Specific serological reactions with pneumococci from different sources : [being a method for demonstrating the presence of specific opsonins and agglutinins in the sera of pneumonic patients, and a description of its application in differentiating the pneumococcus into distinct groups] / by F.S. Lister.

THE SOUTH AFRICAN INSTITUTE FOR MEDICAL RESEARCH.

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SPECIFIC SEROLOGICAL REACTIONS WITH PNEUMOCOCCI FROM DIFFERENT SOURCES.

BY

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PUBLISHED BY THE INSTITUTE,
JOHANNESBURG,
December 22nd, 1913.

Printed by W. E. HORTOR & Co., LTD.
Johannesburg.

Price Two Shillings and Sixpence.

SPECIFIC SEROLOGICAL REACTIONS WITH PNEUMOCOCCI FROM DIFFERENT SOURCES.

Being a method for demonstrating the presence of specific opsonins and agglutinins in the sera of pneumonic patients: and a description of its application in differentiating the pneumococcus into distinct groups.

By F. S. LISTER, M.R.C.S., L.R.C.P.

During the latter part of 1911 and the early part of 1912 I was engaged as an assistant to Sir Almroth Wright, who was then carrying out researches into lobar pneumonia as it affected the native labourers on the Witwatersrand. Whilst so engaged one was struck with the comparative rarity with which pneumococci recently isolated from cases of lobar pneumonia were satisfactorily opsonised, even by the serum of pneumonic patients. On two occasions, however, I obtained opsonic preparations of a quite exceptional character, which showed massive agglutination of cocci, together with such a degree of phagocytosis that opsonic enumeration was impossible.

In June of the present year I set myself to prepare, if it were practicable, sensitised vaccines of the pneumococcus. For this purpose it was desirable to obtain a highly immune serum, or at least a serum having the properties which I had found on the two occasions referred to above. It occurred to me that a serum possessing these characteristics might be secured from a patient at, or about, the time of his crisis. The sera of five such patients all failed to give any reaction, such as described above, when tested with a stock culture of pneumococcus which had been recently isolated, by blood-culture, from a case of pneumonia. This culture was also tested with four normal sera with similarly negative results; it was, however, pathogenic to mice. Casting about for an explanation of this phenomenon, it occurred to me that I might obtain the reaction which I sought for if I were to make use of the patient's own pneumococcus and employ his own serum in carrying out the test. In this investigation I have obtained cultures of the pneumococcus by lung-puncture in twenty cases of lobar pneumonia, and I have also obtained samples of serum

from the same patients at different periods of their disease. The reactions of these various pneumococci and sera, one with the other, form the principal subject of this communication. It should be remarked that my twenty cases yielding the pneumococcus were not necessarily consecutive cases of pneumonia; for the performance of lung-puncture was not always productive of a growth of the pneumococcus, and such cases were, therefore, of no use for my purpose.

Technique. Wright's opsonic method(1) as carried out by means of the "teat and capillary glass tube" is the one which I have adopted in my enquiry. By one practised in the technique the testing of a large number of sera can be quickly performed; this is an important consideration, as the number of specimens to be examined increases rapidly with each fresh case investigated. An additional advantage of the method is that it enables both agglutination and phagocytosis to be observed in the same preparation.

In order to secure uniformity, and to exclude as far as possible the chance of a reaction occurring with normal sera, the following conditions were adhered to throughout:—

(1) The pneumococci were grown on human-blood-agar. I have noticed that such cultures are less susceptible to phagocytosis than those grown on serum or ascitic-agar.

(2) The emulsions of cocci were made in 0·85 per cent. saline, and were so prepared as to be free from "clumps" and "chains." If distilled water be employed a much greater degree of phagocytosis is liable to occur, especially with certain strains of the organism.(2)

(3) The phagocytic mixtures were incubated for fifteen minutes.

(4) The phagocytic mixtures consisted of equal volumes of blood corpuscles, emulsion of pneumococci, and unheated serum.

(5) The blood corpuscles were obtained in every case by pricking the skin of my own finger.

(6) The blood was collected into 1·5 per cent. sodium citrate solution, the mixture was centrifuged and the deposited corpuscles were washed twice with 0·85 per cent. saline.

(7) The sera from patients and the "control" were collected by means of Wright's blood-capsules after puncturing the skin of the finger.

(8) The source of "control" serum was always my own blood.

(9) The cultures of pneumococci made use of have been obtained by lung-puncture only.* That the cultures should be obtained either

* Without expressing any opinion of the safety of this procedure as a routine method of obtaining a bacterial sample, I may state that in only one instance out of a total of thirty-three was the operation followed by any untoward result. In this case a somewhat alarming hæmoptysis supervened, and the respiration became embarrassed. These symptoms, however, subsided within six hours, and the patient made an uneventful recovery from his pneumonia.

by lung-puncture or blood cultivation is a condition which I consider to be of considerable importance, for a culture which is obtained from the sputum may possibly represent no more than the pneumococcus which is frequently present in the throats of healthy persons; the latter event is certainly possible if the sputum has not been thoroughly washed. Pneumococci from the nose and pharynx are presumably less virulent than those obtained from pulmonary lesions.

It might be thought permissible to make use of cultures obtained by inoculating mice with the pneumonic sputum, but the fact must be borne in mind that the pneumococci from the noses and throats of even healthy people occasionally prove fatal to these animals.

Throughout the experiments which I shall now briefly record each case of pneumonia was given a serial number, and the same serial number was also used to designate both the serum and the pneumococcal growth yielded by the same patient.

Case I. "Tropical" Native. Lobar Pneumonia. Pneumococci isolated by lung-puncture (Culture I.). Serum (Serum I.) collected immediately after crisis.

The serum of the patient and also the "control" (normal) serum were tested with Culture I.—that is, the patient's own organism. With the "control" serum there was practically no phagocytosis (0/50), neither was there any agglutination. With the patient's serum, phagocytosis was so excessive as to be uncountable, whilst agglutination was very marked. The agglutination took the form of areas of compacted cocci, and some of these areas were so large that they covered the entire microscopic field.

Case II. Pondo Native. Lobar Pneumonia. Pneumococci isolated by lung-puncture four days before crisis (Culture II.). Serum collected and tested daily.

The serum on the four days preceding the crisis produced no obvious reaction with the organism, but on the day on which the temperature began to fall massive agglutination together with uncountable phagocytosis occurred. The temperature on the following morning was normal, and the patient presented all those symptoms of improvement which are usually associated with recovery by crisis. The "control" serum did not exhibit at any time the slightest degree of opsonic or agglutinative action.

Although I have stated that the patient's serum presented no obvious reaction on the four days preceding the crisis, yet a sample which was collected twelve hours before the fall in the temperature commenced showed some signs of agglutination, there being many clumps of five to eight cocci distributed along the edge of the opsonic film. A similar phenomenon did not occur in the "control" preparation.

The critical serum of Case I. when tested with Culture II., and the critical serum of Case II. when tested with Culture I., gave no evidence of reaction.

Cases III. and IV. Pondo and Baca Natives respectively. The pneumococcus was obtained from both of these some days before the crisis. The behaviour of these organisms, as to their serological reactions, was similar to that described under Case II. The serum from Case I. again failed to produce any reaction with either Cultures III. or IV., and Sera III. and IV. failed to react with Culture I., despite the fact that they had developed the power to act with Cultures III. and IV. I found, however, that any of the Sera II., III., or IV., reacted typically with any of the Cultures II., III., or IV. Such a result suggested that one had to deal with groups or strains of pneumococci. It appeared that these strains of pneumococci differed from one another in their reactions with immune sera, a difference which

was both striking and easily recognisable under the microscope. The application of such a method to the identification of different groups of pneumococci would have very definite advantages, as the criterion would be the presence or absence of very marked phenomena, and not the estimation of slight gradations or variations in a picture common to all the observations. The error which inevitably arises from the personal equation is therefore avoided.

After my experiments with the four cases as described above, my objective became definitely settled in the direction of the search for: (1) The existence of groups of pneumococci having distinct serological reactions; (2) the presence in " critical " sera* from pneumonic patients of specific opsonins; and (3) the presence of specific agglutinins in " critical " sera from pneumonic patients.

Upon a perusal of the accessible literature a number of references bearing upon these subjects have been found. Thus I note that:—

J. H. Musser and G. W. Norris(3), referring to Rosenow, state that he was unable to find phagocytosis of virulent pneumococci by normal or by the patient's serum until the organism had been attenuated by cultivation. He states that pneumococci which had been isolated from the patient's blood, before, during, or after crisis, are not *taken up* (*sic*) either by normal or the patient's serum *in vitro*.

W. H. Park and Anna W. Williams(4) have suggested the possibility of separating pneumococci into groups by means of differences in their morphological characteristics; they also place Streptococcus mucosus capsulatus amongst the pneumococci; and referring to agglutination and quoting Collins, conclude that the phenomenon is so variable as to be of no practical valu .

L. Cotoni and C. Truche(5) found that the agglutinative power of different sera for different strains of pneumococci varies greatly, and that there is no regularity in the results obtained: that the agglutination method is therefore unsuitable for the diagnosis of pneumococcal infections in man. They found agglutinative reactions in 25 per cent. of sera from cases of human lobar pneumonia. Certain paradoxes occurred; for instance, some sera agglutinated a strain of pneumococcus when the serum of the patient from whom the organism was obtained failed to do so. The results obtained with normal and immune horse serum were similarly paradoxical.

Rufus Cole,(6) reviewing the method of recovery in lobar pneumonia, discusses the cause of the crisis; he finds that attempts to demonstrate an increase of the ordinary bactericidal substances, which act in conjunction with complement, have failed. He points out that univalent immune serum protects only 40 per cent. of animals in

* It will be seen from the charts that opsonins and agglutinins are also present in the sera of patients recovering by lysis; the designation " critical " as applied to sera is a mere verbal convenience, and should not be taken literally.

experimental inoculations; he infers from this that at least 60 per cent. of cases are due to organisms other than those of the type strain.

A. B. Wadsworth(7) in an analysis of some agglutination experiments with pneumococci, and dealing with the sera of forty-six cases of pneumonia and fourteen "controls," found that ten out of the fourteen "controls" agglutinated the pneumococcus. From such a result he concluded that the agglutination test in pneumonia is of very little practical value. The same author satisfied himself experimentally that virulent pneumococci are practically insusceptible to phagocytosis. He quotes Hektoen's opinion that insusceptibility to phagocytosis is an indication of virulence and that the opsonic index may therefore be the measure of the virulence. Wadsworth, however, found that virulence may be lowered without any increase in susceptibility to phagocytosis.

