Pathological Section.

February 20, 1912.

Mr. S. G. SHATTOCK in the Chair.

The Relation of Salvarsan Fever to other Forms of Injection Fever.

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WHEN injections of salvarsan in saline began to be widely employed, it was soon found that fever might ensue. Although many theories have been advanced to account for this accident none of them will explain all the facts. On the contrary, we find that salvarsan fever is so closely allied to other forms of injection fever that we thought that this relationship might afford an interesting subject for discussion. In order, however, to make clear the points we wish to make a slight historical digression is necessary.

In 1906 it was shown by Kottmann [4] that injection of saline into man might give rise to fever, and this was confirmed by Schaps [6] in 1907. Since then many workers have shown that injection of animals with saline may have the same effect. Salt fever, therefore, as it was called, became established in the literature, and numerous theories sprang up as to its meaning, all based on the belief that salt was the active pyrogenetic agent. Under this heading also really belongs the fever that may follow the injection of saline in intravenous anæsthesia, surgical shock, hæmorrhage, cholera, and so forth, though often the condition demanding treatment has been held responsible for the fever. Fever after injection of sea-water is a recent addition to this group. In 1910 one of us found [1] that the injection of animals with sterile water also caused fever, which therefore came to be known as water fever. In the belief that the water was absolutely pure this worker maintained that water fever was an auto-intoxication, and in August, 1911, suggested [2] that salt fever and salvarsan fever were in reality only different forms of water fever. In the last few years it had also

been found that the injection of certain other substances, such as sugar or tissue extracts in water or saline, would give rise to fever. Accordingly carbohydrate fever, tissue fever, protein fever, ferment fever, and so forth, all took their place in the literature as definite clinical types. In each case the substance injected was credited with specific pyrogenetic properties. And finally came salvarsan fever. Here, then, we have all these types—water fever, salt fever, sugar fever, ferment fever, tissue fever, anæsthesia fever, surgical fever, sea-water fever, and salvarsan fever, each apparently the result of different causes.

In December, 1911, we showed [3], so far as we know for the first time, that there is good reason to believe that some of these different

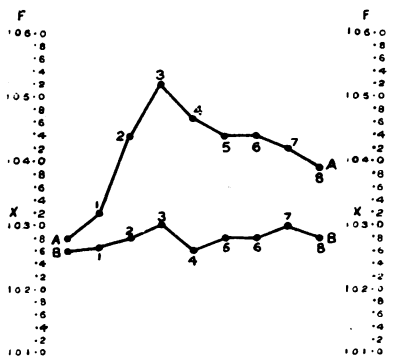

CHART 1.

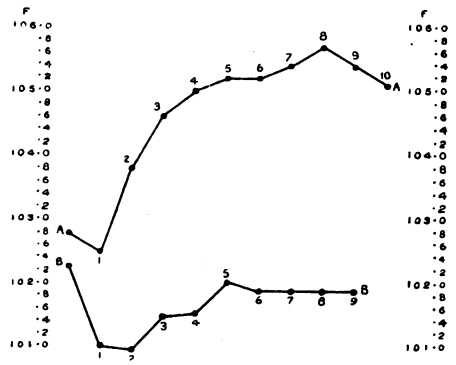

CHART 2.

Chart 1.—**A**, Rabbit, 2,083 grm. injected intravenously with water containing fever toxin and from which 73,000 organisms per cubic centimetre have been removed by centrifuge. Injection ratio 1 in 211. **B**, Rabbit, 2,983 grm. injected with deposit in pure water of 6,000,000 organisms thrown down from above water after thirty minutes in centrifuge tubes. Quantity of water injected 0·50 c.c. Observations every 30 minutes.

Chart 2.—**A**, Rabbit, 2,500 grm. injected intravenously with *c.* 40 million heated *Bacillus typhosus* organisms. **B**, Rabbit, 2,448 grm. injected intravenously with *c.* 50 million saline-grown organisms after heating and centrifuging from saline injection of which in ratio 1 in 50 produced no fever. Interval between observations 30 minutes.

