplasm. In an infected subject the primary source of supply of these substances is therefore, we are taught, one foreign to that subject, whether the agents affecting their liberation be of an autolytic or heterolytic character. Moreover, since antigen and pyrogen are supposed to be genuine derivatives of bacterial protoplasm in solution, the theory lays down that it is only dead organisms that can supply them. The living bacterium as such has, we must infer—if we accept the theory—no antigenic or pyrogenetic values. Its potential worth, in terms of the production of antibodies and of fever, only becomes kinetic when bacterial death followed by bacterial solution and absorption has occurred.

So far as antigen is concerned, the theory that it is

* The protocols for many of the experiments referred to in this paper have already been published. In most of the experiments detailed in such protocols I was aided by Dr. W. J. Penfold. Full data of the additional experiments referred to in this paper will be published elsewhere, and for these I accept sole responsibility.

derived from dead bacterial protoplasm has in the main been accepted without question for many years. And incidentally it is the bedrock of that form of vaccine-therapy—the use of dead vaccines—which is under trial in this country to-day. So far as fever is concerned, the theory that the pyrogen of infective disease is a derivative of dead bacterial protoplasm in solution has also, as a rule, been accepted without serious question till comparatively recent date.¹

In consequence of the general acceptance of this hypothesis as to the source of antigen and pyrogen in infective disease it might reasonably be supposed that it rests on a sound experimental basis. Whether this supposition be well grounded in the case of antigen is a question that cannot be discussed here. In the case, however, of pyrogen I shall show that the evidence hitherto relied on in favour of the view that it is a genuine derivative of dead bacterial protoplasm can no longer be accepted. On the contrary, I shall cite experimental data which suggest—if they do not conclusively prove—that the fever of infective disease is the direct result of absorption of pyrogen derived from the medium offered by the tissues of the infected host in consequence of the activity of the living organism or its products on such medium. And as a corollary I shall indicate the practical bearing of this displacement of current theory on the administration of dead vaccines for prophylactic or therapeutic purposes, as well as on all such test-tube experiments as involve the use of bacterial emulsions of the same nature.

The experimental basis of the theory that dead bacterial protoplasm is the direct source of the fever of infective disease is the undoubted fact that fever does frequently follow the injection into healthy subjects of dead cultures of certain organisms. At first sight this evidence would seem to be conclusive, especially if fever were found to follow the injection of all known forms of dead pathogenic bacteria and protozoa. It has, however, for some years been recognized that there are exceptions to what is by many still believed to be a general rule. For example, injection of dead cultures of the bacillus of Koch, *B. anthracis* and *B. diphtheriae* into healthy subjects has from time to time been found not to be followed by fever. In the case of the bacillus of Koch it was believed that the "waxy envelope" prevented bacterial solution and consequent extraction of the pyrogen with which this organism was credited. In the case of *B. anthracis* and *B. diphtheriae*, however, no explanation was forthcoming as to the absence of fever after injection of the killed organism. Nevertheless, these exceptions were looked upon as unimportant, and were to a great extent forgotten. When the practice of administering dead bacterial vaccines became general it was soon found that their injection might give rise to mild forms of intoxication, including fever. Prophylactic injections into healthy subjects of dead vaccines of *B. typhosus* are a case in point. Belief in dead bacteria as the primary source of pyrogen became therefore more firmly rooted than ever, though again no explanation was given—beyond a quantitative one—of the frequent experience that fever did not follow, for example, injections of certain staphylococcal vaccines. The subject, in fact, does not seem to have been investigated.

Early in 1911, therefore, I examined, with the aid of my laboratory colleague, Dr. Penfold, a large number of dead and living bacterial vaccines by injecting them, for the most part intravenously, into healthy rabbits. The organisms examined in respect of their pyrogenetic properties are given in the subjoined list. They are divided into two groups, according to whether they did or did not on intravenous injection produce immediate fever if the organisms were first killed. The character of the list given is, I think, sufficient answer to the very reasonable suggestion that the absence of immediate fever after injection of an emulsion of organisms might be due to difficulty of lysis of the particular organism. In all cases the vaccines injected were made from laboratory cultures. Many different strains of several of the organisms were examined, an important point, as will be seen, in the study of the relative pyrogenetic properties of any bacterial emulsion. Attention is drawn to the fact that the list of the organisms from laboratory cultures which did produce fever is a list also of Gram-negative organisms. On the other hand, the list of organisms injection of which did not

produce fever is a list also of Gram-positive organisms. The parallelism is a striking one, though its interpretation is at present obscure.

PYROGENETIC VACCINES.

B. typhosus,
B. coli,
B. influenzae,
B. pestis,
B. melitensis,
M. catarrhalis,
V. cholerae,
Acne bacillus,
S. albus (certain strains),
S. citreus (certain strains),
S. aureus (certain strains).

APYROGENETIC VACCINES.

