A Critical Study of Experimental Fever.

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(Communicated by Dr. C. J. Martin, F.R.S. Received February 17,—Read March 14, 1912.)

(From the Lister Institute of Preventive Medicine.)

The injection of solutions of salt into man and animals by the subcutaneous or intravenous route has in recent years become a common practice. In many cases the procedure is followed by fever, as was shown in man by Kottmann (1)* and by Schaps (2). If distilled water alone be injected in large quantities the same accident may happen, as shown (3) by E. Bergmann in 1868. In 1910 the injection of small quantities of water into animals was found (4) to have the same effect.

The cause, however, of what are known as "water fever" and "salt fever" has till recently (5) not been satisfactorily explained, though many theories have been advanced.

In 1911 it was suggested by Wechselmann (6) that the fever so often met with in man after injection of sterilised saline containing salvarsan is due to gross bacterial infection of the saline, demonstrable just before sterilisation. This view was based on the discovery of numerous organisms in his unheated solutions, and on the fact that fever no longer followed injection if he dissolved his salt in freshly-distilled water. This suggestion as to the cause of salvarsan fever was also adopted by McIntosh, Fildes, and Dearmont after independent confirmation in Dr. Bulloch's laboratory of Wechselmann's observations. The theory appeared to us to have an important bearing on the wider question of water fever, and of salt fever in general, and we therefore conducted several experiments on animals, publishing (5) our results in December, 1911.

We were able then to confirm the statement that solutions of salt are apt to exhibit pyrogenetic properties that do not belong to solutions made with freshly-distilled water. We found, however, that in the case of water these properties bore an inverse relation to the number of organisms present in the specimens we examined, and by control experiments in animals we showed that to a great extent they were primarily due to the presence in the water of a substance indestructible by prolonged heating at 120° C. We could not remove this fever-producing substance either by filtration through white

* Numerals in brackets refer to List of References at end of paper.

Doulton filters or by the use of the centrifuge, as shown by subsequent injection of the water. The actual presence therefore of organisms at the time of injection of sterilised saline did not appear to be the sole cause of salvarsan fever, nor of " salt fever " in general.

Since reporting these results we have studied the question more fully. Injection of the centrifugalised deposit from 250 c.c. of water shown to be pyrogenetic, and to contain 73,000 organisms per cubic centimetre, produced no fever. This was also true of a deposit from 75 c.c. of saline shown to be pyrogenetic, and to contain before the use of the centrifuge 950,000 organisms per cubic centimetre. In both cases the medium employed for injection of the organisms was first shown to be free from the heat-stable fever-producing body referred to. On the other hand injection of suitable quantities of water containing this body, but only 40 to 160 organisms per cubic centimetre, produced fever whether salt were subsequently added or not. This body is held back by Martin's* gelatine filter, a fact which shows that it is a colloidal substance in a fine state of dispersion. We conclude, therefore, that contamination by this body of water before mixture with salt is a more important factor in salvarsan fever than we had realised.

The unexpected presence of this hitherto unrecognised body must to some extent vitiate deductions drawn from previous work on the causation of fever which is the sequel to injection of a variety of substances dissolved or suspended in water.

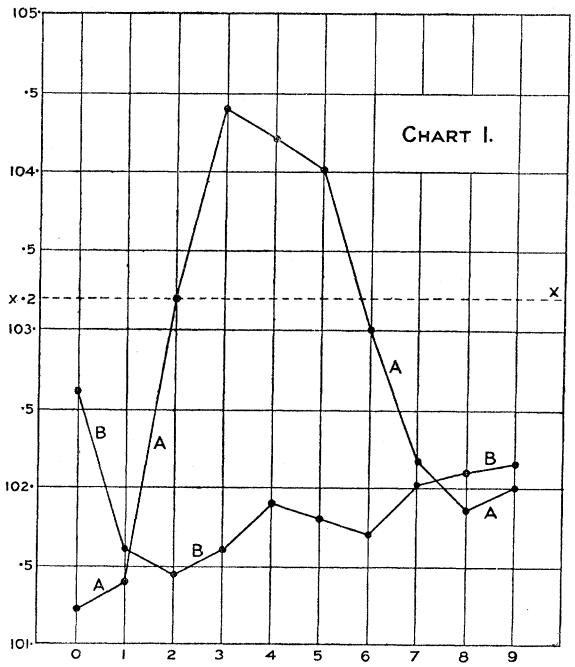
We have therefore determined to what extent the fever which had been known to follow injection of salt, sugar, fibrin ferment, and tissue extracts was due to this contamination.