types of fever are due to a common cause. Our experiments leading to this result were suggested by a theory advanced by Wechselmann [7] that salvarsan fever is due to gross infection, demonstrable at the time of injection, of the solutions of salt and salvarsan. Wechselmann's theory as to the cause of salvarsan fever was subsequently endorsed by McIntosh, Fildes, and Dearden, working in Dr. Bulloch's laboratory at the London Hospital, as a result of independent observation. The theory at first sight seemed to afford a clear explanation of salvarsan

fever, because the liquids examined, either before or after the addition of salt, were reported to contain large numbers of organisms. Moreover, if saline made with freshly distilled water were used, it was found that salvarsan fever did not occur. This was in one instance [5] also true after injection of a filtered sample of sterilized saline made with water that contained before filtration large numbers of organisms, and the apparent effect of the filter in preventing fever was therefore used as an argument that salvarsan fever and salt fever are necessarily due to the actual presence of micro-organisms. No control experiments, however, were cited by these observers to show that organisms grown in pure saline are, if injected immediately after heating, in actual fact themselves capable of producing fever. And from the evidence adduced [5] in support of the statement that filtration will render toxic saline atoxic, it is not clear whether the toxicity, in terms of fever, of the unfiltered

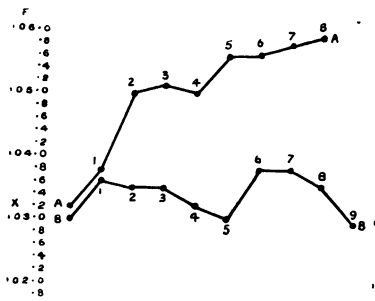

CHART 3.

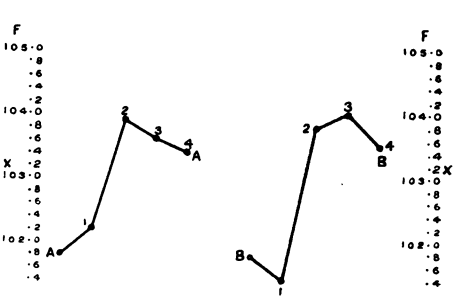

CHART 4.

Chart 3.—**A**, Rabbit, 2,324 grm. injected intravenously with 40·0 c.c. water containing filtrable fever toxin and 40 organisms per cubic centimetre. Injection ratio 1 in 58. **B**, Rabbit, 2,448 grm. injected intravenously with 49·0 c.c. saline containing 950,000 organisms per cubic centimetre, but no filtrable fever toxin. Injection ratio 1 in 50. Both liquids injected immediately after heating. Interval between observations 30 minutes.

Chart 4.—**A**, Rabbit, 2,278 grm. injected intravenously with 10·80 c.c. water containing 40 organisms per cubic centimetre and filtrable fever toxin. Injection ratio 1 in 211, before filtration. **B**, Rabbit, 2,335 grm. injected intravenously with 11·0 c.c. from the same water. Injection ratio 1 in 211, after filtration. Interval between observations 30 minutes.

sample of water with which the saline was made up had or had not been demonstrated. The theory, in fact, appeared to be based on the assumption that the presence of a large number of dead organisms is in itself sufficient to cause fever, whatever their source.

We therefore conducted control experiments to see if unbroken bacterial protein is necessarily pyrogenetic, and if the ordinary bacterial filter does actually render toxic solutions atoxic. We centrifuged 225 c.c.

of heated water, and 54 c.c. of heated saline, containing respectively 73,000 and 950,000 organisms per cubic centimetre, and injected the deposits. In neither case (Charts 1 and 2) did fever result. We also injected 50 c.c. of a specimen of saline containing nearly 50 million organisms (Chart 3). This saline was autoclaved immediately before injection. Again no fever resulted. Finally, we injected samples of water and of saline, both grossly infected, before and after filtration through white Doulton candles, and found that their fever-producing properties were practically unimpaired by passage through the filter (Chart 4).

Clearly, therefore, the theory that salvarsan fever is necessarily due to the actual presence in the solutions of dead micro-organisms

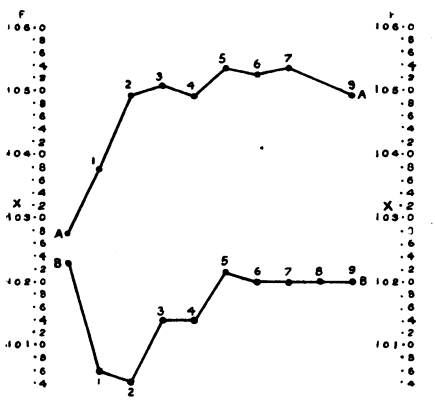

CHART 5.

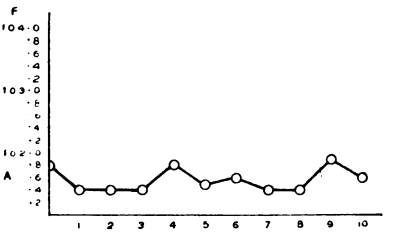

CHART 6.

