SOME EXPERIMENTS ON THE FILTRATION OF CATTLE PLAGUE BLOOD.

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(Seven charts.)

The outbreak of cattle plague in Egypt and the establishment of the Serum Institute at Abbassieh offered a suitable opportunity for investigating some points concerning the nature of the causal agent of the disease, and especially the important question whether the virus is, or is not, capable of traversing a filter candle. On this point the literature is strangely contradictory.

Semmer (*Deutsche Zeitschr. f. Tiermed. u. vergl. Path.*, 1896, vol. XXII. p. 32) found that infective materials after passage through a Chamberland filter were non-virulent.

Nencki, Sieber and Wyznikiewicz (*Centralbl. f. Bakt.*, 1898, Abt. 1, vol. XXIII. p. 535) found that the filtrates from both Berkefeld and Chamberland filters were non-virulent.

Kolle and Turner (*Zeitschr. f. Hyg.*, 1898, vol. XXVIII. p. 361) state that the microbe does not pass through Pasteur, Chamberland or Berkefeld filters, and that when virulent blood is passed either slowly or quickly through such filters the filtrate, even in large quantities, is not infectious, whilst the material remaining on the filters is highly so.

To eliminate the possibility of this result being caused by the microbe being an obligatory intracorpuscular parasite and so never being really free in the blood, the corpuscles were haemolysed by the addition of 0·2% sodium chloride solution, and it was found that this had no influence on the result.

Kolle and Turner are of opinion that the parasite is not so small as to be invisible to modern lenses, but at the same time hold that the probability of seeing it is very small as it must be at any rate more minute than an influenza bacillus.

Kolle (*Zeitschr. f. Hyg.*, 1899, vol. XXX. p. 36) showed that if defibrinated cattle plague blood—either haemolysed or not—be centrifuged at a speed of from 2900 to 3000 revolutions per minute for 20 to 30 minutes, the parasite is entirely driven down with the deposit, which is highly infectious, the supernatant fluid being quite free from infection.

Nicolle and Adil-Bey in their first paper (*Ann. Inst. Pasteur*, 1899, vol. XIII. p. 323), agree completely with the above results; they say: "Si l'on filtre le sang, defibriné et étendu au dixième, sur le filtre Chamberland ou sur la bougie Berkefeld, le liquide se montre inoffensif, mais il ne vaccine pas," but in a subsequent paper (*ibid.*, 1902, vol. XVI. p. 56) the authors state that the virus is capable of traversing Berkefeld and also, with greater difficulty, Chamberland F candles, but only under certain conditions. They conclude that the microbe is commonly intraleucocytic.

Yersin (*ibid.*, 1904, vol. XVIII. p. 429), working with the fluid obtained by washing the peritoneum in animals suffering from the disease (he injected salt solution intraperitoneally and removed it after four hours), found that the virus passed through Chamberland filters (mark F) but was stopped by mark (B).

Memmo, Martoglio and Adani (*Ann. d' Ig. Sper.*, 1904, vol. XIV. p. 256), working in Eritrea, come to the conclusion that the virus passes the Berkefeld candle, but is stopped by the Chamberland filter.

There is thus a very wide discrepancy in the results of the different workers. Semmer, Nencki, Sieber and Wyznikiewicz, and Kolle and Turner all agree that the virus does not pass through a filter, and the last-named authors find that it is not only incapable of passing through an ordinary Berkefeld filter, but is comparatively easily removed from suspension by centrifugalisation.

Memmo, Martoglio and Adani, on the other hand, find that it passes the Berkefeld candle but not the Chamberland filter, while Nicolle and Yersin find that it passes a Chamberland filter.

Filtration experiments, in the case of a disease so highly infectious as cattle plague, are surrounded by many possibilities of error. Apart from the question of a flaw or other weakness in the filter candle itself, which can be more or less easily controlled, there is the great danger of accidental infection of the inoculated animals. Unless the strictest precautions are taken this may take place through channels which would hardly be suspected by persons who had not had an actual acquaintance with the disease.

For this reason one is inclined to place much more reliance on a few negative results, carried out under strict conditions, than on a larger number of experiments giving positive results.