B. diphtheriae,
B. anthracis,
Koch's bacillus,
B. mallei,
Streptococcus,
Pneumococcus,
Staphylococcus aureus (certain strains),
(Tuberculin),
(Mallein.)

Clearly, then, the list of organisms that apparently do not contain any pyrogenetic substances is much longer than has previously been realized. And no doubt further examination will reveal yet other laboratory-grown organisms injection of which does not produce fever if they have been first killed. So far I have not examined for the presence or absence of pyrogenetic function such protozoa as can be grown on laboratory media. It should also be noted that repeated injection at suitable intervals into the same animal of a dead vaccine of the apyrogenetic type does not produce fever. This observation seriously affects the question of anaphylactic fever in the sense employed by Continental observers. We have gone more fully into this subject in a paper read at Liverpool in the summer of 1912.²

In the face of the experiments referred to here,³ it must be admitted that if some laboratory-grown organisms appear to contain a protoplasmic pyrogen—because they produce immediate fever when injected dead—it is difficult to understand why so many others do not.

In further experiments, however, published⁴ in the autumn of 1912, we showed that certain strains of organisms which after growth on agar are primarily apyrogenetic when injected dead appear to acquire pyrogenetic properties if subcultivation on fresh agar is carried out. For example, some subcultured strains of *S. aureus* readily exhibit the power of provoking fever if the heated organisms be injected. This is particularly the case if animal passage has taken place before subcultivation, the rabbit being selected as the medium of passage. In other words, subcultivation on laboratory media, especially if preceded by passage, appears to select out a race of organisms trained to extract from the culture medium a pyrogen that the original strain either could not extract or had become incapable of extracting. Control observations showed that the uninoculated media used produced no fever in the case both of agar and of broth. The suggestion therefore arises that when the subcultivated heated organism is injected pyrogen extracted or synthesized from the medium by the action of the living organism is unavoidably injected at the same time. It is possible, therefore, that the pyrogenetic properties of all those organisms injection of which when dead produces fever are in reality due not to the possession of pyrogenetic properties of their own, but to adsorption of something elaborated by the action of the living organism on the medium of growth. As will be seen, it has so far not been possible to detach completely this substance from organisms that have picked it up, possibly owing to its inclusion in interstices in the bacterial wall. Adsorption on the free surface of the bacterial wall appears not to prevent partial detachment of this adventitious pyrogen, since its presence can readily be demonstrated in the washings by the fever that follows their injection. If this theory—that the pyrogenetic effect of dead vaccines is due merely to adsorption of some substance manufactured from the medium of growth—is true, we ought to find that an inoculated medium when it has been deprived of its organisms is still pyrogenetic. And experiment proves this to be the case, provided that the centrifuge is used as the means of separation and not the bacterial filter.

A large number of observations have from time to time been made on the toxicity of bacterial filtrates expressed in fever.⁵ And numerous observers have shown that many filtrates are practically non-toxic and produce no fever. At the same time it was shown that the heated material

held up by the filter was often in these cases found to be strongly pyrogenetic as well as the heated unfiltered cultures. These findings naturally gave additional support to the view that the fever of infectious disease was caused by dead bacterial protoplasm. As, however, we showed⁶ in 1911 the use of the filter is quite inadmissible in determining the presence or absence of pyrogenetic substances in culture media. And for two reasons. In the first place, it is difficult—if not impossible—to avoid contamination of the filtrates with antipyretic salts derived from the filter itself. Calcium salts, for example, were, we showed, traceable in the filtrate even after repeated passage of freshly distilled water. And calcium salts have been shown by many observers to inhibit fever. In the second place, although in view of our observation that pyrogen appears to be a body of small molecule⁷ there is no guarantee that filtration of a colloidal liquid containing bodies of small molecule as well as of larger molecule, such as bacteria, will allow the filter to hold back the large and let through the small. On the contrary, larger bodies must tend to block the filter and prevent to an undetermined extent passage of the smaller.

Furthermore, apart from the question of filtration, if we wish to demonstrate the presence or absence of pyrogen by injection, it is always necessary to be sure that a sufficient volume of pure water is used as the diluent or vehicle of injection. This is necessary because increase of the volume of the diluent increases the amount of fever produced by injection of pyrogen, whilst injection of the same quantity of pyrogen in a smaller volume of diluent may produce no appreciable effect. Again, if normal saline be used as diluent instead of water, injection of one animal may produce high fever, whilst injection of a second animal of the same weight with the same quantity of pyrogen in the same quantity of saline may produce none. Experiments⁸ dealing fully with all these points we have published elsewhere, though at present it is not possible to explain them. All observations, therefore, that deal with the pyrogenetic properties of bacteria, or of emulsions of bacteria, must be regarded as suspect (a) if the filter is used, (b) if saline be employed as the diluent, (c) if the relation of the volume of pure water and of the quantity of pyrogen containing material to body weight be not clearly stated, (d) if the water used for injection be not freshly distilled each day and used at once. The numerous fallacies introduced into the study of fever by absence of knowledge as to the necessity for this last precaution we have fully dealt with in other papers.^{2, 3, 4, 7} Here it will be sufficient to point out that the experimental evidence hitherto relied on in favour of the existence of such distinct entities of fever as water fever, salt fever, salvarsan fever, lactose fever, glucose fever, saccharose fever, protein fever, nuclein fever, tissue fever, fibrin ferment fever, serum fever, blood cell fever, haemolytic fever, transfusion fever, and anaphylactic fever no longer exists, since we have reproduced fever charts typical of all of these by injecting pyrogen-containing water alone, whilst the injection of each of these substances in pure water produced no fever.