S. Strouse(8) refers to Neufeld's work, by which the latter claims to have demonstrated the presence of "immune opsonins," or bacteriotropins, in almost every sample of pneumonic serum. In some later work, however, strains of pneumococci were found which were not acted upon by sera which ordinarily gave a positive result with other strains; such pneumococci were, nevertheless, acted upon by the post-critical sera of the pneumonic patients from whom they had been derived.

Seligmann and Klopstock(9) and Boettcher(10) were unable to confirm these results. Strouse, following Neufeld's technique, was never able to find opsonins in heated sera which had been taken after the crisis, and tested with virulent pneumococci which showed no spontaneous phagocytosis. From further animal experiments he was able to show that phagocytic immunity is specific, to a high degree, for the organism used in immunisation, and that the amount of opsonin produced largely depends on the virulence of the organism.

A. R. Dochez(11) found that the serum of pneumonic patients at their crisis protected mice, and that the presence of protective substances is more evident when the homologous organism is used for the test. In only four cases—out of, apparently, fourteen cases examined—were protective substances demonstrated before the crisis. Quoting Neufeld, he says that pneumococci have not as yet been differentiated into clearly defined biological groups, though it is known that a serum derived from a certain typical strain may fail to protect animals against an infection by other strains which are morphologically and culturally characteristic.

Bezançon and Griffin(12) state: "Agglutination is not obtained with all strains of the pneumococcus; it is, in a way, an individual and

not a specific property; very often agglutination is obtained with the micro-organism recovered direct from the infected person while laboratory strains give negative results."

The conclusions which I draw from such references are—(1) That agglutination, although of frequent occurrence, is too variable a phenomenon to be of practical use. (2) That virulent pneumococci are not susceptible to phagocytosis in any circumstances. (3) That immunity, as studied by animal experiments, is specific, in great measure, for the particular strain of pneumococcus with which the animal has been inoculated.

Having dealt with the experiments and conclusions of other workers, I have now to refer to my own observations. Both the agglutinins and the opsonins in the serum of pneumonic patients appear to be specific for certain classes of the pneumococcus. The serum of patients suffering from lobar pneumonia seems to possess marked agglutinative and opsonic properties with respect to the particular organism responsible for the infection: provided always that the serum is collected about the time that the temperature falls, and provided also that this fall of temperature is not an indication of approaching death. There appear to be occasional exceptions to the rule, but the probable infrequency of such exceptions can be seen by reference to the tabulated statement of my experiments.

In addition to this specific reaction of a critical serum with its particular organism, a cross reaction can be obtained with other organisms and other sera. Thus a critical serum when tested with a number of strains of pneumococci, isolated from other cases of pneumonia, will affect some of these pneumococci in a similar manner, whilst with others there will be no trace of reaction. If now the sera of the patients from whom the reacting organisms were obtained, be tested with the particular organism which was clinically associated with the critical serum first mentioned—provided that these sera be collected at a time when they react to their own particular organism—a similarly positive result will be obtained. To put the matter in another way. If a, b, c, and d are four cultures derived from four different cases of lobar pneumonia, and if a′, b′, c′, and d′ represent the sera of these patients—and granting for argument's sake that the serum of each of these patients has developed an agglutinative and opsonic reaction towards his own organism—then, if the serum a′ show a reaction with organisms b and c, but not with d, it will also be found that the sera b′ and c′ react with organism a. Sera a′, b′, and c′ will not react with organism d, nor will serum d′ react with any of the organisms a, b, or c. In other words, the reciprocal action of organism and serum is specific.

I applied such a method of serological investigation to the twenty strains of pneumococcus to which I have already referred; the technique was as I have previously described. By such means I found it possible to classify eighteen of the strains into four definite groups. Two of the strains were, however, quite refractory and gave no indication of being affected either by the serum of the patients from which they had been derived, nor by any of the sera in my possession. Both of the cases which yielded these refractory strains came from the same compound; both recovered, one after a particularly mild attack, and the other after an illness of exceptional severity—in fact it seemed to only just escape a fatal issue. Both patients were members of a tribe whose susceptibility to and mortality from pneumonia, at any rate on the Rand, is very high. I have no explanation to account for the irregularity of my results with these two cases.

Among the twenty cases referred to there were four, from whom cultures were obtained, which terminated fatally without any evidence of crisis; the sera of these patients did not at any time exhibit any agglutinative or opsonic properties.

Two of my series of twenty died after exhibiting a crisis—one three weeks after, of tuberculosis of both lungs, the other two days after, with renewed pyrexia. The sera of both these cases exhibited the usual marked reaction shortly before the crisis and until death.

It will be seen, therefore, that there were sixteen cases, including the two exceptional cases referred to above, which terminated by either crisis or lysis, and that the sera of fourteen of these developed agglutinins and opsonins.

In carrying out the investigation a culture was tested as soon as it had been definitely isolated, an event which, in most cases, occurred several days before the serum from the case had devloped any activity. The cultures were also tested with sera derived from other cases, and which were known to have developed activity towards their own organisms. In every case so tested, where a positive reaction was obtained, the serum of the case of origin subsequently developed agglutinins and opsonins, not only for its own organism, but also for the organisms from all cases whose sera affected it.

From a consideration of these phenomena it seems reasonable to assume that the sera of the four cases which terminated fatally, and whose particular organism gave a reaction with one or other of the potent sera, would have developed their own agglutinins and opsonins had the patients survived. It may be that these phenomena are very closely associated with recovery, although one cannot, at the present, define the changes of which they are the indicators.

In the earlier cases an attempt was made to estimate the degree of phagocytosis, in both the sample and " control " preparations, by ascertaining the bacterial content of 50 leucocytes on each slide. It was found that the preparation made with normal serum usually contained not more than one phagocyted coccus in fifty cells, and that very rarely was this number raised to ten. In preparations made from the patient's serum, however—when such had become active—the intra-cellular collection of cocci were, in all cases, so numerous as to render accurate estimation impossible. Although empty cells were not uncommon, yet cells each of which contained from ten to forty cocci were numerous.

As it appeared of no practical value, for the purpose of this experiment, to attempt an opsonic estimation, and as, moreover, the probable error would have been enormous, systematic enumeration was not attempted. It may be stated, in general terms, that fifty leucocytes with " control " serum contained from nought to ten cocci, whilst with the patient's serum cocci to the number of two hundred and fifty and upwards would have to be enumerated.

It has been urged that agglutination occurring in a phagocytic mixture leads to misinterpretation when the film comes to be enumerated, for in such circumstances the appearance of from ten to twenty cocci in a single cell may be due to masses of agglutinated cocci lying either above or below the leucocyte. I am satisfied that this contention is, to a large extent, unwarrantable, and for the following reason. During my investigations agglutinins have often been demonstrated to be present before opsonins have appeared, and they have persisted after the latter have disappeared. Opsonic films prepared in such circumstances have not shown any indications of apparent phagocytosis.

It seems probable that agglutination may in part account for the irregularity of the phagocytosis in films in which many empty cells are seen. Its action in this matter may be purely physical and due to the fact that by causing the collection of the cocci into large clusters it withdraws them entirely from the field of action of some of the leucocytes.

In my observations I have found no difficulty in deciding on the presence or absence of agglutination, for whenever it was present the appearances were unmistakable. Some sera which were obtained when agglutinins were only just appearing provided films containing numerous groups of from five to eight cocci scattered throughout the field. It was the rule, however, for further samples of such sera, taken within twenty-four hours of the first and sometimes earlier, to possess the power of causing massive agglutination.

The term *Virulent*, as applied to pneumococci, appears to be used in current literature with variable and uncertain meaning. The majority of authors appear to refer the quality of virulence of the pneumococcus to its capacity to kill the mouse, while others seem to consider any pneumococcus virulent which can or has produced pneumonic changes. I leave this terminological dispute undecided and merely use the word as an epithet to organisms of the latter class. It is doubtful whether virulence for mice and capacity for producing lobar pneumonia are parallel characteristics, and it is also doubtful—at all events to me—whether a virulent pneumococcus is ever phagocyted or agglutinated, *in vitro*, by a normal serum.

With regard to the twenty cultures of the pneumococcus which I have studied, and which were all derived by lung-puncture from cases of pneumonia, the following facts may be recorded. None of them were susceptible to phagocytosis, either by the control serum used, or by any of the ten other normal sera which will be subsequently referred to. Nine were inoculated subcutaneously into mice, and all proved fatal in from eighteen to sixty hours. One culture, originally insusceptible to phagocytosis by normal serum, subsequently developed this property in a marked degree. Before this change came about it had been subjected, however, to many subcultivations on human-blood agar, over a period of two months. After it had become opsonisable by normal serum it was found to be still virulent for mice, although a larger dose was now necessary, and the period of survival was prolonged.

For the sake of brevity in describing the following experiments the expression " the reaction " is used as referring to the presence of obvious agglutination and phagocytosis. An opsonic film was made use of to demonstrate the presence or absence of the reaction, and the conditions of the preparation of the phagocytic mixture have already been described; in two instances, however, the sera, after being tested in the usual manner, were diluted forty-fold, with the result that phagocytosis was completely inhibited, although some agglutination still remained apparent. No systematic study was, however, made of the effect on the character of the reaction of variations in the degree of dilution of the sera.

The following table shows the presence or otherwise of the reaction in the sera of fourteen pneumonic patients, *all of whom recovered*. In every case the serum was critical or post-critical, and the organism with which it was tested was always derived from the patient who yielded the serum. The " control " observation on each occasion was made with normal serum and the organism of the case.

TABLE I.

Case No.	Tribe.	"Reaction."	"Control reaction."
1	Mozambique	+	—
2	Pondo	+	—
3	Baca	+	—
5	Pondo	+	—
7	M'Sutu	+	—
8	Quilimane	+	—
9	,,	—	—
10	M'Sutu	+	—
11	Zambesi	+	—
12	M'Sutu	+	—
13	Zulu	+	—
17	Nyambaan	—	—
19	Pondo	+	—
20	Zulu	+	—

The results of testing the sera of six cases, *all of which terminated fatally*, are given in Table II. The conditions of the test were the same as those recorded in Table I.

TABLE II.