Nicolle and Yersin, who both found that the virus passes a Chamberland filter, are by no means in agreement as to the ease with which this takes place. Nicolle says:—"On réussit *rarement* et encore n'arrive-t-on qu'à vacciner les animaux," whilst Yersin finds that "Le virus traverse *constamment* la bougie F." The latter author unfortunately only quotes one experiment, and in this does not give the temperature chart of the animal inoculated, so that it is impossible to form an opinion. Nicolle gives complete details of three animals inoculated with Berkefeld filtrates—two of these remained well but were afterwards found to be immune, the third contracting a fatal attack of the disease after a long incubation period. The initial rise of temperature took place on the morning of the eighth day, and Nicolle attributes this long incubation period to the small number of germs inoculated and to their dilution. This explanation is, however, somewhat difficult to accept, and is certainly not in agreement with the experience at Abbassieh, where it was found that with very small doses of virulent blood (down to $\frac{1}{500}$ c.c.) the temperature rose after exactly the same incubation period as in the case of larger doses.

This constancy of the incubation period is most definite, at any rate in Cyprus cattle, and on comparing the temperature charts of a large number of cases of the experimentally produced disease one is struck by the remarkable similarity of the temperature curves. The most characteristic point is the almost constant incubation period before the initial rise of temperature. These facts are well shown in the annexed charts.

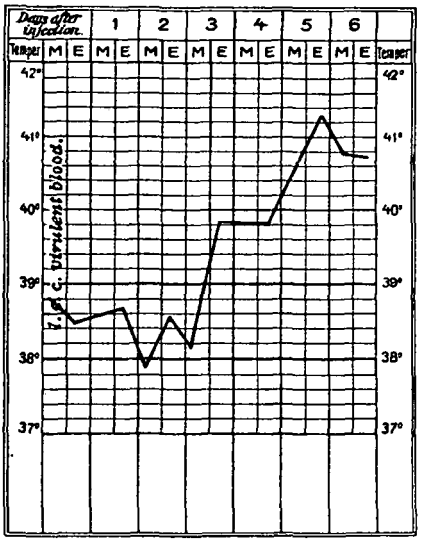
To eliminate small accidental variations the temperature charts of groups of four consecutive animals (without any selection) were taken. Beginning with the first day, the morning temperatures of each of the four animals were added together and divided by four, thus giving the average temperature of the four animals for that morning. This was repeated for the morning and evening observations of six days and the average temperature charted on one chart, which was the average temperature chart for four animals.

Chart 1 (*a*, *b*, *c*, *d*) shows a group of four of these charts, each representing the average of a group of four animals. The similarity is very striking.

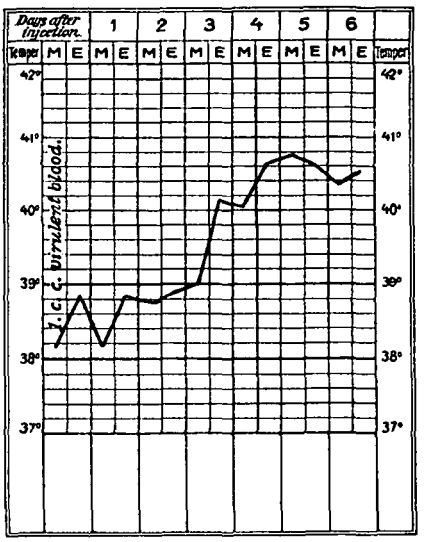
Chart 2 is a chart similarly obtained by averaging the temperature

CHART 1.

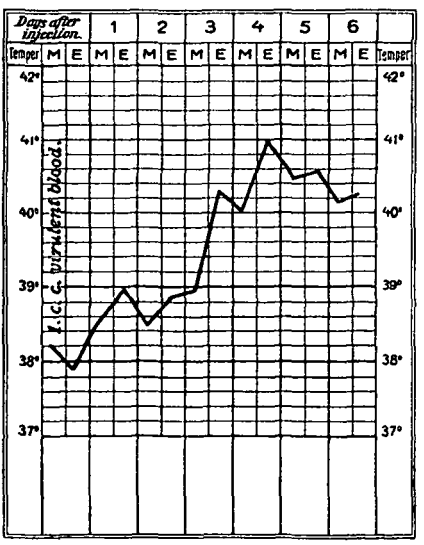
(*a*)