Chart 5.—**A**, Rabbit, 2,324 grm. injected intravenously with *c.* 42·0 c.c. water containing filtrable fever toxin and 40 dead water-grown organisms per cubic centimetre. Injection ratio 1 in 50. **B**, Rabbit, 2,448 grm. injected intravenously with 49 c.c. pure saline containing no filtrable fever toxin but *c.* 1 million dead saline-grown organisms per cubic centimetres. Injection ratio 1 in 50. Interval between observations 30 minutes.

Chart 6.—Distilled water, 1 in 5,180; 2,526 grm., **A**, 0·48 c.c. water.

capable of removal by hardware filters is not supported by our experiments. We do not, of course, assert that what we found to be true of organisms grown in water or saline in our laboratory is necessarily true of all organisms grown in water or saline. Injection of heated organisms that have been grown on nutrient media is known to give rise to fever, and from this fact no doubt arose the assumption that injection of organisms grown on water or saline and then heated must necessarily have the same effect. Since, then, this theory of salvarsan fever appears incomplete, explanation of its cause must be looked for elsewhere.

In December last [3] we showed that the pyrogenetic function of ordinary distilled water is often inversely proportional to the number of organisms present. Water, for example, containing forty dead organisms per cubic centimetre produced high fever, whilst an equivalent quantity of saline containing nearly a million dead organisms per cubic centimetre produced none (Chart 5). We also pointed out that an important factor in the production of fever after injection of sterilized water or saline is the presence of a heat-stable substance, of unknown source, incapable of removal by the centrifuge or the ordinary bacterial filter (Charts 6—11).[1] We now find that this pyrogenetic body is to a great extent, though not entirely, held up by Martin's gelatine filter. It is therefore a colloidal substance of small molecule. Since we could demonstrate its presence both in liquids grossly infected and in liquids practically bacteria-free, the problem is to explain how the

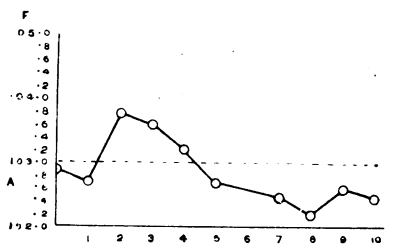

CHART 7.

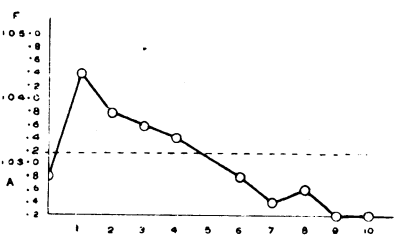

CHART 8.

Chart 7.—Distilled water 1 in 2,000 ; 2,250 grm., **A**, 1·10 c.c. water.

Chart 8.—Distilled water 1 in 1,375 ; 2,363 grm., **A**, 1·71 c.c. water.

contamination arises. We find that if water be collected from the ordinary distilling apparatus found in laboratories and pharmacies and at once injected after sterilization, marked fever may follow, in spite of the fact that the water is sterile. This also applies to saline made from this water. We also find that water freshly distilled from a glass retort, and at once injected, does not contain this filter-passing pyrogen, and therefore does not cause fever, whether salt and salvarsan be added or not.

One possible suggestion, therefore, as to the explanation of salvarsan fever is as follows: The receiving tank in a good closed distilling apparatus is impervious to air infection except through the joint with the condenser, which being generally of cork should prevent entry of

[1] Charts 6—10 show the progressive amounts of fever produced by increasing quantities of toxic sterile water when injected in strict relation to body-weight, Chart 6 showing no fever owing to the small quantity injected.

organisms in the absence of reverse currents. When the apparatus is in use there is, of course, no reverse current. As soon, however, as distillation for the day is ended cooling takes place, and a reverse current, the action of which can be watched, is set up. Water in the receiving tank in consequence becomes infected from the air by aspiration. The next time the apparatus is used, unless the tank be meanwhile emptied, the freshly distilled water coming from the condenser is liable to contamination by passage through a residue that is readily shown to be pyrogenetic. Very few organisms can be found in the latter, because if the still is in daily use they are destroyed by the high temperature of the water coming from the worm when the condenser has become hot. The net result is that through repeated small increments of infection, when the apparatus is cooling, the water in the receiving tank becomes charged with this heat-stable toxin. This

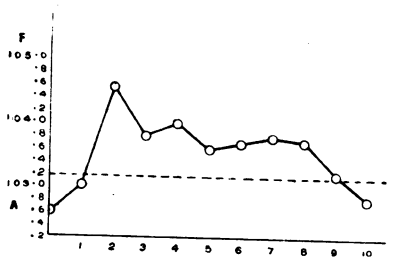

CHART 9.