Case No.	Tribe.	"Reaction."	"Control reaction."
4	Pondo	+	—
6	X'osa	—	—
14	X'osa	+	—
15	M'Chopie	—	—
16	M'Chopie	—	—
18	Mozambique	—	—

Of the two cases which yielded the reaction, patient No. 4 died three weeks after the crisis of acute pulmonary phthisis, and patient No. 14 died two days after crisis with a fresh elevation of temperature. None of the other four cases afforded the reaction, and none of them had reached the stage of defervescence.

Table III. records the results, with respect to the presence or absence of reaction, of testing a series of pneumonic sera with a series of pneumococci derived from the same cases.

TABLE III.

No. of case providing test serum.	Case number of origin of culture.									
	1	2	3	4	5	7	8	11	12	Control.
1	+	—	—	—	—	—	—	+	—	—
2	—	+	+	+	+	+	—	—	—	—
3	—	+	+	+	+	+	—	—	—	—
4	—	+	+	+	+	+	—	—	—	—
5	—	+	+	+	+	+	—	—	—	—
7	—	+	+	+	+	+	—	—	—	—
8	—	—	—	—	—	—	+	—	—	—
11	+	—	—	—	—	—	—	+	—	—
12	—	—	—	—	—	—	—	—	+	—

It will be seen from this table that all the organisms tested arrange themselves into four groups according to their reaction with the sera. These groups, which will, hereafter, be designated A., B., C. and D., comprise the organisms from the following cases. Group A., from cases 2, 3, 4, 5, and 7; Group B., from cases 1 and 11; Group C., from case 12; and Group D., from case 8.

In subsequent work I have been content to test any new culture of the pneumococcus by its reaction with four sera, each of which was representative of one of these four groups. Though many experiments were done, from time to time, on the extended basis shown in Table III., yet no serum was ever found which transgressed the boundaries of the group-specifity which I have found to exist.

The cultures from cases 9 and 17 were tested with every available active serum and also with the sera obtained from twelve apparently normal Europeans and Natives. None of the sera produced any reaction with these organisms, and, as already stated, no demonstrable agglutinins and opsonins appeared in the sera of the cases from which they had been derived. For these reasons it was impossible to classify them in any of the first four groups; a fifth group, which I have called "E," was therefore set aside for the reception of such anomalous strains.

Out of the twenty cultures tested by the above method, eighteen were found to be classifiable into one of four groups, as follows:—

A. 2. 3. 4. 5. 7. 10. 13. 14. 19.
B. 1. 11. 16. 18. 20.
C. 12.
D. 6. 8. 15.

The numbers which designate the cultures in the above statement represent also the serial order in which the cases from which they were derived came under my observation. It will be seen that a repre-

sentative of each group was discovered in the first twelve cases examined, and that in the eight subsequent cases no new group was discovered. This fact suggests that amongst the population dealt with, and at the present time, the number of groups is limited.

The cases were drawn from Native mine labourers employed on various mines on the Rand, and from Native "house and outside boys" working in Johannesburg.

When these groups had been established a representative culture from each was tested with each of the sera from five healthy Natives and five healthy Europeans, and the reaction was found to be absent on all occasions.

In the application of this method of investigation it is of great advantage to have access to a large number of pneumonia patients within a short period, for the opsonins and agglutinins seem to disappear from the freshly drawn serum at the end of a few weeks. With respect to the retention of these substances by serum *in vitro*, I have a sample which was collected at the time of the crisis and which is now ten weeks old. No special care has been taken of this sample as it has been lying on the bench. I find that the opsonic power has now disappeared, but that sufficient of its agglutinating powers remain to enable me to use the sample in detecting any organism belonging to Class B. Serum from blood which has been collected in the ordinary manner, in a Wright's capsule, and allowed to stand and clot, is still quite active after several days. I have found that some cultures, after many transplantations, become susceptible to phagocytosis by normal sera, although I have not found any cultures which developed liability to agglutination; the phenomenon of agglutination is, therefore, of greater service than that of phagocytosis for the purpose of classifying organisms.

The opsonic power of serum is, with few exceptions, destroyed by heating at 55° C. for twenty minutes. The agglutinating power, however, survives this treatment, although its activity appears to be somewhat reduced.

If the validity of this method of classifying pneumococci be confirmed it will have more than one useful application. The possibility of identifying the particular strains of the organism will provide a clue as to the source of infection in different cases, and will help to elucidate the rôle of those pneumococcal organisms which are found so commonly in the throats of healthy individuals. The prevalence of different groups of pneumococci in different circumstances—industrial, tribal, geographical, etc.—will be capable of investigation. The suggestion that second attacks of pneumonia are due to invasion by another strain of the organism can be put to the test. These and many similar enquiries may be rendered feasible.

The prophylactic pneumococcal vaccine now so extensively used on these fields may, in the light of these studies, become modified; it is obvious that the efficacy of such vaccine would be enhanced if use were made of the particular strain of organism to infection by which the labourers will be exposed. It may also be found advantageous to use certain definite combinations of strains. Again, the efficiency of sensitised vaccines will probably be secured by employing an organism belonging to the same group as that which has infected the patient.

The serum therapy of pneumonia, which has not hitherto afforded notable results, may derive additional importance when it can be used in so specific a manner as the results of these investigations lead one to hope.

CONCLUSIONS.

The sera of patients recovering from lobar pneumonia usually acquire opsonising and agglutinating properties for the particular strain of pneumococcus which is responsible for the infection.

"Virulent" pneumococci—by which I mean pneumococci which have been derived from a hepatized lung—are readily phagocyted and agglutinated in such circumstances.

Most pneumococci can be classified into groups by means of these specific serological reactions.

Certain strains of the pneumococcus cannot, at present, be classified by such means as the serum of the patients from whom they are obtained develops no opsonising or agglutinating powers.

My work in carrying out this brief study has been much facilitated by the co-operation of Dr. W. Watkins-Pitchford, the Director, and it could not have been so advantageously conducted but for the generosity of the management of the Bantjes and Durban Deep Gold Mining Company in permitting me the necessary leave. Many of the cases investigated were kindly placed at my disposal by medical colleagues in the General Hospital and on the mines, and for this I am indebted to them. The photomicrographs are the work of Mr. O. Slawkowsky.

Postscript. Since committing the above study to paper my attention has been drawn to some recent work by A. R. Dochez and L. J. Gillespie, and which has appeared in the Journal of the American Medical Association, for September 6th, 1913 (pages 727 to 730). Although these workers have adopted different methods of investigation, yet their observations seem to have led them to conclusions somewhat similar to my own.

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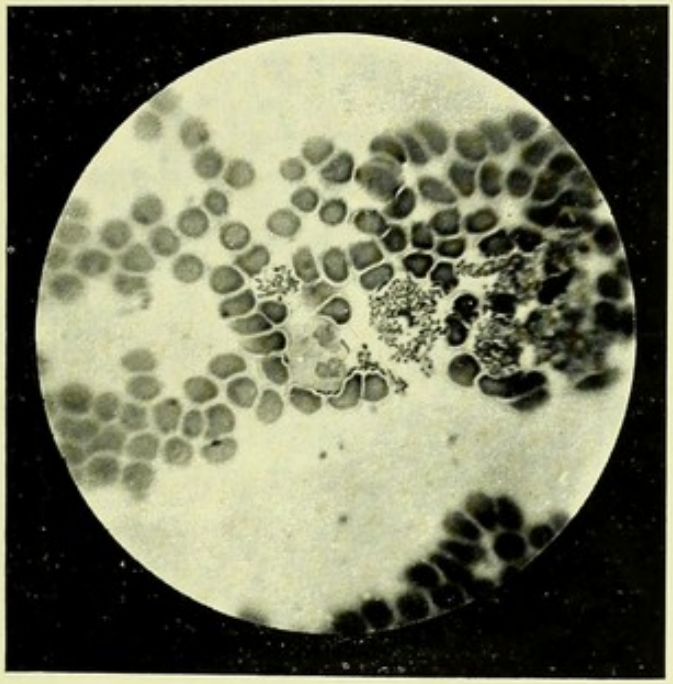
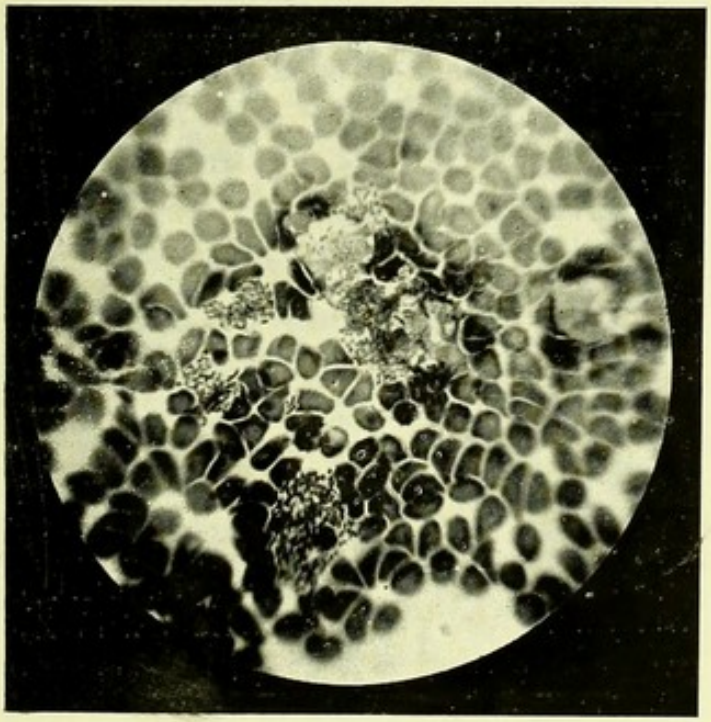
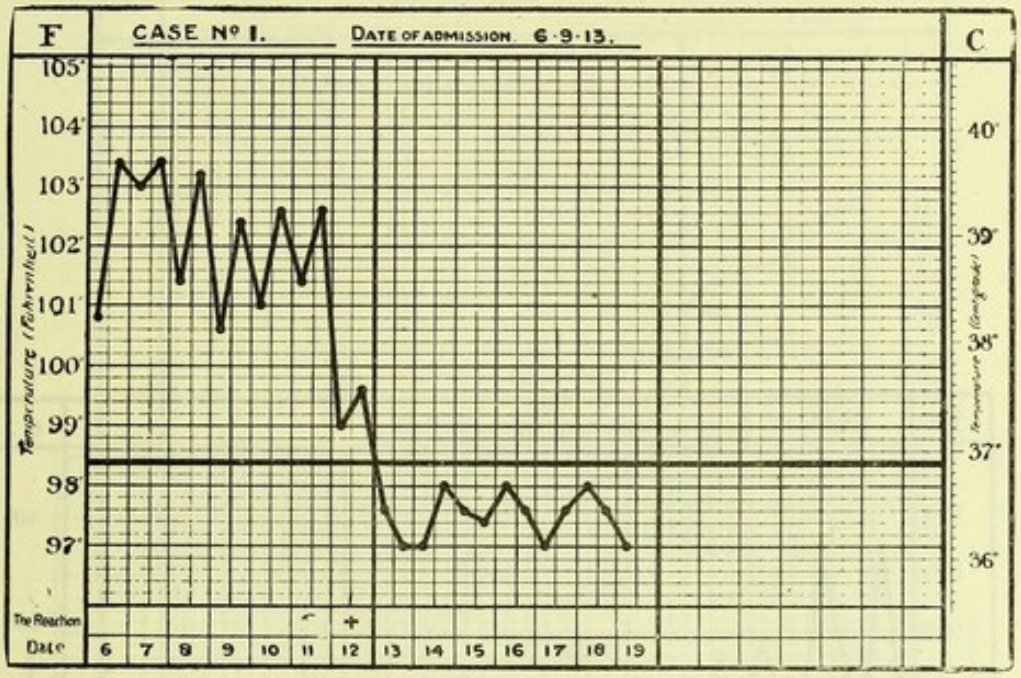
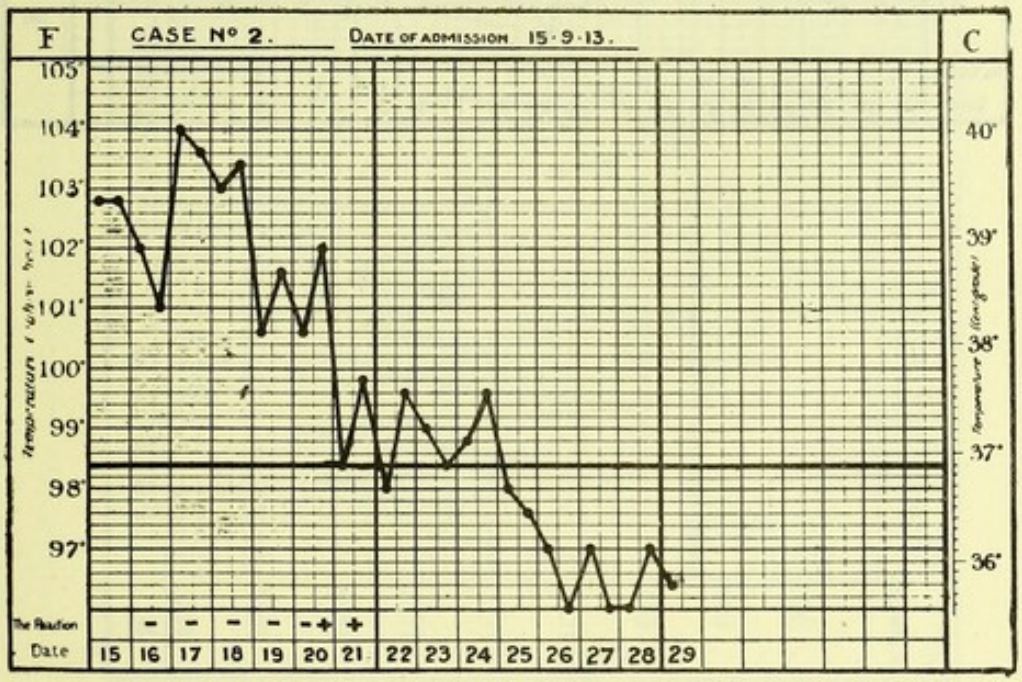