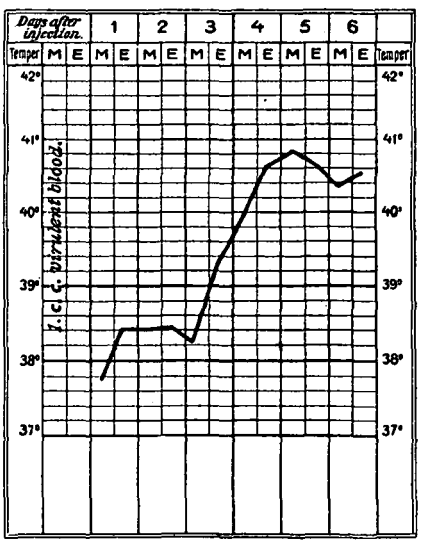
(*b*)



(*c*)



(*d*)



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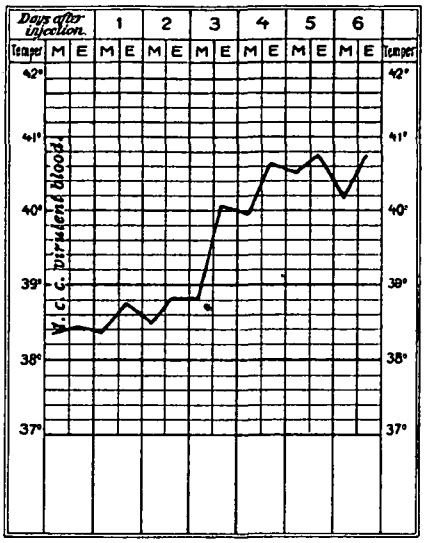
charts of a series of 20 consecutive Cyprus animals, and may be taken as the typical chart of the experimentally produced disease in the Cyprus animal.

From this it will be seen that the first rise of temperature takes place with great regularity on the evening of the third day, *i.e.* 72 hours after the inoculation.

CHART 2.



To further establish this a series of 637 charts of experimentally infected Cyprus animals was examined, and a note made of the exact period at which the first rise of temperature occurred. The results are as follows:—

Rise of temperature	No. of animals	Per cent. (approx.)
After 1 day	0	0
,, 2 days	97	15
,, 3 ,,	437	69
,, 4 ,,	94	14·5
,, 5 ,,	9	1·5
,, 6 ,,	0	0

The injections of virulent blood were unfortunately not made at the same hour every day during the period under observation, but varied from 9 a.m. to 6 p.m. But for this fact the results would probably have been even more striking. The few cases in which the rise did not occur

until after five days were probably largely accounted for by some accidental circumstance, *e.g.* the thermometer not being left sufficiently long in the rectum; moreover in winter the Cyprus stables were very much exposed to the cold south wind, and, as the animals were usually in very poor condition, they not unfrequently showed a subnormal temperature.

This typical temperature curve is most valuable in the diagnosis of the disease, and in cases where an experimental inoculation is made for diagnostic purposes it is the temperature chart as a rule which gives the most useful evidence. That this is the case in other parts of the world is shown by Yersin's statement with regard to the epizootic in Indo-China; he says (*loc. cit.*):—

"Les seuls symptoms constants, que nous avons presque toujours observés chez les animaux infectés par nous, ont été la durée de l'incubation, la periode d'hyperthermie sans rémission et la diarrhée."

From the above it is seen that out of 637 animals inoculated with virulent blood in no case did the initial rise of temperature occur later than five days after inoculation. One is therefore inclined to regard the animal referred to by Nicolle as having contracted the disease accidentally at some date subsequent to the injection of the filtrate.

This accidental infection is by no means easy to avoid, as was realised in the first filtration experiment made at Abbassieh.

In this case two Cyprus bulls were inoculated with 200 c.c. of the filtrate of virulent blood diluted to 1 in 4 with saline solution before filtration. The animals were placed outside the compound in a separate stable and ordinary precautions were taken to avoid infection, but they were attended to by members of the staff of the Institute. The temperatures of both animals began to rise on the fifth day after the injection, and both animals developed typical cattle plague. As it is exceedingly rarely if ever that the incubation period in Cyprus animals lasts so long as this after subcutaneous inoculation, while after infection by the mucous membranes (*e.g.* smearing the nostrils with infective materials) this period is the rule, it was almost certain that these animals had in some way become infected naturally, probably during handling in the course of injection. Further experiments were therefore postponed until they could be done under thoroughly strict conditions.