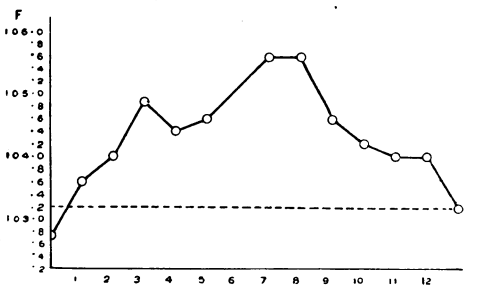

CHART 10.

Chart 9.—Effects of distilled water, 1 in 490; 2,450 grm.; **A**, 5·0 c.c. water injected.

Chart 10.—Distilled water, 1 in 58; weight of rabbit, 2,926 grm.; **A**, 50·40 c.c. water.

substance appears, in fact, to be a degradation product of air-borne organisms, themselves no longer to be found. Subsequent infection of this water in the laboratory, or of saline made from it, may of course occur if good conditions are not observed. Unless, therefore, control injections be made before the onset of secondary infection, a totally erroneous interpretation may be put on the fever that may follow injection of saline made with this water after secondary infection has occurred. If, on the other hand, water freshly distilled from glass, and proved to be free from this pyrogenetic substance by injection, be mixed with salt and exposed to laboratory air for several days, and injected immediately after autoclaving, fever may or may not result. If, however, a sample of this saline, if infected, be autoclaved, and after an interval of days be then injected, fever follows, apparently because in

the interval degradation products have now been extracted. The conclusion, therefore, appears to be that protein extractives derived from air-borne organisms are not present in sufficient quantity to cause fever if a sufficient time has not been allowed to elapse to permit of extraction. . If, on the other hand, the organisms are killed, and if adequate time be allowed for extraction, degradation products make their appearance and fever will then follow injection (Chart 12).

Whether this explanation prove eventually to account for all the facts or not, it is certain that demonstration of the sterility of any given sample of water or saline affords no guarantee of the absence of this heat-stable pyrogenetic body. The presence or absence of this substance can be readily shown in an hour's time by the injection of rabbits, but

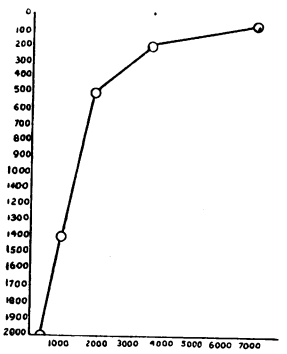

CHART 11.

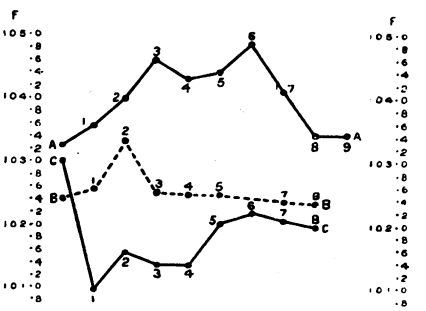

CHART 12.

Chart 11.—A rough curve illustrating the points shown in Charts 6—10.

Chart 12.—**A**, Rabbit, 1,970 grm., injected intravenously with 78·0 c.c. of water. **B**, Rabbit, 2,083 grm., injected intravenously with 85·0 c.c. of water. **C**, Rabbit, 2,112 grm., injected intravenously with 87·0 c.c. of water. Injection ratios in all three, 1 in 25. Interval between observations 30 minutes. In **A** the water was taken direct from the receiving tank. In **B** the water was collected direct from the worm. In **C** the water was collected direct from a glass retort without the intervention of worm or receiving tank.

unless the volume of injection be graduated according to body-weight no reliable inference can be drawn, as our charts show.

It is, we believe, this substance, whatever its origin, that is largely responsible for salvarsan fever, salt fever, water fever, and many of the other types of injection fever referred to. This belief is based partly on the work we have outlined. It is also based on numerous experiments by which we find that it is very difficult to produce injection fever of any kind, apart from bacterial fever, if the water and saline used as

solvent or vehicle do not contain this pyrogenetic substance. These experiments, however, we are not at liberty to refer to further here as they have been submitted to another Society. The importance of recognizing its existence is, however, obvious. Although the explanation we have offered of its appearance in water and saline seems to be supported by the facts we have quoted, we cannot as yet be absolutely certain that this filter-passing toxin is of bacterial origin.