Hence the only satisfactory method of determining by injection if an infected culture medium, after separation of the infecting organisms, is or is not toxic in the sense of producing fever is to use an efficient centrifuge and to employ as diluent freshly-distilled water in constant and stated volume. When these precautions are taken, the reputed atoxicity of many organism-free culture media that have been submitted to infection entirely disappears. The results of the large number of experiments we have carried out on this subject may be briefly illustrated.

METHOD OF EXPERIMENT.

An eighteen-hours culture, for example, of a stock strain of *B. typhosus* on broth is examined in the following ways:

A.—Unheated.

1. A measured quantity—sufficiently small not to kill—of the whole broth is injected intravenously in a suitable quantity of water into a healthy rabbit of known weight. Fever rapidly follows, reaching its maximum in two to four hours, and disappearing in six to eight hours. Observations are taken every thirty minutes. Observations taken at much longer intervals than this are useless. The next day and on several successive days observations are again repeatedly taken, but fever does not return within the limits of observation.

2. The centrifuged deposit of an equal quantity of the same culture is repeatedly washed and is then injected in water into a second animal, and observations are taken as before. The results are precisely the same as in the first experiment, allowing for the slight variations in the degree of fever produced as a result of the varying personal equation on the part of the animals injected.

3. The supernatant fluid not injected in Experiment 2 before washing is now injected in water into a third animal. Once more the same essential features are recorded.

B.—Heated (to 100.0° C.).

Precisely the same experiments described under A are now repeated with the heated culture, the heated centrifuged and washed organisms, and with the heated supernatant broth. And again the same results are recorded in every case. Examination of the heated supernatant broth shows that no organisms are present.

The heated and unheated centrifuged deposits from a fresh broth culture of the same strain are then washed with a solution of hydrogen peroxide. This procedure entirely removes their pyrogenic properties, as was shown by injection into several animals. Microscopic examination of stained films of the deposit before and after this treatment reveals no differences. The heated and unheated centrifuged deposits from fresh samples of the culture are repeatedly washed with pure water, and the washings pooled. The washings produce on injection slight fever, the deposits produce high fever which is immediate. The heated and unheated centrifuged deposits from yet other samples of the culture are repeatedly washed with 1.4 per cent. sodium citrate solution in pure water and the washings pooled. These produce more fever than do the water washings. The deposits, however, are still markedly pyrogenic. Finally, the heated and unheated centrifuged deposits from the remainder of the culture are repeatedly washed with pure ether and the washings again pooled. These are then evaporated in a water bath and the residue taken up in citrate solution and injected. The procedure is followed by high fever. The original deposit of organisms, however, is still somewhat pyrogenic, possibly because it is not easy to get rid of all the other soluble pyrogen present. Owing to contamination of the bottom and sides of the test tube with the residue of pyrogen left after evaporation of the last remnants of the ether unavoidably left behind in removal of the supernatant fluid this appears to be the probable explanation. Examined by the microscope the ether washed organisms appear intact but moniliform, and they stain badly.

The points brought out by these experiments with ordinary laboratory strains may be thus epitomized:

B. typhosus grown in broth produces, if the heated or unheated organism be injected, an immediate and fugitive fever characterized by a sharp rise and fall. The animal does not become infected after injection of the living organism, and does not show any late fever after injection of either the living or the dead organism. Precisely the same events take place on injection of the culture-medium after separation of the organisms. The pyrogen therefore is present in the separated broth, and in or on the surface of the bacteria. This pyrogen cannot be completely washed away from the organisms, but it can be better detached by citrate solutions than by pure water, and better still by ether. It is, in short, feebly soluble in water, more soluble in citrate solution, and markedly soluble in ether. It cannot therefore, be a protein. It is apparently oxidized by peroxide since washing with this subject renders it inert, and standing for twelve hours in the semi-dry state has the same effect. We find in addition that it is active after incubation with a strong solution of tryptic ferment. Chloroform appears to some extent to inhibit its activity, but it is difficult to determine if this action is reversible owing to the difficulty of accurate determination of relative degrees of fever in the rabbit.