A very striking point in Nicolle's results is the number of cases in which he obtained "vaccinating filtrates," *i.e.* the animal remained well after the injection of the filtrate, and when tested with virulent blood,

from 10 to 15 days later, resisted infection. This in itself is a most interesting fact, and it is a pity that the temperature charts are not given.

It would be interesting to know if the animals employed (animals from Anatolia, Crimea, etc.) are not sometimes immune.

In an earlier paper Nicolle states that he has tested the comparative susceptibility of the various races most commonly found in Turkey, and that the Crimean, Anatolian and Egyptian animals are very susceptible, and that in them death constantly takes place after the injection of virulent blood. For the Egyptian animals, in Egypt at any rate, we know that this is by no means the case.

In repeating these experiments on the filtration of virulent blood the greatest precautions were taken to obviate all possible chances of accidental infection. The filtrates were tested on cattle imported directly from Cyprus. These Cyprus cattle were very highly susceptible to cattle plague—out of 1098 inoculated with virulent blood in the course of the routine work of the Institute only one proved to be immune; this was a very old cow, whose previous history was of course unknown.

The Cyprus cattle which were used remained in the stables where the non-infected Cyprus cattle for the Institute were kept, and so were together with a considerable number of clean Cyprus animals, which served as controls. These stables were about a mile from the Institute and had an entirely different staff, which had no connection with that of the Institute.

Virulent blood for the experiment was defibrinated by whipping, diluted with four times its volume of 0·8% salt solution and divided into two parts. One part (*a*) was passed through a large Berkefeld filter, which was somewhat close-pored, and the filtration was done as slowly as possible. The other part (*b*) was filtered as rapidly as possible through a large and very porous Berkefeld filter, and in this case ran through very rapidly. Filtration was carried out by suction from an ordinary laboratory water-pump, and the difference in pressure was therefore less than 1 atmosphere. In both cases a culture of an exceedingly small bacillus was mixed with the blood before filtration, but the bacillus did not pass the filter.

50 c.c. of each of the filtrates were injected subcutaneously into two Cyprus animals, care being taken that no one who had anything to do with cattle plague should come in contact with the animals. The temperature of all the four cattle remained normal—except one, which had a mild attack of red-water—until 10 days later, when they were

each inoculated with 10 c.c. of virulent blood. In each case the animals developed typical cattle plague, the initial rise of temperature occurring in two animals on the third day and in the other two on the fourth day. All the four animals were bled to death for virulent blood, and the diagnosis of cattle plague verified by post-mortem examination.

Three control animals inoculated with $\frac{1}{5}$ c.c. of the virulent blood before it was filtered developed the disease typically, the initial rise of temperature occurring in two of the animals on the third and in the other on the fourth day.

The temperature charts of the four cattle which received the filtrate are given (Charts 3, 4, 5 and 6) on pp. 578, 579.

This experiment shows that highly virulent cattle plague blood, when diluted to five times its volume with saline solution, is rendered absolutely non-virulent even by rapid filtration through a porous Berkefeld candle. This being the case, it appears obvious that the microbe must be unable to traverse the much closer grained Chamberland candle, but it was thought interesting to try an experiment to establish this.

With this idea about 40 c.c. of citrated virulent blood was introduced by means of a pipette into the interior of a sterilised Chamberland filter F. The greatest care was taken to keep the filter aseptic and to avoid touching the neck with the virulent blood. A sterile cork was then placed in the neck of the candle, which was finally sealed with sealing-wax. The filter containing the virulent blood was then introduced with all surgical precautions into the peritoneal cavity of a Cyprus animal through a small incision in the abdominal wall, which was afterwards very carefully and thoroughly closed with sutures.

The operation succeeded well, and was followed by practically no rise of temperature. The wound healed by first intention, and the animal remained perfectly well for 13 days, when it was tested by an injection of virulent blood. This gave rise to a typical attack of the disease, death occurring eight days after the injection of virulent blood, showing that not only did the virus fail to pass the filter but that no immunity whatever resulted.

The chart of this animal is given (Chart 7) on p. 580.

Reviewing the work which has been published on this question and the results of the experiments cited above, one is led to the conclusion that there is insufficient evidence in favour of the active agent of cattle plague being capable of passing through a filter.

FILTRATION EXPERIMENTS.

CHART 3.