F
CASE Nº 1.
DATE OF ADMISSION 6·9·13.
C
105°
104°
103°
102°
101°
100°
99°
98°
97°
40°
39°
38°
37°
36°
Temperature (Fahrenheit)
The Reaction
Date
6 7 8 9 10 11 12 13 14 15 16 17 18 19
+


F
CASE Nº 2.
DATE OF ADMISSION 15·9·13.
C
105°
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102°
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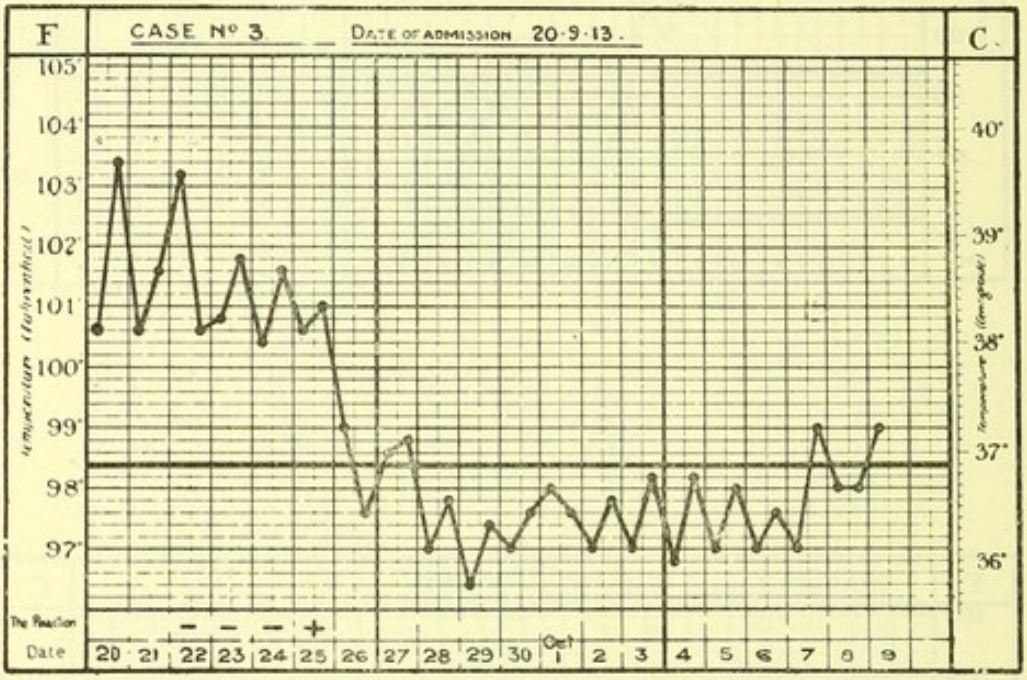
36°
The Reaction
Date
15 16 17 18 19 20 21 22 23 24 25 26 27 28 29
- - - - -+ +