Experimental Methods.

Before dealing with our evidence, it is necessary to refer to the current belief that it is impossible to study experimental fever with any approach to accuracy. The number of variants is admittedly great. Full experimental data dealing with the most important will be published in due course. They include the effect on body temperature of disease, age, sex, weight, breed, food supply, environment, exercise, rest, repeated thermometric observation, and other factors. Prolonged study has taught us that to establish broad effects, such as the ability of any given substance to produce fever on injection, knowledge of all the variants is necessary but sufficient. On the other hand, no comparative observations can be relied on unless the ratio of the volume of an injection (including weight of material dissolved or suspended in injection fluid) to body weight is kept constant. In every

* C. J. Martin, "Gelatine Filter," 'Journ. Physiol.,' 1896, vol. 20.

experiment already reported by one of us this injection ratio is stated. In all experiments here cited the same rule is observed. In our experiments we found it necessary to select animals as far as possible of the same age and weight, and to observe constant conditions of food supply and external temperature. All thermometric observations were taken in the rectum every 30 minutes, instruments of tested accuracy being always employed. Every observation has been taken by us, and never entrusted to assistants. The upper normal limit of the daily range of temperature in 150 healthy rabbits was found to be 103·2° F. All animals with a temperature more than slightly above this point were rejected, as well as those showing any apparent departure from health in other ways. Animals less than 1500 grm., or more than 3000 grm., in weight are unsuitable for experiment if the rabbit be used, the former weight indicating too unstable a temperature, the latter too resistant. Reference is here made only to early fever occurring within five hours of injection, and statements as to the



A. Rabbit, 2448 grm., injected intravenously with 11·50 c.c. water containing F.P.B. Injection ratio, 1/211.

B. Rabbit, 2478 grm., injected intravenously with 11·80 c.c. water containing no F.P.B. Injection ratio, 1/211.

(Interval between observations, 30 minutes.)

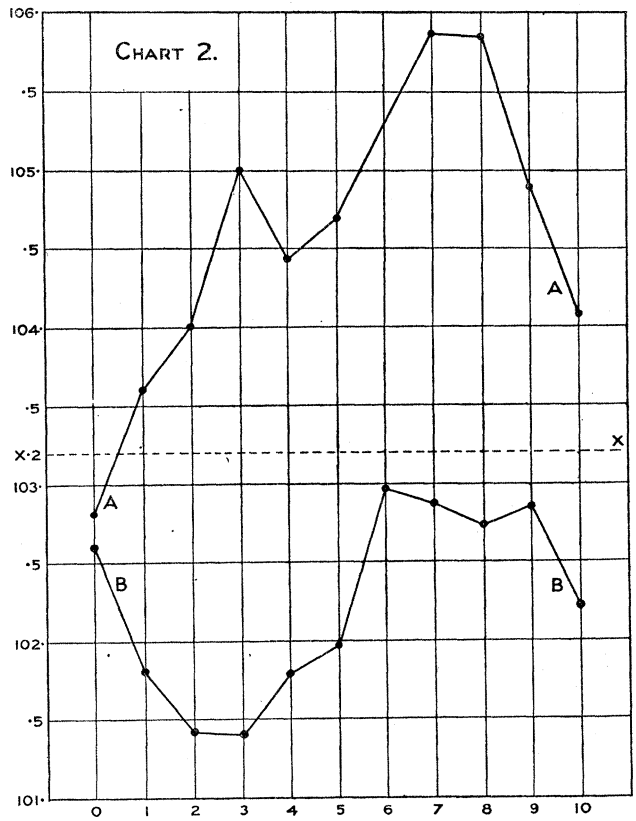
X corresponds to upper normal limit of range of temperature in healthy rabbits. This letter occurs throughout the charts.

presence or absence of fever in any given case are only made in this sense. Unless otherwise stated, all injections were made into the marginal veins of the ear.