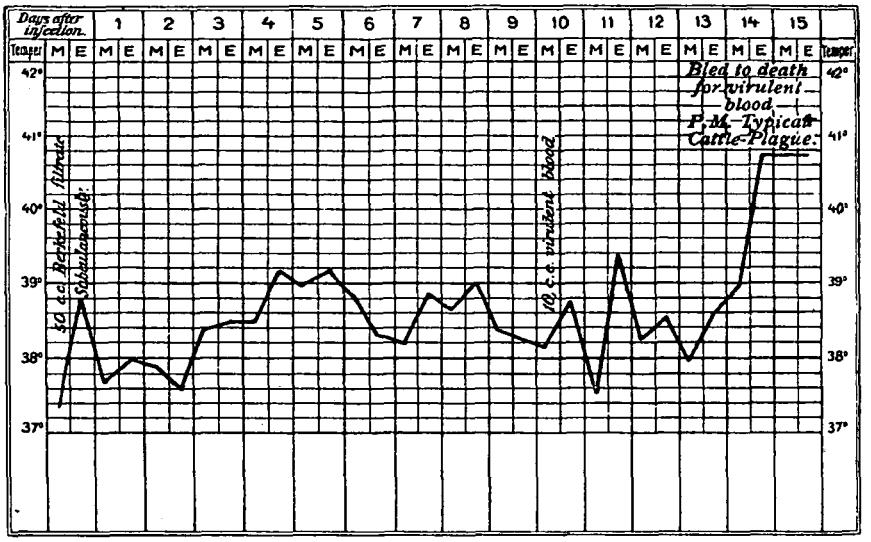


CHART 4.

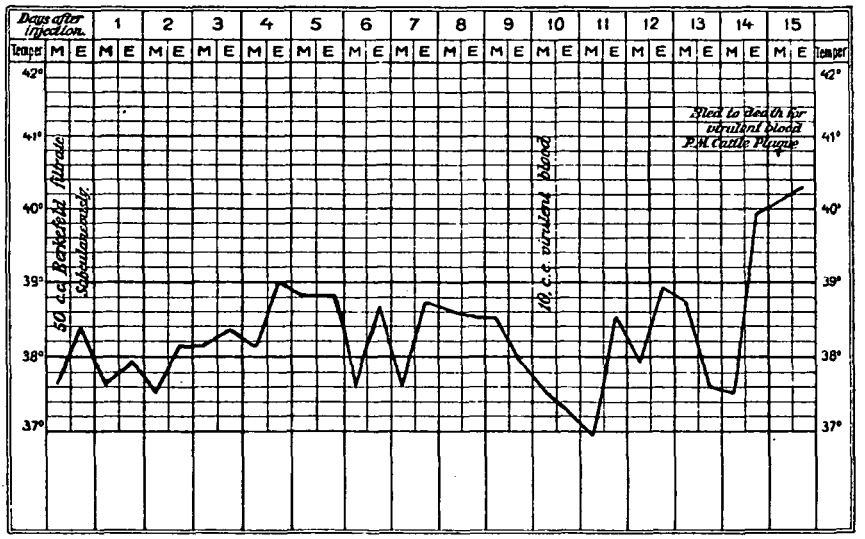


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CHART 5.

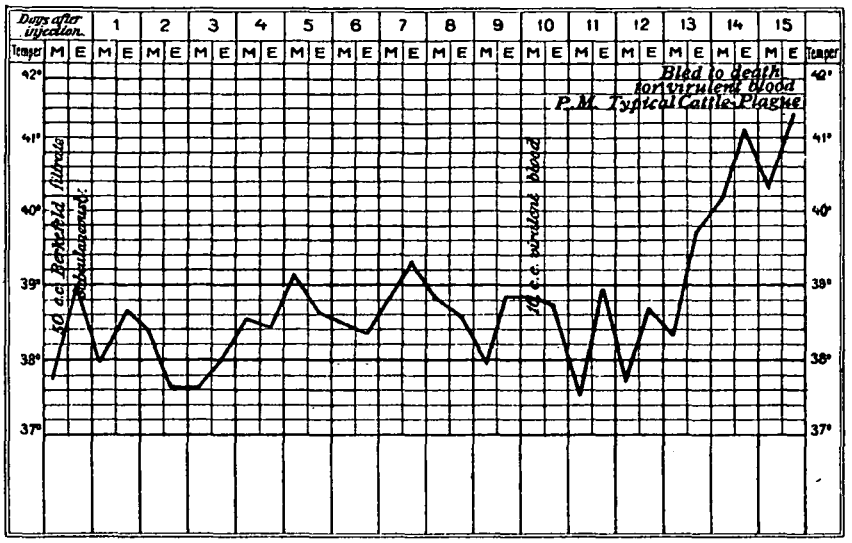

CHART 6.