F
CASE Nº 3
DATE OF ADMISSION 20·9·13.
C.
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Tho Reaction
Date
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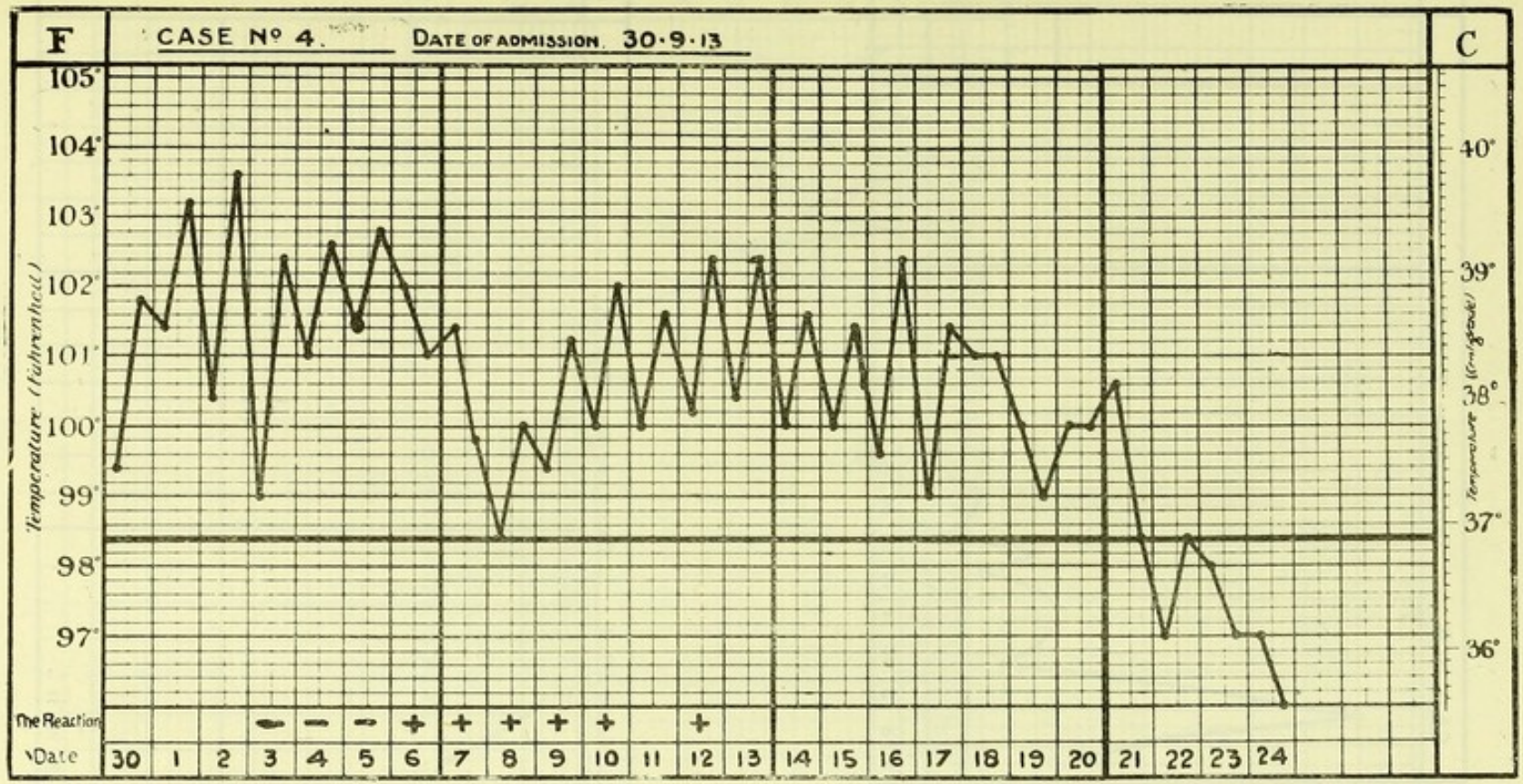
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DATE OF ADMISSION. 30·9·13
C
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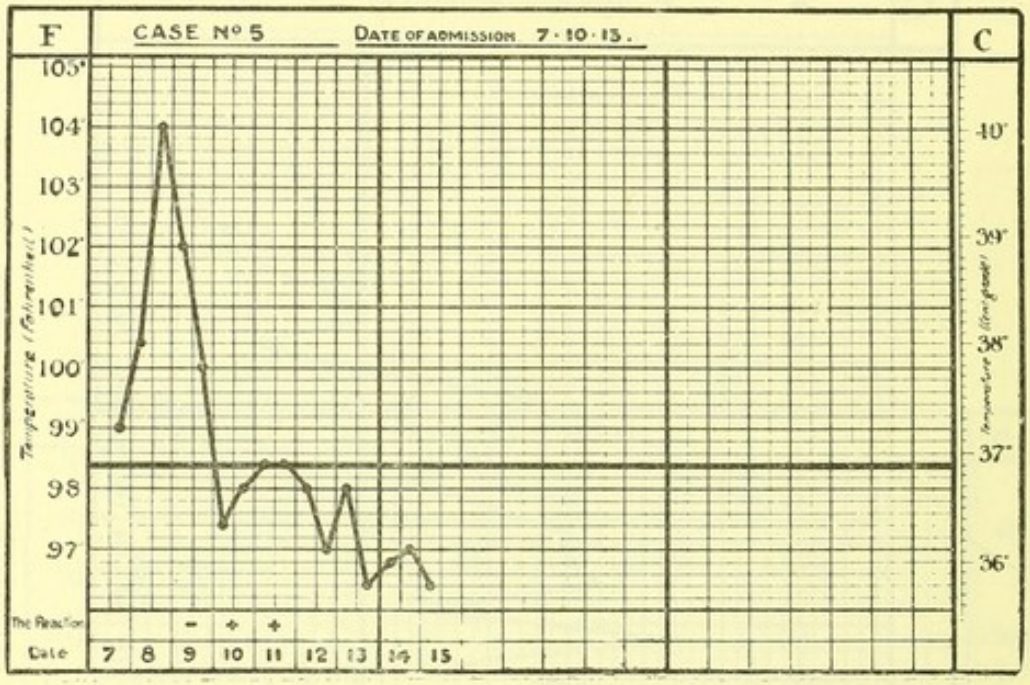
97°
Temperature (Fahrenheit)
40°
39°
38°
37°
36°
Temperature (Centigrade)
The Reaction
Date
30 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24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CASE Nº 5
DATE OF ADMISSION 7·10·13.
C
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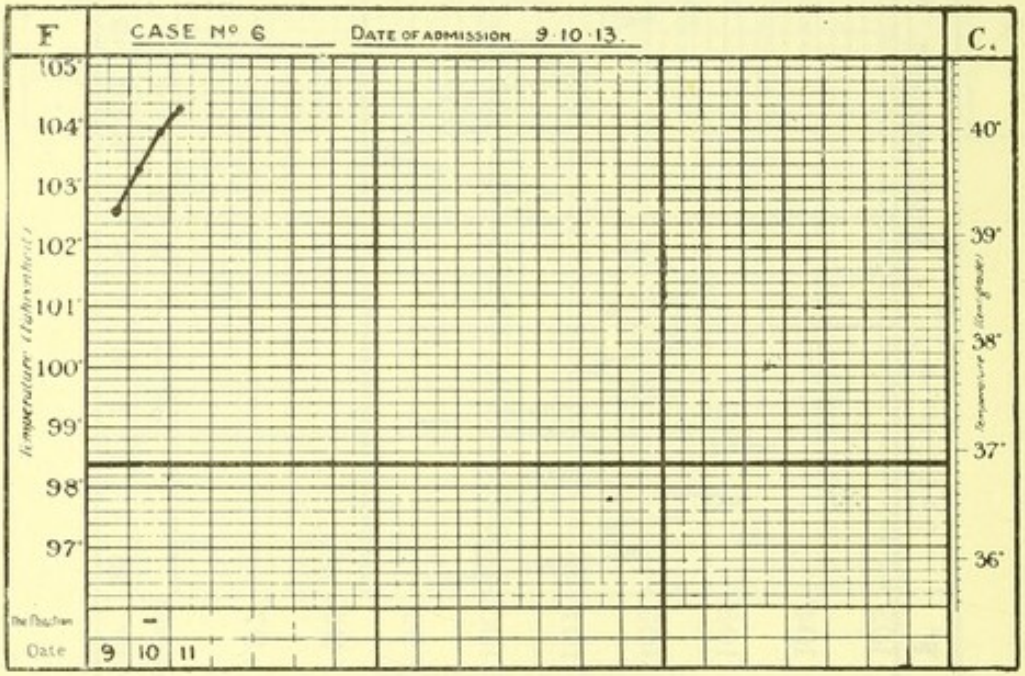
36°
The Reaction
Date
7 8 9 10 11 12 13 14 15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CASE Nº 6
DATE OF ADMISSION 9·10·13.
C.
103°
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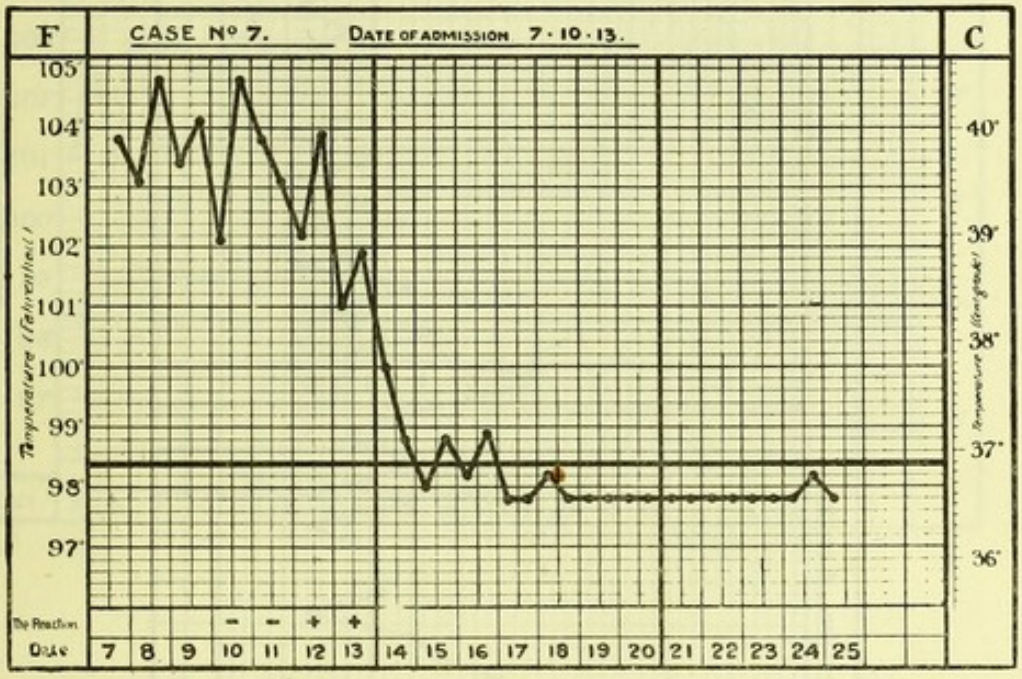
37°
36°
The Reaction
Date
9 10 11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CASE Nº 7.
DATE OF ADMISSION 7·10·13.
C
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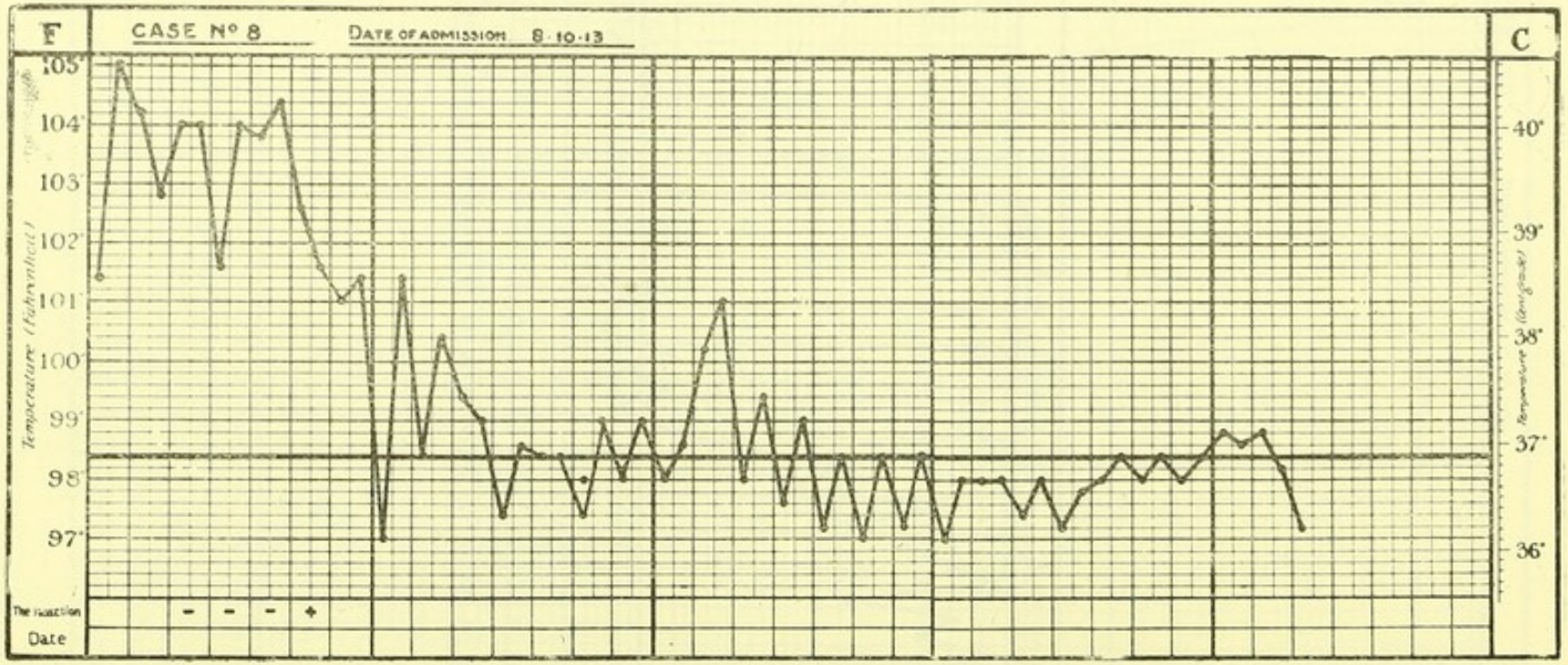
Temperature (Fahrenheit)
Temperature (Centigrade)
Tuberculin Reaction
Date
7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25
- - + +