The points we wish to establish are illustrated by charts. For the sake of brevity, we designate the fever-producing body under discussion by the letters F.P.B.

Water Fever.

Charts 1, 2, 3 are typical instances of experiments showing the fever-producing capacity of ordinary distilled water, and its absence in the case of water freshly distilled from a glass retort and injected at once. The essential features of these charts occurred in every one of several hundred experiments in which different volumes per kilogramme were injected. Special stress is laid not only on the absence of fever in the control animals, but also on the

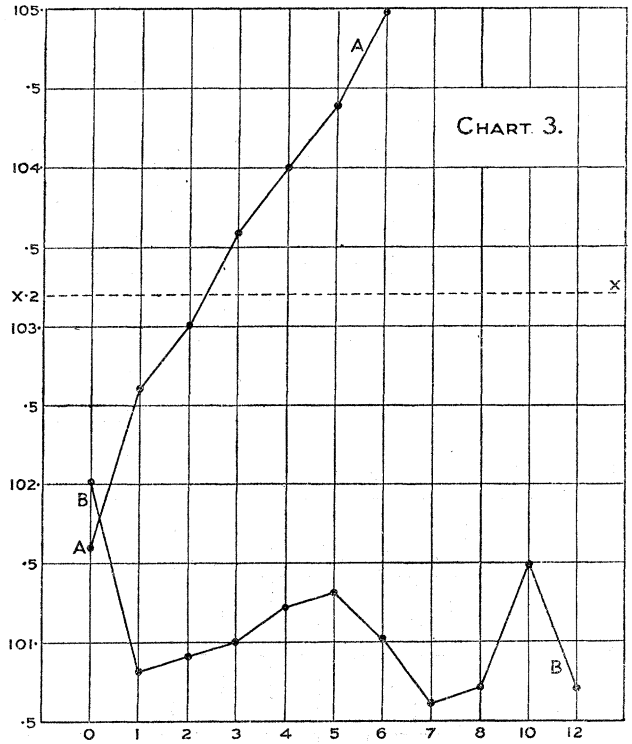


A. Rabbit, 2926 grm., injected intravenously with 50·40 c.c. water containing F.P.B. Injection ratio, 1/58.

B. Rabbit, 2500 grm., injected intravenously with 50 c.c. water containing no F.P.B.

(Interval between observations, 30 minutes.)

marked fall of temperature. The different extent of fever excited by different injection ratios when one sample of water containing F.P.B. is used, is clearly seen in nearly all the charts here shown. In 1910 one of us (4) advanced the theory that water fever is largely an auto-intoxication



A. Rabbit, 2083 grm., injected subcutaneously with water containing F.P.B. Injection ratio, 1/40.

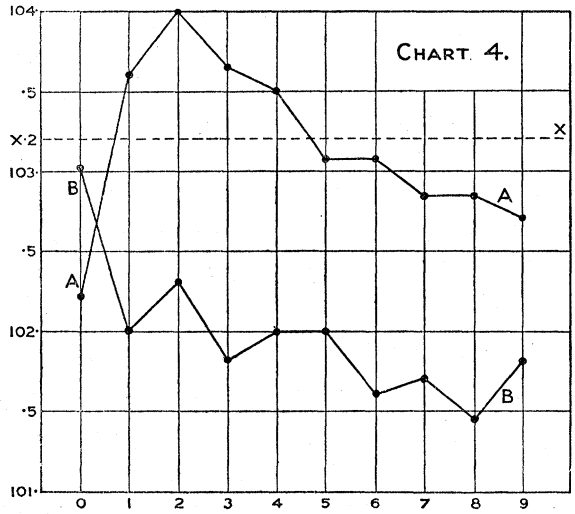
B. Rabbit, 2080 grm., injected subcutaneously with water containing no F.P.B. Injection ratio, 1/40.

(Interval between observations, 30 minutes.)

due to absorption of lytic products liberated locally by the injection of water. The evidence given in support of this view now disappears, since water supposed to be pure, and known to contain only 160 organisms per cubic centimetre, may actually contain F.P.B.

Salt Fever.