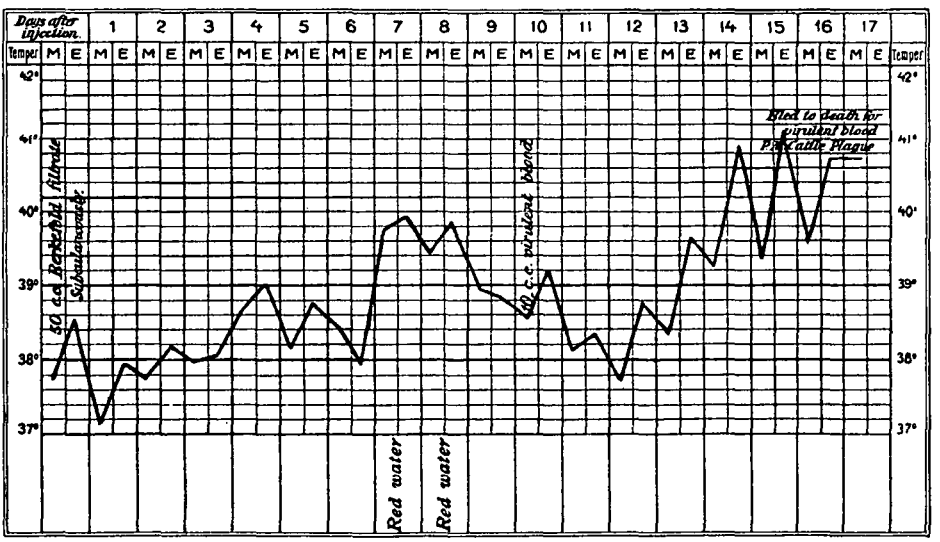

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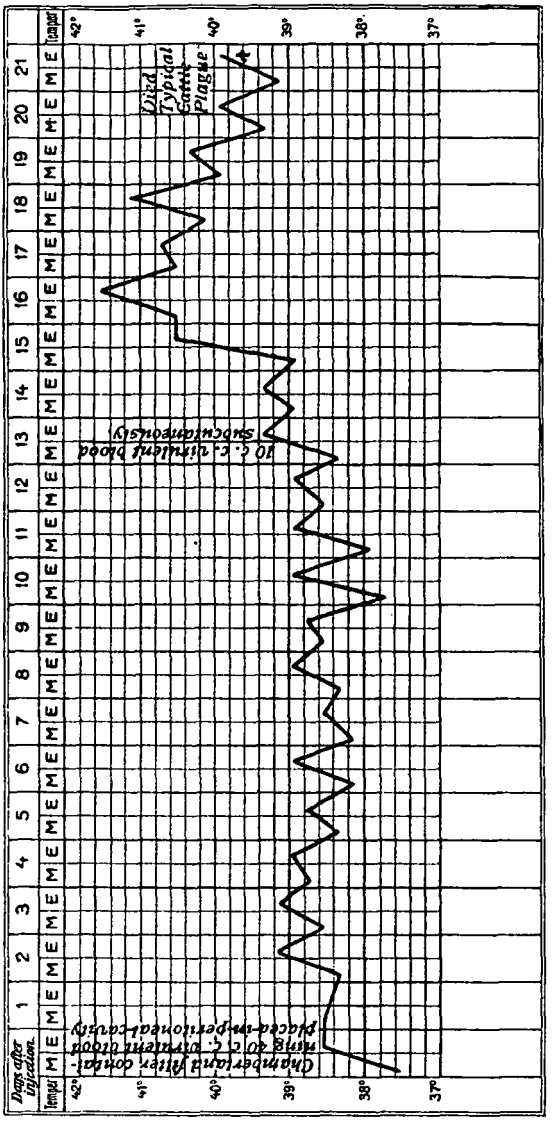
CHART 7.



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