This pyrogen appears to be a preformed pyrogen when injected and to bear in its effects no relation to any known form of infection-fever occurring naturally in man and animals. After the immediate fever has passed off there appear to be no after-effects and there is no secondary fever, and this is also true after injection of the living or dead organism that has been allowed to stand in minimal quantities of water or saline. The pyrogen is, in addition, remarkably heat stable.

Such are some of the characteristics of a pyrogen that has hitherto been believed to be the purely bacterial product of a laboratory-grown organism that is known not to be pathogenic to the rabbit. Clearly, then, *B. typhosus* is not an organism in which it is possible to study natural pyrogen as it occurs in the body. Examination of other strains of *B. typhosus* on broth produced the same results, as also did examination of this organism on agar.

In order, therefore, to determine whether the pyrogen of infective disease is or is not a true bacterial product it is necessary to select for examination an organism that is pathogenic to the rabbit, and which at the same time has not become contaminated by a preformed pyrogen derived from the medium employed in artificial culture.

Certain strains of *S. aureus* satisfactorily meet these conditions. The absence of contamination in such strains is readily proved by injection. If, for example, the heated or unheated agar or broth culture be injected without separation of the organisms from the agar scrapings or broth no immediate fever is produced. The same negative result is obtained if the washed centrifuged organisms, or the supernatant fluid in the case of broth, or the washings in the case of agar, are injected. If, moreover, repeated observations are taken for several days of all the animals injected, it is found that fever only occurs in those which have received the living organism. In these animals, provided that the dose is not large enough to produce early death, or small enough to prevent infection, fever appears in a day or two, and continues till death or recovery takes place. If the virulence of the organism injected has not been reinforced by recent subcultivation, or by subcultivation *plus* animal passage, recovery is the rule. The fever generally begins within twenty-four hours of injection, but the latent period—if avirulent strains have been selected—was well marked in all the numerous cases examined. Here, then, is a very different picture to that produced by injection of *B. typhosus*. The fever set up is late in appearing instead of early, occurs always after injection of a suitable number of the living organisms, and never after injection of the dead; it has a slow instead of a rapid rise, and continues for several days instead of from one to eight hours. Further, it is not provoked by injection of the culture media from which the organisms have been separated, in marked contrast to the result obtained by injection of *B. typhosus* cultures deprived of that organism. The fever curve produced is that of a natural infection instead of one that bears no resemblance thereto. And finally, the substance—enzymic or what not in nature—which in the living organism liberates or synthesizes pyrogen is heat labile, whereas the pyrogen derived from laboratory media is remarkably heat stable.

Nevertheless the experiments so far quoted do not reveal the true source of the pyrogen in actual infections. A laboratory product, of course, natural pyrogen cannot be, as appears to be the case in the artificially produced fever set up by injection of cultures of *B. typhosus* and other members of the same group. Since, therefore, the source of natural pyrogen is not dissolved bacterial protoplasm, it can only be the substrate offered to the living organism by the tissues of the host.

The next step in the solution of the problem, therefore, was to discard laboratory media and to employ, as far as possible, the natural media of the animal body. For this purpose red cells from the blood of healthy rabbits were washed free of plasma with pure solution of sodium citrate, and were at once lysed in freshly distilled water. Samples of this blood-cell extract medium were exposed for one hour to air infection, and were then placed in a sterile flask, which was at once hermetically sealed and then incubated for twelve hours at 37.0° C. At the end of this time a copious growth of a staphylococcus was present, and demonstrated by film preparations from the centrifuged deposit. Control blood cell extract medium prepared at the same time and treated in the same way, with the exception of exposure to air, was found to be sterile. Injections of this control extract were followed by marked depression of temperature. Injections were then made of the infected extract into different animals as follows: (a) The heated washed centrifuged organisms; (b) the unheated washed organisms; (c) the unheated supernatant blood cell extract proved by control inoculations of agar to be free from organisms capable of growth. In each case immediate fever resulted of a precisely similar nature to that obtained by injection of *B. typhosus* cultures or of staphylococcal cultures after subcultivation preceded by passage. The animals injected with the heated organisms or with the supernatant liquid on the other hand failed to show any secondary fever. The animals, however, that were injected with the living organism

exhibited in addition to immediate fever the late continued fever of a natural infection with a much shortened latent period.