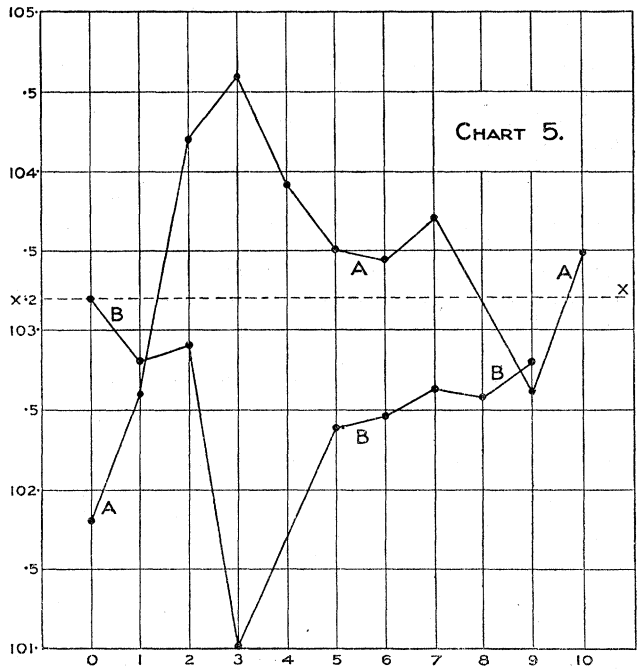
Charts 4 and 5 show that solutions of salt made with ordinary distilled water give rise on injection to fever, but that when freshly distilled water is the solvent a fall of temperature results. These effects were obtained in all our experiments up to 25-per-cent. concentrations of sodium chloride.



A. Rabbit, 1970 grm., injected intravenously with saline made with water containing F.P.B. Injection ratio, 1/211.

B. Rabbit, 1800 grm., injected intravenously with saline made with water containing no F.P.B. Injection ratio, 1/211.

(Interval between observations, 30 minutes.)



A. Rabbit, 2551 grm., injected intravenously with saline made with water containing F.P.B. Injection ratio, 1/350.

B. Rabbit, 2316 grm., injected intravenously with saline made with water containing no F.P.B. Injection ratio, 1/211.

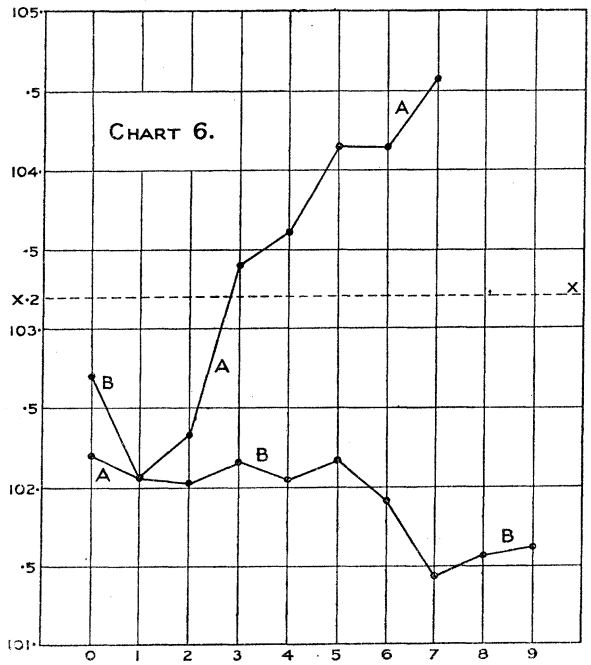
(Interval between observations, 30 minutes.)

In the experiments illustrated by the curves A the water injected was ordinary distilled water and contained 40 organisms per c.c. In the case of the experiments illustrated by curves B, the saline was made with freshly distilled water and at once injected. The results here recorded occurred in all essentials in all of the 27 experiments made. Numerous papers dealing with salt fever have appeared (7–14) in which various theories based on the view that salt was the pyrogenetic agent have been advanced. The evidence in support of these views no longer holds good.

Carbohydrate Fever (Charts 6, 7, 8).

Charts 6 and 7 show that the injection of glucose and saccharose produced no fever when the solutions injected were made in pure water.