F
CASE Nº 8
DATE OF ADMISSION 8-10-13
C
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40°
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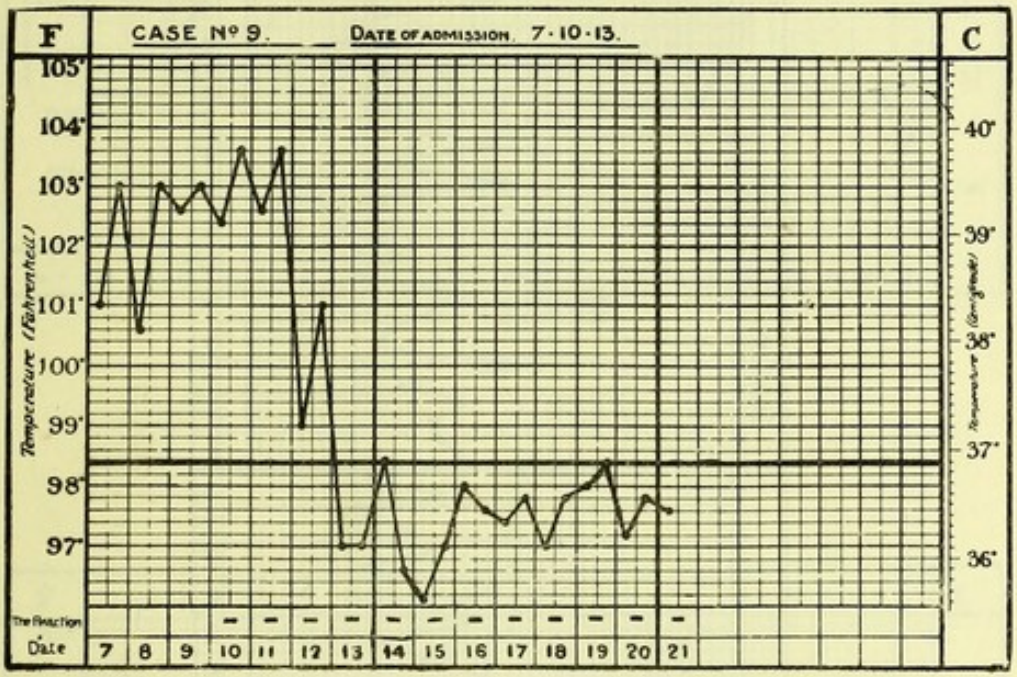
36°
Temperature (Fahrenheit)
Temperature (Centigrade)
The reaction
Date
- - - +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CASE Nº 9.
DATE OF ADMISSION. 7·10·13.
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36°
Temperature (Fahrenheit)
The Reaction
Date
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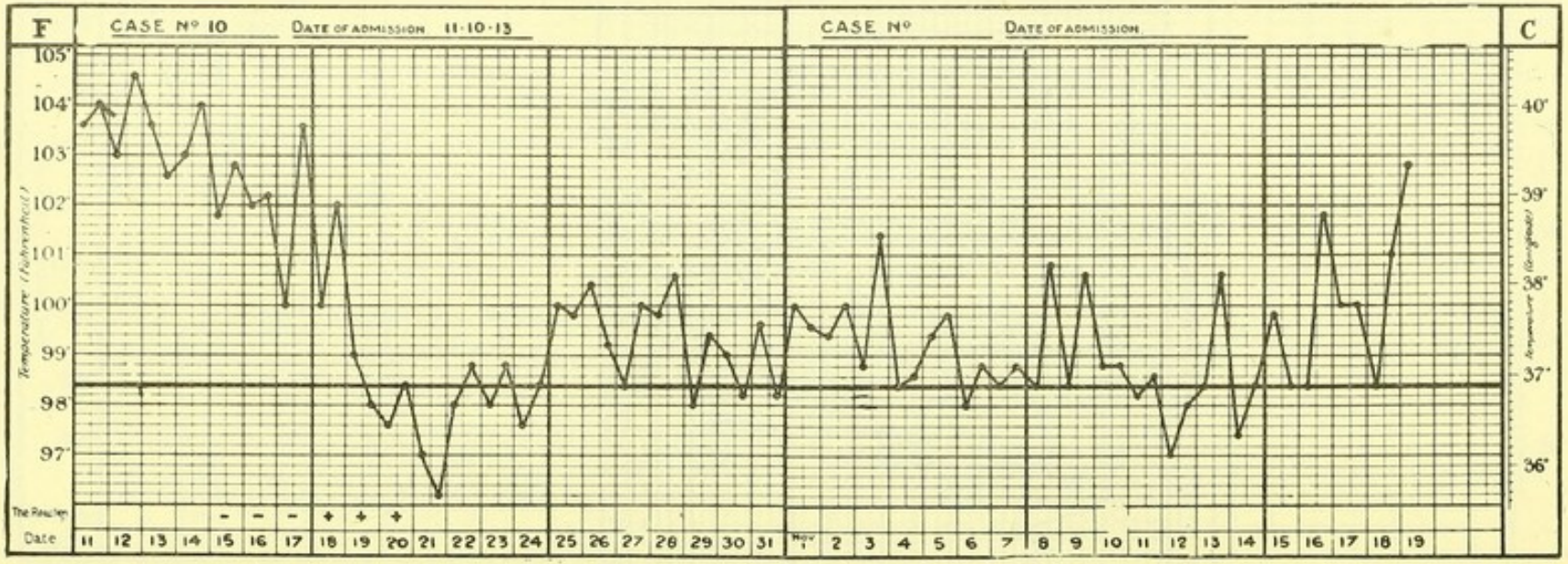
F
CASE Nº 10
DATE OF ADMISSION 11·10·13
CASE Nº
DATE OF ADMISSION
C
105°
104°
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102°
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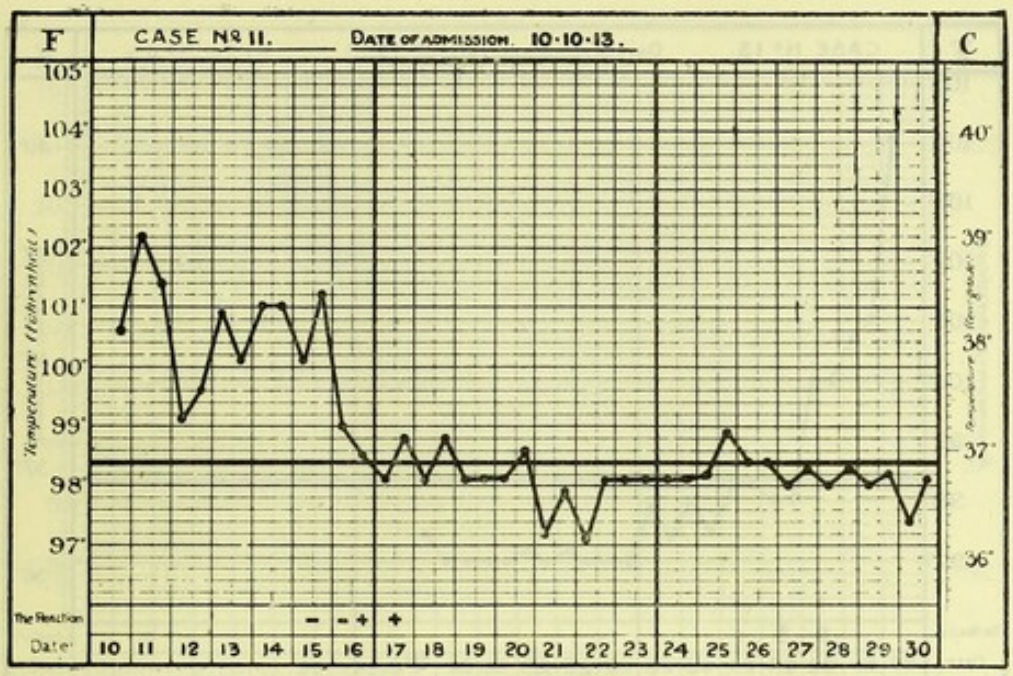
36°
Temperature (Fahrenheit)
Temperature (Centigrade)
The Reaction
Date
11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31
Nov 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19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CASE Nº 11.
DATE OF ADMISSION. 10·10·13.
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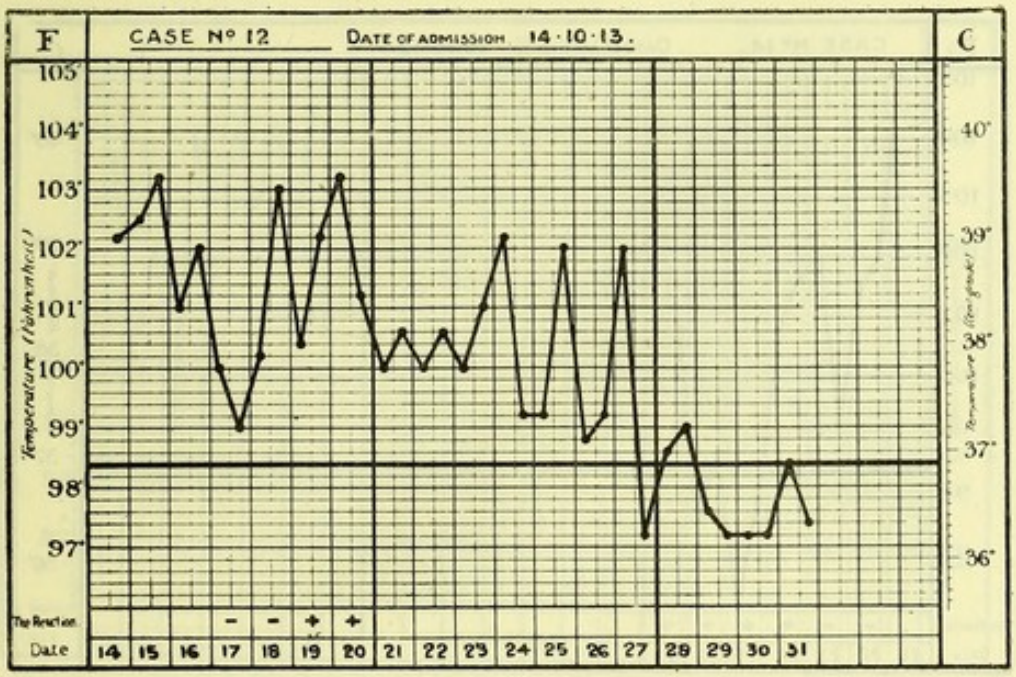
Temperature (Fahrenheit)
Temperature (centigrade)
The Reaction
- - + +
Date
10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30