From these experiments it is clear that a pyrogen clinically indistinguishable from the pyrogen elaborated from agar or broth can be readily obtained if blood-cell extract be used as the medium. It is also clear that the immediate fever produced after injection of the heated organism or the supernatant fluid or the unheated organism was due to injection of preformed pyrogen—elaborated from the blood extract in the test tube—whilst the late fever produced after injection of the living organism was due to a second pyrogen—elaborated from the circulating blood or other tissues. The fact that the organisms were obtained direct from the air excludes the possibility of transference of any contamination from the ordinary laboratory media. These experiments appear to support the view that an organism that is unable to produce immediate fever dead or alive can do so both dead and alive if the living organism is first able to extract and adsorb from the medium on which it grows a pyrogen elaborated from that medium. We have elsewhere shown that organisms derived from the air, and not allowed to grow on the ordinary laboratory media, produce no immediate fever on injection provided that pyrogen-free water or saline is employed as the vehicle of injection. The pyrogen of blood-cell extract is just as heat stable as is the pyrogen derived from agar or from broth.

Owing to individual variation in the degree of fever produced in rabbits even after injection of constant quantities of pyrogen in constant quantities of water relatively to body weight, it has not been found possible accurately to measure the increase of "test tube pyrogen" from day to day in living cultures. The concentration, however, of "test tube" pyrogen is certainly high after twelve hours' growth, and remains high, in broth cultures, for many weeks. Abstraction of pyrogen taking place spontaneously is, of course, impossible, and no great destruction occurs within reasonable limits of observation. On the other hand, in the living body, during a natural infection we should not expect to find the same conditions. The concentration of pyrogen in the circulating blood of a feverish subject, for example, should be relatively low, since abstraction, if not destruction as well, must continually be going on. We should expect, therefore, to find that in order to produce fever in a healthy animal by injecting blood from a feverish animal it would be necessary to inject large quantities of blood. And on experiment this proves to be the case. For example, I injected into a series of healthy animals varying quantities of a 40 per cent. centrifuged citrated blood plasma from an animal exhibiting the continued fever of an acute genuine infection. The mixture of plasma and 1.4 per cent. citrate solution was centrifuged in order to get rid of any organisms that might be present. Injections up to 5.0 c.cm., equivalent to one four-hundredth of the weight of the animal injected, produced no fever. Injections of 10.0 c.cm. provoked slight rise of temperature, whilst injection of 15.0 c.cm. produced slight but definite fever. Control injections of healthy citrated plasma produced no rise of temperature. Clearly, therefore, the amount of pyrogen circulating in the blood in such case is much less than that contained in blood-cell extract in the test tube, which only requires injection of 0.50 c.cm. of a 25 per cent. extract, from which the organisms have been separated by the centrifuge, to produce high fever. The next step, therefore, was to determine if the injection of heated organisms after recovery from the body of a feverish animal does or does not produce immediate fever. If it were found that injection of such organisms did produce an immediate fever similar to that produced by injection of heated organisms after test tube growth on agar or broth or on blood cell extract, the fact would appear to make demonstration of the true source of pyrogen in infective disease by this method of investigation an almost impossible achievement. If, on the other hand, injection of heated organisms recovered from infected subjects produced no fever whatever the number injected, the theory that dissolved bacterial protoplasm is the primary source of the pyrogen of infective disease would be clearly untenable. Demonstration of this would, in conjunction with the experiments already described, leave no alterna-

tive but acceptance of the view that fever is the expression of the activity of living organisms, or their products, in and on the infected body.

Further experiments were therefore carried out (a) with organisms recovered from the fixed tissues of infected subjects, including pus, (b) with organisms recovered from the blood in subjects of septicaemia.

Recovery of Organisms from Infected Tissues.

Organisms were recovered from infected tissues in the following way:

An unheated laboratory strain of *S. aureus* that produced no immediate fever was injected into a series of healthy rabbits. A late continued fever resulted in all cases. After a week's illness with fever one of the animals was killed, and from the multiple abscesses present in the kidneys large numbers of organisms were heated before injection to 57° C. No fever whatever followed the injection early or late. The experiment was repeated with another animal which was killed at an earlier stage of the infection and the same results were obtained. In another experiment an unheated laboratory strain of a pneumococcus of low virulence was injected into a healthy rabbit. No immediate fever followed injection, but a late fever set in. On the eighth day, the fever still continuing, the animal was killed. An emulsion of splenic pulp, shown by film preparations to contain enormous numbers of organisms, was injected. Again no fever followed injection. In this experiment, as well as in the first series, control cultivations from the kidney and spleen showed that living organisms were present in the material examined, since copious growth was obtained on agar. Injection of the heated and unheated agar organisms produced high immediate fever in every case, and the injections in the case of the living organisms led next day to a fatal issue.

These observations therefore appear to show that organisms recovered from tissues other than the blood, and injected at once, possess no pyrogen, but that when grown on laboratory media they readily elaborate a contaminating pyrogen from such medium, which they subsequently adsorb. They also show that the concentration of body pyrogen cannot be high in the infected organs, since the tissue material injected in some of the cases was considerable in amount. Owing to the difficulty, however, of freeing organisms recovered in this way from tissue debris, their enumeration cannot be satisfactorily carried out. Moreover, it is conceivable that absence of pyrogen in the dead organisms after recovery from the body might be explained by exhaustion of any pyrogen they might have contained whilst in the body during the continuance of fever. The low concentration of pyrogen, however, already demonstrated in the blood plasma during high fever, and the fact that injection of organisms recovered from the body at an earlier stage of an infection shows them also to be pyrogen free, make such a view improbable.