Chart 8 shows temperatures obtained in two experiments made with lactose from different sources. The solutions were made in pure water. The upper curve shows the result with a sample of commercial lactose. The lower curve shows the result with lactose made from a catheter sample of cow's

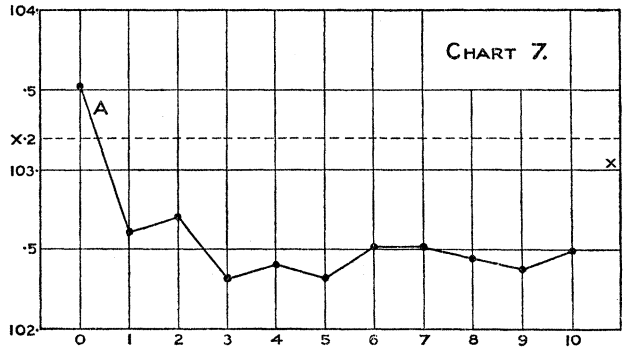


A. Rabbit, 1545 grm., injected intravenously with 8 c.c. 5-per-cent. glucose in water containing F.P.B. Injection ratio, 1/193.

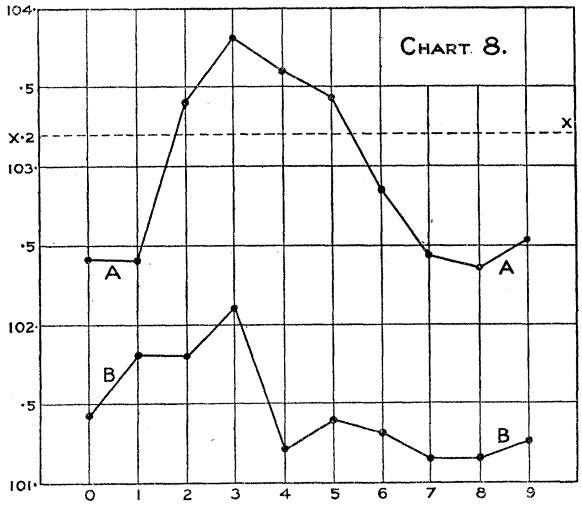
B. Rabbit, 1718 grm., injected intravenously with 8—9 c.c. 5-per-cent. glucose in freshly distilled water. Injection ratio, 1/193.

(Interval between observations, 30 minutes.)

milk. In its preparation all the reagents used were made with freshly distilled water, and the crystallisation was carried out at 0° C. For this we are indebted to Dr. McLean at the Lister Institute. With these precautions the rise of temperature was insignificant.



A. Rabbit, 1747 grm., injected intravenously with 8·8 per cent. cane sugar dissolved in 8·20 c.c. water freshly distilled from glass retort. Injection ratio, 1/211.
(Interval between observations, 30 minutes.)

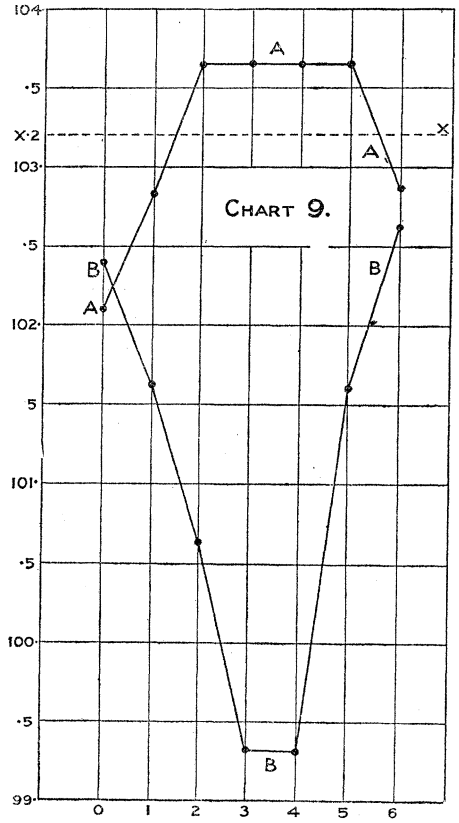


A. Rabbit, 1647 grm., injected intravenously with isosmotic solution of commercial lactose in pure water. Injection ratio, 1/211.
B. Rabbit, 1605 grm., injected intravenously with isosmotic solution of lactose obtained from a catheter sample of milk in the method described in text.
(Interval between observations, 30 minutes.)