The method of preventing contamination of water or of saline by this fever toxin is fortunately simple. The use of freshly distilled water that has been collected from a receiving tank in connexion with the ordinary metal still is, as we have seen, not permissible, unless the tank is emptied and cleaned before use. If water be used immediately after distillation from a glass retort, fever does not follow the injection of salvarsan in saline made from it. If storage be desired, water distilled from glass should be at once collected direct into sterile vessels, which should then be immediately hermetically sealed and autoclaved. Samples of water prepared in this way on examination several weeks afterwards did not produce fever, either alone or in combination with salt. The use of sealed ampoules containing sea-water or saline has been practised in France for some years. In the case of saline, and often in the case of sea-water, each ampoule is labelled to the effect that it has been autoclaved. We recently examined some of these, and found that many of the ampoules contained saline and sea-water that were pyrogenetic. Either, therefore, the water when inserted already contained this pyrogenetic substance, or delay occurred between distillation and autoclaving. If delay does occur, or if the water be taken from an infected still, autoclaving is a distinct disadvantage, because the extraction of toxic degradation products appears to be then facilitated.

In discussing the causes of salvarsan fever it has often been suggested that a pyrogenetic substance may be set free from the spirochætes destroyed, and that salvarsan fever may be, wholly or in part, a spirochæte fever. Before the latter view can be accepted it will be necessary to inject a suspension or extract of washed spirochætes in pure saline or water. Until, however, this be done it is not possible to explain salvarsan fever by contamination of the water or saline alone, as Dr. McIntosh and his colleagues have pointed out. The evidence they have so far brought forward of spirochæte fever, though highly suggestive, is not convincing, because the two cases they quote were children. It is therefore possible that the fever observed in these two cases after injection of salvarsan was due to slight toxicity of the

solutions which was not detected by injection of adults. In other words, as we have shown, it is not safe to assume that any given saline is atoxic because it contains no organisms, and produces no fever when injected into a man weighing, say, 14 st. Injection of the same volume of the same saline into a child weighing, say, 6 st., may, if the water has been taken from the ordinary closed still, contain the pyrogenetic substance. Constant volume and inconstant weight are a fruitful source of error. On the question of spirochæte fever it is perhaps worth notice that Wechselmann met with no fever after injecting 150 cases with salvarsan in pure saline, a fact which throws some doubt on the ability of spirochætes to produce fever.

We conclude, therefore: (1) That there is at present no evidence that salvarsan fever is necessarily due to injection of organisms grown on water or saline; (2) that the presence in water or saline of a filter-passing pyrogenetic substance, which may or may not be a product of bacterial protein, is an important factor in the production of salvarsan fever and of many other types of injection fever.

In saying this, however, we wish to make it clear that our ability to demonstrate the fever-producing properties of this unsuspected contamination of watery solutions is the direct outcome of the observations made by Wechselmann and his supporters. Speaking for myself alone, if I had been aware of its existence when I advanced the autolytic theory of fever, I should not now be in the unenviable position of demonstrating to be false what I then believed to be true. To save other workers from a similar fate I am bound, therefore, to disclose the fallacies involved.

Note.—In speaking of fever throughout this paper we refer only to the presence or absence of fever occurring within one to six hours of injection.

REFERENCES.

[1] HORT, E. C. "Autotoxæmia and Infection," *Proc. Roy. Soc.*, 1910, lxxxii, pp. 529-45.
[2] *Idem.* "Fever without Physical Signs," *Brit. Med. Journ.*, 1911, ii, p. 800.
[3] HORT, E. C., and PENFOLD, W. J. "The Dangers of Saline Injections," *Brit. Med. Journ.*, 1911, ii, p. 1510.
[4] KOTTMANN. "Bestimmung der Blutmenge b. Menschen u. Tier," *Arch. f. exp. Path. u. Pharm.*, Leipz., 1906, liv, p. 375.
[5] MCINTOSH, FILDES, AND DEARDEN. "Salt Fever and the Treatment of Syphilis by '606,'" *Zeitschr. f. Immunitätsforsch., u. exper. Therap.*, Jena (Originale), 1912, xii, p. 164.
[6] SCHAPS. "Salz. u. Zuckerinjektion, b. Saugling," *Berl. klin. Wochenschr.*, 1907, xliv, p. 597.
[7] WECHSELMANN. "Neuere Erfahrungen ueber intravenöse Salvarsan-Injektionen, ohne Reactions-Erscheinungen." *Münch. med. Wochenschr.*, 1911, lviii, p. 1510.