Fresh Human Pus.

Pus was first taken from an abscess believed to be tuberculous in a patient with a temperature of 100° F. In film preparations no organisms could be found. Intravenous injection of small quantities of this pus in water was not followed by fever, early or late, whether heated or unheated. Samples of fresh pus were then taken from several patients with fever, and were examined bacteriologically and by intravenous and subcutaneous injection. Only those specimens were selected for injection which were found to contain large numbers of micro-organisms indicating the presence of recently-formed pus, or of pus from the periphery of abscess sacs. Control cultivations on agar gave in all cases selected copious growth. The organisms injected in different specimens included staphylococci, diplococci, and streptococci. One specimen contained both staphylococci and streptococci. In no case did injection of quantities of heated pus from 0.1 gram in 1 c.cm. of water to 2.50 grams in 2.50 c.cm. of water produce fever early or late. On the other hand, injection of sufficient quantities of unheated pus in this series never failed to produce a continued fever with a much shortened latent period.

These experiments are of interest in view of the numerous observations that have been published to show that injection of pus is constantly followed by fever. Such observations can only be explained by injection of pus in pyrogenetic saline, or by injection only of unheated pus. The experiments here quoted, however, lend additional support to the view that fever can only be produced during a natural infection by the living organism. In the case, however, of pus injections it is again impossible to carry out satisfactory enumeration of the organisms injected, and this method was therefore abandoned.

The only convenient method remaining of determining whether organisms recovered from the body during an infection with fever do or do not produce immediate

fever when injected dead is to obtain them direct from the blood stream in cases of septicaemia. If the circulating blood in septicaemia be received into pure sodium citrate solution and the mixture at once centrifuged, the deposit can, after lysis of the red cells in pure water, be readily examined. Any organisms present can then be counted in the ordinary way. In laboratory experiments, however, as is well known, it is a difficult matter to produce a genuine bacteriaemia, since the injection of organisms after growth on ordinary laboratory media is followed by their rapid disappearance from the blood stream as estimated by ordinary cultivation methods. And the cultivation of serum-fast strains has certain disadvantages. I have, however, overcome these difficulties by growing, for example, a laboratory strain of pneumococcus on the rabbit blood cell extract above described for forty-eight hours. Injection of the living organism from such extract into a healthy rabbit produces an infection with fever that can be demonstrated for some days. And injection of known numbers of organisms recovered from the blood during this fever can be readily carried out. Injection of 3,090 million dead organisms recovered in this way failed to produce any fever early or late, whilst injection of 10.0 c.cm. of a 40 per cent. citrated solution of plasma from the same animal, after removal of any organisms present by the aid of the centrifuge, produced slight but definite rise of temperature, and injection of the living organisms produced a typical continued fever.

Demonstration of the absence of pyrogen in dead bacterial protoplasm of organisms recovered during natural infections appears therefore in the cases examined to be complete. Whether this will prove also to be true of all organisms recovered from infected subjects, including man, is a question that only further study can decide.

In the meanwhile, in view of the cumulative evidence here presented, it is difficult to resist the conclusion that a rising temperature is not—as we have paradoxically been led to believe—a sign of increase of bactericidal and bacteriolytic function, but is on the contrary an expression of the activity of living organisms. Dead organisms, in short, do not in natural infections give rise to fever.

The next question that arises is the practical bearing of these observations on immunity problems in general and in particular on the administration of dead bacterial vaccines in the diagnosis, prevention and treatment of infective disease.

It will, I think, be clear that the non-bacterial pyrogen derived from laboratory media which is so misleading a factor in the experimental study of fever is also one that we cannot afford to ignore in practical medicine.

There are in connexion with prophylactic and therapeutic vaccines two possibilities to be considered. On the one hand contamination of bacterial vaccines with pyrogenic substances of purely laboratory origin may greatly enhance their prophylactic and therapeutic value. In this connexion the observations made some years ago on the effect of injecting nucleinates are of interest, though they cannot be dealt with here. If comparative experiments with the vaccines at present in use and with detoxicated vaccines prove that the presence of non-bacterial substances is an advantage, steps will have to be taken by subcultivation or in other ways to ensure that all vaccines are toxic. At present some vaccines are toxic in this respect, and some are not. Some of the causes for these differences I have explained. On the other hand, contamination of vaccines with laboratory material may be a distinct disadvantage. If this prove to be the case, vaccines, before they can be justifiably used in man, must first be tested for the absence of medium derivatives by injection of animals with the precautions mentioned, or in some other way. And such vaccines as are found to be toxic must then be rendered atoxic by one or other of the methods given, or by some other method if shown to be more convenient. It will not be forgotten that the injection of contaminated vaccines into healthy subjects for purposes of prophylaxis may be a less serious affair—if disadvantage be proved—than therapeutic injection of subjects already gravely ill. In these the question of dosage will necessarily be a matter of special concern, unless vaccines shown to have been detoxicated, or to be primarily atoxic, are employed.