F
CASE Nº 12
DATE OF ADMISSION 14·10·13.
C
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104°
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40°
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37°
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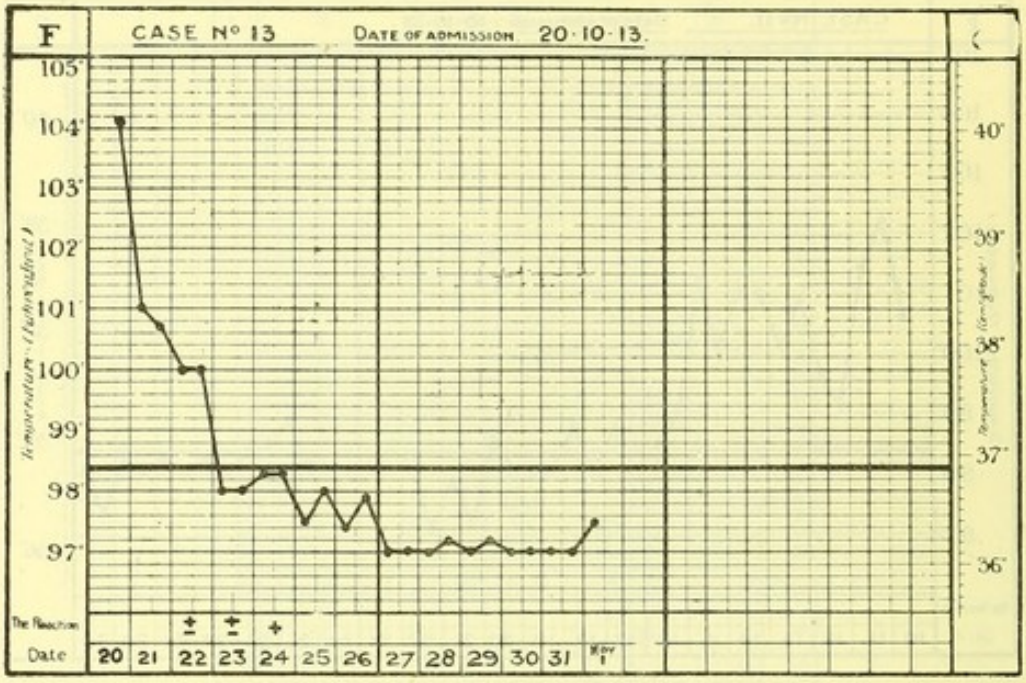
Temperature (Fahrenheit)
Temperature (centigrade)
The Reaction
- - + +
Date
14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31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CASE Nº 13
DATE OF ADMISSION 20·10·13.
C
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Temperature (Fahrenheit)
Temperature (Centigrade)
The Reaction
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Date
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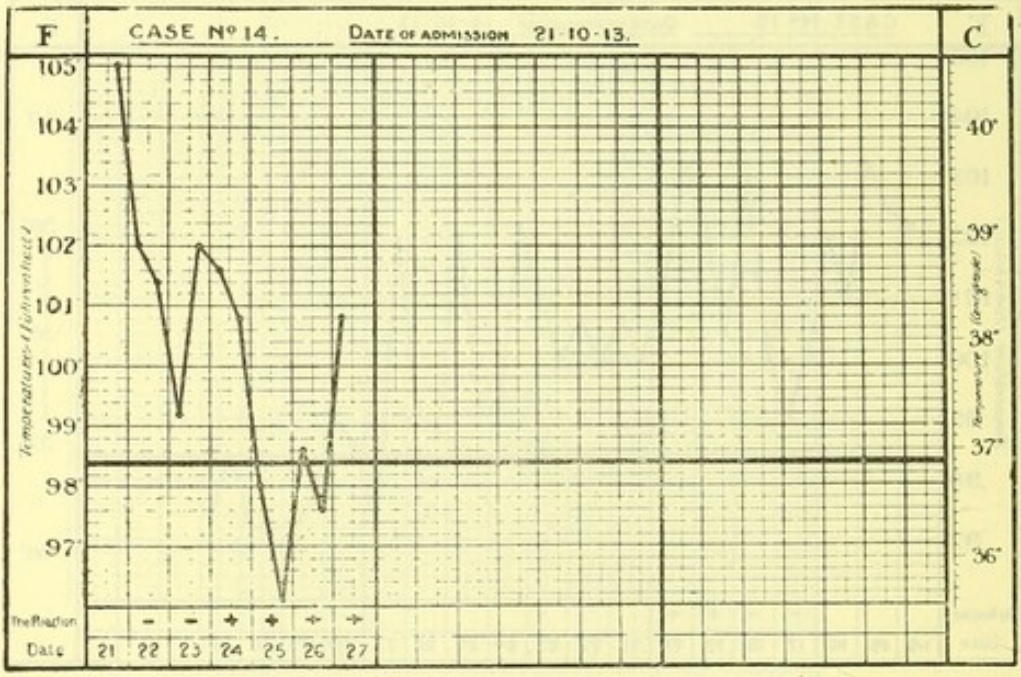
F
CASE Nº 14.
DATE OF ADMISSION 21·10·13.
C
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Temperature (Fahrenheit)
Temperature (Centigrade)
The Reaction
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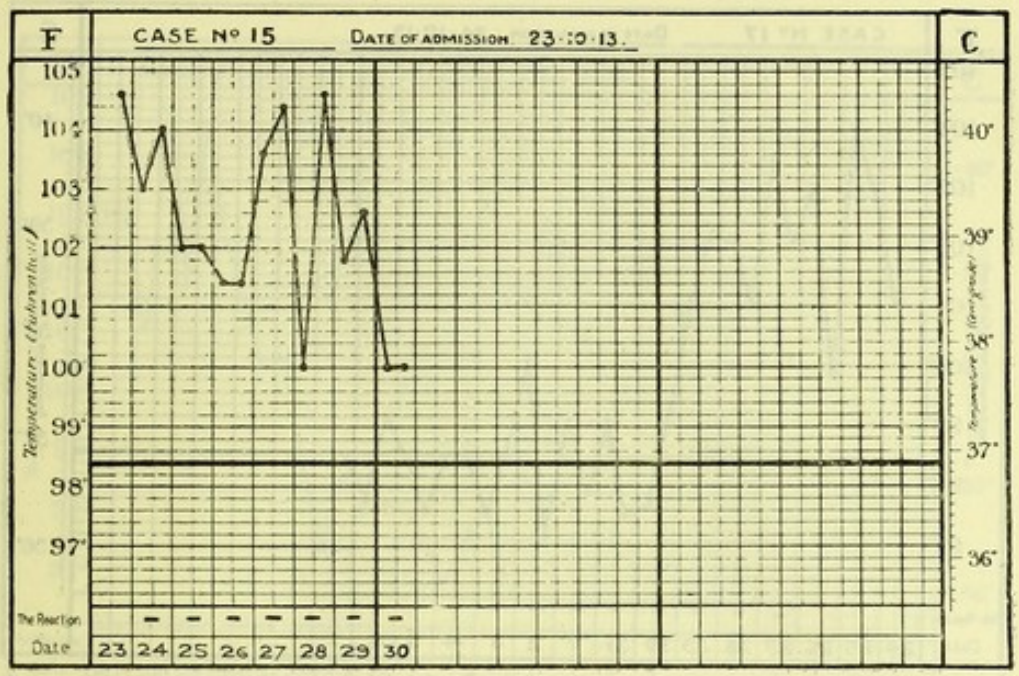
Date
21 22 23 24 25 26 27


F
CASE Nº 15
DATE OF ADMISSION 23·10·13.
C
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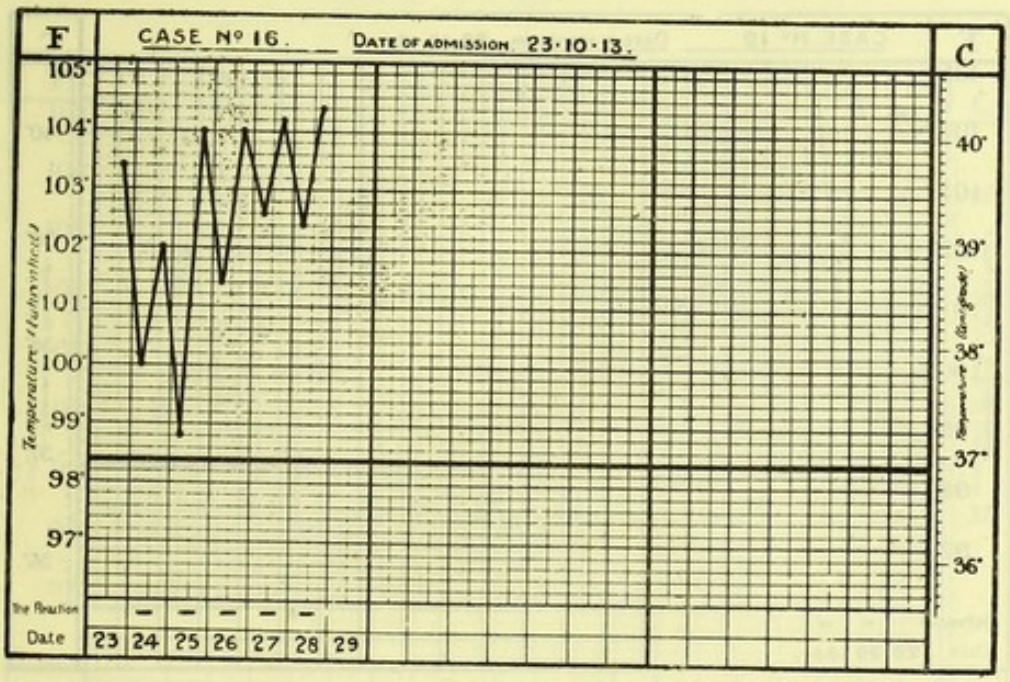
36°
Temperature (Fahrenheit)
Temperature (Centigrade)
The Reaction
Date
23 24 25 26 27 28 29 30