The existence of carbohydrate fever (10, 15, 16), which has long been looked upon as a definite clinical type, is not supported by the above experiments.

Tissue Fever (Charts 9, 10, 11).

Chart 9 shows that the presence of F.P.B. is also responsible for the fever that follows the injection of blood laked with water, and that injection of similar quantities of blood laked with pure water produces marked fall of temperature. This fall, as will be seen, is due to the depressant effect of two distinct factors, pure water and extract of red cells.



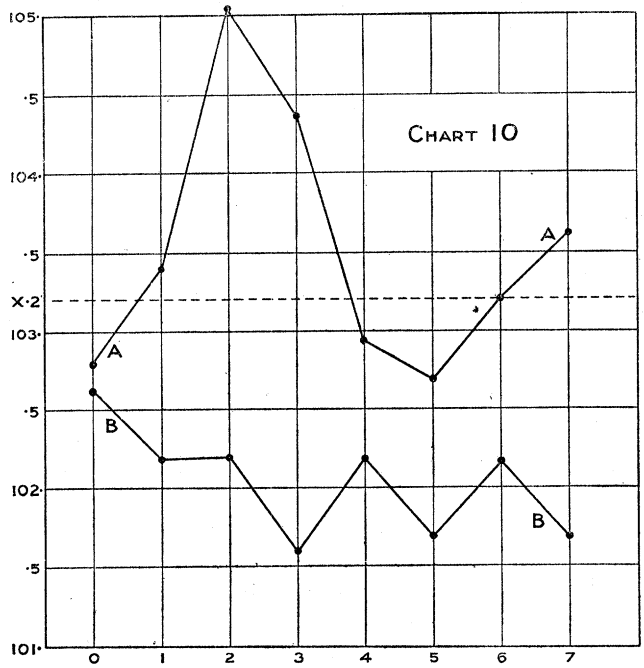
A. Rabbit, 2590 grm., injected intravenously with 4·6 c.c. rabbit blood in 15·3 c.c. water containing F.P.B. Injection ratios, 1/563 and 1/169.

B. Rabbit, 2197 grm., injected intravenously with rabbit blood in pure water. Injection ratios as in A (slightly less).

(Interval between observations, 30 minutes.)

Chart 10 shows the relative temperatures after injection of two animals with extract of red blood cells taken from another animal of the same species. The extract was made in the case of animal A by lysis of the cells in water shown to contain F.P.B., the extract for animal B being made in pure water. This chart destroys the value of the evidence of fever

due to injection of extract of red cells of the same species. The same result followed the injection of red-cell extract of another species, as well as that of red-cell extract made from the cells of the animal injected. In all cases where fever resulted the water used for the preparation of the extracts was the same and contained 40 organisms per c.c. on injection. The water employed in the preparation of the sodium citrate solutions into which the blood was shed, the saline in which the cells were washed, and for lysis of the



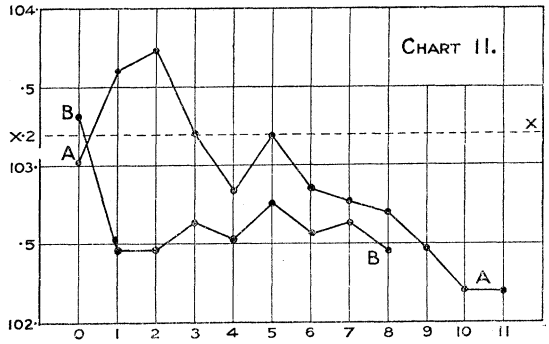
A. Rabbit, 2785 grm., injected intravenously with red-cell extract in water containing F.P.B. Injection ratios of water and extract same as in B (figures lost).

B. Rabbit, 2370 grm., injected intravenously with red-cell extract in water containing no F.P.B. Injection ratios of water and extract same as in A.

(For details of preparation of extract, *vide* text. Intervals between observations, 30 minutes.)

blood corpuscles was a sample containing F.P.B., and 40 organisms per c.c. The control extracts were made throughout with solutions containing pure water. The extent of fever shown is in excess of that which follows injection of a similar quantity of water containing F.P.B. This increase is perhaps due to absorption of F.P.B. during preparation of the extracts, perhaps to the action of F.P.B. on the stromata resulting in their acquisition of pyrogenetic function not previously possessed. The point requires quantitative investigation.