Of more importance, however, even than the question of the positive good or harm effected by injections of contaminated vaccines is the larger question of to what extent the specific antigenic values of vaccines in general depend on the presence of this contamination. (In passing it may be well to point out that there is some evidence that the toxic substances elaborated from culture media by the action of living organisms on such media are to a certain extent specific to those organisms.*)

In other words this problem presents itself: Have we at present unimpeachable evidence that a pure dead vaccine has any antigenic values in the prophylaxis or treatment of general or local infections? It is worth notice that the vaccines for which the highest protective and therapeutic values are claimed are just those in which the content of laboratory pyrogen is highest. Cases in point are *B. typhosus*, *B. coli*, possibly *B. pestis*, and the acne bacillus. I am not here suggesting that this relation is more than accidental, or that dead vaccines have no specific antigenic values apart from their laboratory contamination. It is obvious, however, that until the point has been fully investigated we are no longer justified in attributing to dead bacterial vaccines antigenic values that may prove to belong—in whole or in part—to substances that are not primarily derived from dead bacterial protoplasm. At present when we employ these vaccines we have no guarantee that any single vaccine provided is a pure vaccine.

In future, therefore, we shall require such guarantee, as well as an assurance that the process of purification has not damaged any genuine antigen the purified vaccine may possess. And in passing it may be well to glance at the question of the so-called autogenous vaccine, which in one important respect is a somewhat misleading name, as I pointed out in 1911. A vaccine made from dead organisms collected from the blood or other tissues of an infected subject and injected without intermediate cultivation on laboratory media is a genuine autogenous vaccine, and its injection in animals appears not to be followed by toxic symptoms, though it remains to be seen if it has any therapeutic effect. A vaccine, however, that is made from organisms collected in the same way, but then cultivated on artificial media and then injected—after death of the organisms has taken place—is not a genuine autogenous vaccine, because, as I have shown, the toxic symptoms that may now arise are due to contamination from the laboratory medium of growth. The organism, in short, has had a new function imposed upon its descendants by such growth, and it is not impossible, therefore, that the increased therapeutic values with which the *soi-disant* autogenous vaccine is credited may prove to be due to this very fact.

It is not stretching the argument too far to point out that it is also necessary in view of the experiments here described to examine afresh the evidence that has hitherto been relied on in favour of the existence of bacterial toxins in general and especially of the so-called endotoxins. The endotoxin of *B. typhosus* is a case in point. In all these cases it will now be necessary before we can postulate the existence of a genuine endotoxin to be sure that the organism selected for purposes of extraction, whatever the method of extraction selected, is first of all deprived of the highly toxic material elaborated from the culture medium. In all experiments so far undertaken with the reputed toxin of *B. typhosus* when cultivated on laboratory media this precaution has for obvious reasons not yet been taken.

Finally comes the question of diagnosis. If, for prophylactic or therapeutic purposes, it is necessary to use pure vaccines, it is equally so in all those test tube reactions in which antigen and antibody are the essential factors. We shall not only have to make certain that only pure vaccines are used for the production of animal immune serums, but we shall also have to ensure that all bacterial emulsions used as diagnostic antigens are equally above reproach. In the case of such organisms as the bacillus of Koch, and of such other members as apparently do not elaborate

* For example, the centrifuged washed organisms of *B. coli* laboratory cultures cannot be deprived of their pyrogenic properties by washing with hydrogen peroxide. This fact appears therefore to indicate that the living *B. coli* elaborates from its culture medium a pyrogen of a different nature to that elaborated by the living *B. typhosus*. Moreover, *B. coli* is able to synthesize a pyrogen from protein-free media which before inoculation with the living organism are apyrogenic.

this contamination from culture media these precautions may prove unnecessary. On the other hand, in the case of all those organisms which we have shown to be characterized by their ability in the living state to elaborate toxic derivatives from the media of artificial growth, these precautions cannot be neglected.

In conclusion, although it is premature to indicate the practical lines of direct immunity research opened up by these experiments, it is impossible to ignore the suggestions they offer as to the meaning of sensitization and the superiority of living vaccines over dead, as well as to the necessity for re-examination of the whole scheme of vaccine-therapy. And in the meanwhile, if they do no more than press home the necessity for fresh investigations into the true source and character of the natural antigens of disease they will faithfully have served their purpose.