F
CASE Nº 16.
DATE OF ADMISSION 23·10·13.
C
105
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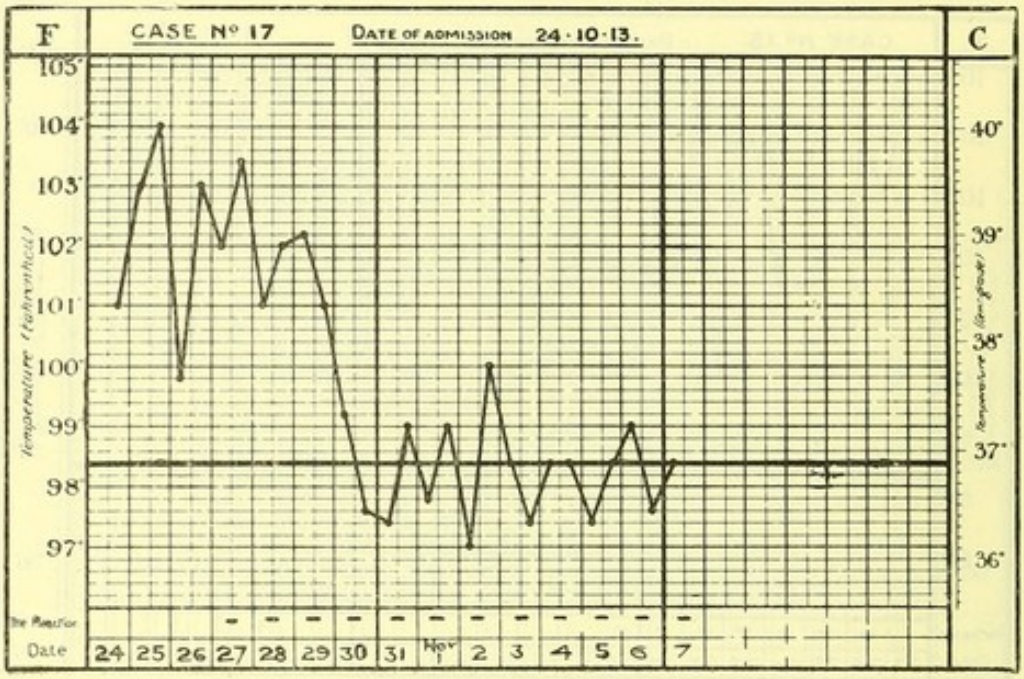
Temperature (Fahrenheit)
Temperature (Centigrade)
The Reaction
Date
23 24 25 26 27 28 29


F
CASE Nº 17
DATE OF ADMISSION 24·10·13.
C
105
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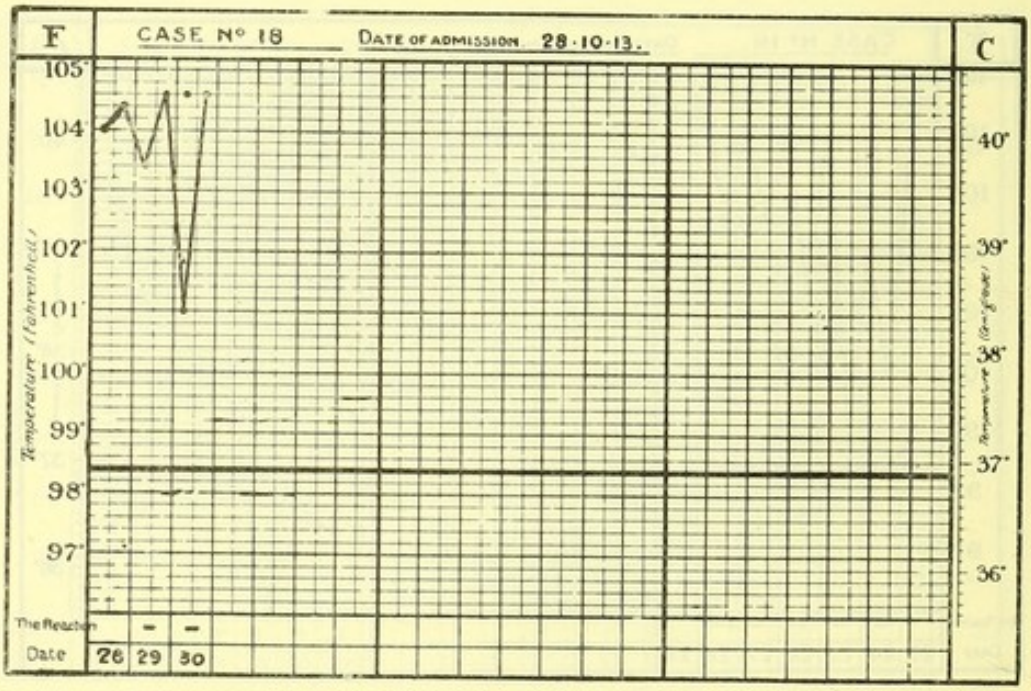
Temperature (Fahrenheit)
Temperature (Centigrade)
The Reaction
Date
24 25 26 27 28 29 30 31 Nov 1 2 3 4 5 6 7


F
CASE Nº 18
DATE OF ADMISSION 28·10·13.
C
105
104
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40°
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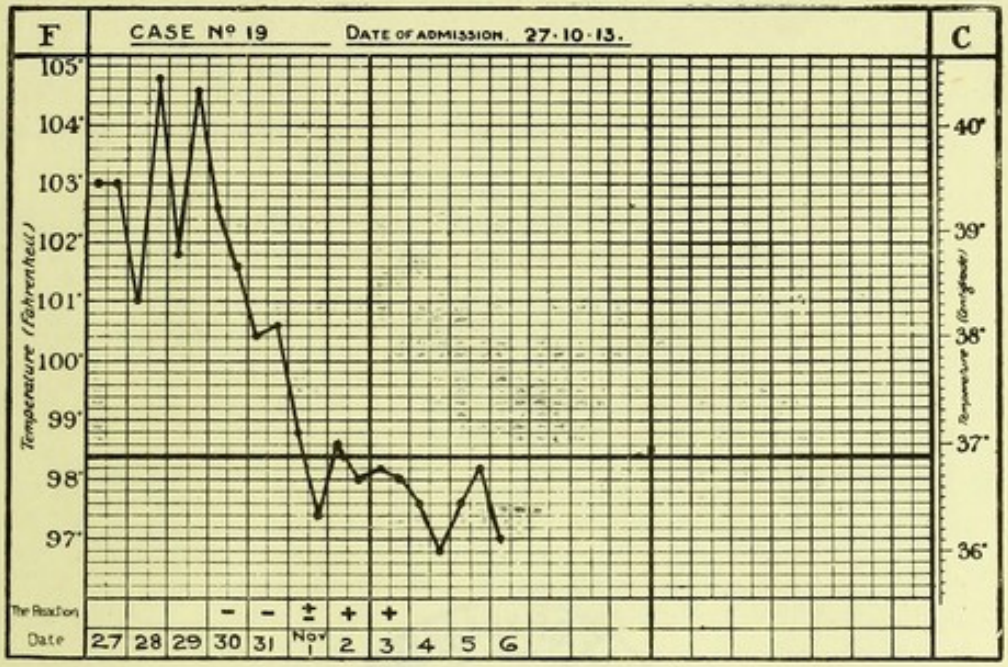
36°
Temperature (Fahrenheit)
Temperature (Centigrade)
The Reaction
Date
28 29 30


F
CASE Nº 19
DATE OF ADMISSION. 27·10·13.
C
105°
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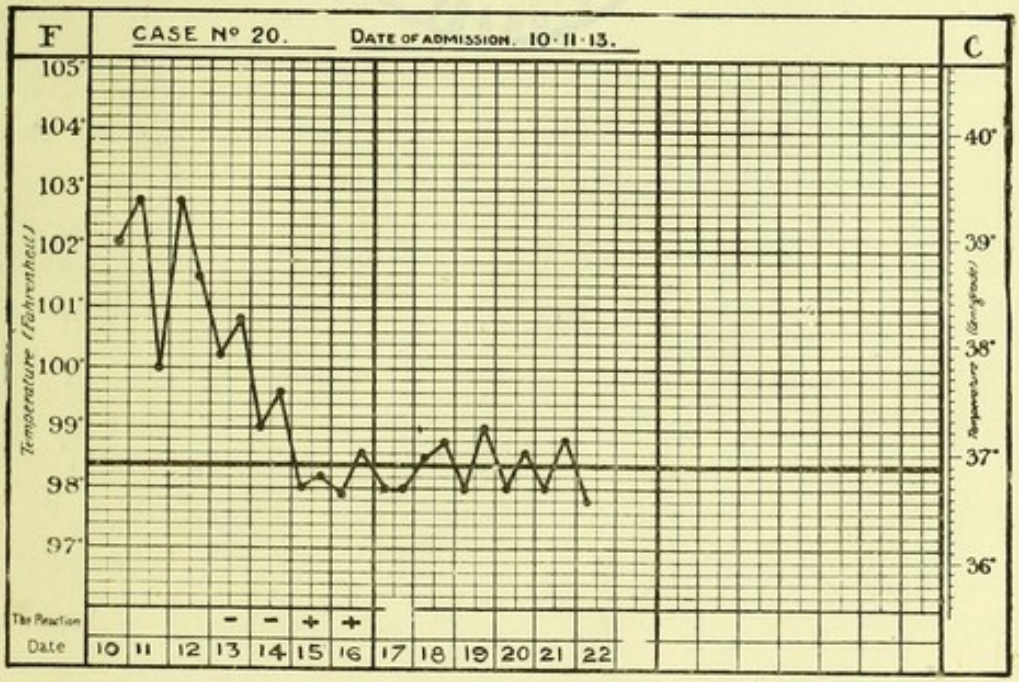
Temperature (Fahrenheit)
Temperature (Centigrade)
The Reaction
- - ± + +
Date
27 28 29 30 31 Nov 1 2 3 4 5 6


F
CASE Nº 20.
DATE OF ADMISSION. 10·11·13.
C
105°
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Temperature (Fahrenheit)
Temperature (Centigrade)
The Reaction
- - + +
Date
10 11 12 13 14 15 16 17 18 19 20 21 22