Chart 11 shows that the injection of fresh normal serum produces a fall of temperature, in spite of the presence of fibrin ferment. This fact gravely affects the theory of fibrin ferment fever (17–22) more especially as all the evidence hitherto accepted in support of this theory is based on injection of extracts in water that we now know to have been liable to contamination with F.P.B. Until, therefore, these experiments on ferment fever, including fibrin ferment fever, have been repeated with extracts in water proved to be free from this substance all observations on this subject require careful scrutiny.



A. Rabbit, 2211 grm., injected intravenously with 3 c.c. normal serum from another healthy rabbit. Injection 4 hours after separation. Injection ratio, 1/737.

B. Rabbit, 1932 grm., injected intravenously with 3·50 c.c. of his own serum. Injection 4 hours after separation. Injection ratio, 1/552.

(Interval between observations, 30 minutes.)

With regard to Charts 9, 10, 11 as a group it is generally believed that the injection of sterile extracts of animal tissues (23–25) may cause fever. This applies to extracts of foreign blood, of red cells, of leucocytes, or of blood serum, as well as to extracts of all foreign tissues. By the term foreign tissue is here meant tissue alien to the animal injected, even if belonging to animals of the same species. Fever is also believed to follow the injection of an animal with extracts of his own blood or blood cells, and the fever following fracture and hæmatomata have been thus explained. In all experiments, however, the extracts were prepared in liquids containing water which must now be regarded as suspect. In view of our experiments the value of much of the evidence, therefore, on which belief in this type of fever rests disappears. If reference be made to Charts 1, 2, 3 in the light of Charts 9, 10, 11 it will be seen that they afford additional evidence that the theory of tissue fever cannot be supported by lysis of cells effected by the injection of distilled water. We find this to be also true of extracts of solid organs, including the brain. The injection of fresh egg albumen also failed in our hands to give

rise to fever, a fact that has some bearing on the belief in protein fever after single injections of this class of substance in an unbroken state.

In conclusion, we submit that the existence of "water-fever," "salt-fever," "sugar-fever," "ferment-fever," and "tissue-fever" no longer rests on secure ground. That future advance in the study of fever is only possible by recognition of all the fallacies inherent to experiments involving the use of water liable to contamination with this fever-producing substance will not, we believe, be questioned.

The fact that one of us has been a victim to these fallacies is sufficient justification for the critical nature of this note.

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On the Systematic Position of the Spirochæts.

By Clifford Dobell, Fellow of Trinity College, Cambridge; Lecturer at the Imperial College of Science, London, S.W.

(Communicated by A. Sedgwick, F.R.S. Received February 21,—Read March 28, 1912.)

This paper is a very brief summary of the chief results of my researches on the Spirochæts and related organisms. I have been occupied with these researches for several years, and I believe that I have now obtained sufficient evidence for it to be possible to form a correct judgment regarding the systematic position of the Spirochæts.

It is well known that various views of this problem have been taken. Hitherto, three different opinions have been expressed,* and more or less vigorously defended, by different workers. They are: (1) that the Spirochæts belong to the Protozoa; (2) that they belong to the Bacteria; (3) that they belong to the Cyanophyceæ. The upholders of the first view suppose that the Spirochæts resemble the Flagellata, especially the trypanosomes. Those who support the second view usually regard the Spirochæts as being closely similar to *Spirilla*. Those who support the third view believe that the Spirochæts show points of resemblance to the spiral forms of Cyanophyceæ (*Spirulina* and *Arthrospira*).†

In view of the existence of these wide differences of opinion and the corresponding mental attitudes of those who have attempted to form a judgment in this matter, I would submit the following statement regarding my own position :—

* There is a fourth view, which I formerly advocated—namely, that the Spirochæts should be regarded as an independent group of organisms. I no longer hold this view, as further research has shown me that it is incorrect.

† These three views may be traced back respectively (1) to Schaudinn (1904); (2) to Ehrenberg (1833), who placed *Spirochæta* in his family Vibrionia—the equivalent of the modern Bacteria; (3) to Cohn (1854), who regarded *Spirochæta* as a colourless form of *Spirulina*.