NOTE.—Since this paper was placed in type two articles on experimental fever have appeared in a German periodical by Thiele and Embleton which might seem at first sight to affect some of the conclusions reached in this communication.⁹ In the articles referred to, the authors—as so many have done before them—have fallen victims to the very fallacies which Dr. Penfold and I have already exposed in the English journals.¹⁰ One of the fundamental errors, for example, in all study of experimental fever produced by injection is the assumption that water which is innocent of demonstrable infection at the time of injection must also be innocent of pyrogen, and that water collected direct from the condenser of a distilling apparatus in daily use and then filtered is necessarily pure water. In consequence, therefore, of failure to recognize this source of error these workers in many of their experiments unwittingly used impure water, as is clearly shown in their own protocols. This accident is alone sufficient to invalidate all the experiments and deductions based thereon, though there are other fallacies of almost equal importance which I have fully described elsewhere.

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APYREXIAL TYPHOID.

BY

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APYREXIAL typhoid, although known to exist, is of excessively rare occurrence, and may therefore readily pass unrecognized, particularly in the absence of an epidemic of the disease. Cases of this type have been described by Gerhardt and Dreschfeld, and of these brief mention is made by the latter in Allbutt's *System of Medicine*.

In typhoid fever we know that, in patients of over 40 years of age, the temperature curve is lower and less characteristic, but although cardiac, pulmonary, and renal complications are more common, splenic enlargement and rose rash are frequently absent. For these reasons, therefore, the diagnosis may be rendered more difficult. The case recorded below will serve to illustrate these points.

The patient was a woman aged 60, who had resided in the same house for more than thirty years, and during that period had never required medical assistance for more than minor ailments. A careful investigation into her previous history precluded all possibility of any previous attack of typhoid fever.

The present illness commenced in the first few days of September, with the usual feelings of malaise, and with gradually increasing diarrhoea. She took to her bed on September 8th.

When first seen, on September 10th, the patient was in an exhausted condition, with a dry, furred tongue and subnormal temperature. The outstanding feature of the case at the time was the exceedingly profuse diarrhoea, which persisted in spite of all the usual remedies. On September 12th (two days later) the patient began to wander, and sank into a semi-comatose state, in which she remained for the ensuing fourteen days. During this period the intestinal symptoms were unchanged, there was a moderate distension of the abdomen, but no spots or enlarged spleen were present then or later. Widal's reaction on September 16th was positive in every dilution. From the onset of the attack until September 25th (that is, the fourth week of the disease), when complete consciousness returned, the mouth and surface temperatures were invariably subnormal, and the rectal temperature, taken twice daily, never exceeded 99.4°. From September 26th to October 4th the mouth and rectal temperatures each reached their maximum of 99.6° and 100.4° respectively, after which they dropped again.

During the fourth week a further specimen of serum was obtained for the purpose of comparative agglutination reactions to *B. typhosus* and other allied organisms, with the following results:

- B. typhosus* agglutinated up to 1 in 2,000.
- B. paratyphosus B* agglutinated up to 1 in 200.
- B. paratyphosus A* agglutinated very slightly.
- B. enteritidis* (Gaertner) agglutinated not at all.

The patient ultimately made a good recovery, and without serious complications.

An investigation of the water-supply of the (isolated) dwelling revealed a privy in close proximity to the well, which constituted the sole supply of drinking water on the premises. This water, on examination by the county authority, proved to be contaminated with sewage.

In the light of all the bacteriological findings, I think there can be very little doubt that in this case there existed a true typhoid infection.

A SIMPLE AND RAPID METHOD FOR THE ADMINISTRATION OF SUBCUTANEOUS SALINES.

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THE administration of saline solution by the method of subcutaneous injection has many points in its favour and should by no means become obsolete. There is no method to equal it when the absorption of a large quantity of fluid is required.

In numerous cases of late years I have made use of an apparatus which, from its convenience and simplicity, has served to replace more cumbersome methods.

The only requisites are (1) a large silver cannula from a Southey's tube apparatus; (2) Southey's rubber tubing; (3) a small piece of glass tubing, narrow at one end to fit this tubing, and larger at the other (for this an ordinary clean fountain-pen filler does very well); (4) a large glass funnel connected to the glass tube by rubber. The various parts are joined together and the whole is sterilized by boiling.

The mode of introduction deserves special notice, for it differs considerably from the usual method. The anterior axillary fold is grasped firmly and drawn outwards. The trocar with its cannula is then passed into the skin in a direction vertical to the chest wall and is pushed steadily through the axillary fold so that its point emerges within the axilla. The trocar is then withdrawn and the cannula is pressed onwards into the axilla until its shoulder rests flush with the skin.

The fluid as it emerges from this cannula spirts in all directions and is poured out into three distinct plains, separated anatomically into watertight compartments by the deep fascial layers. Into the most superficial of these

G1, Glass funnel. R1, Large bore rubber tubing. G2, Glass tube. R2, Southey's rubber tubing. T, Trocar. C, Cannula.