Thesis

for

The Degree of Doctor of Medicine

by

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SUBJECT.

Researches on the agglutinating and prophylactic properties of blood serum in cases of Plague, with observations on the Haematology and Bacteriology of the disease.

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Within a short period of years - covered approximately by the last decade of the century just closed - many and varied lines of investigation have been followed out in the attempt to gain a fuller and more accurate conception as to what underlies the phenomenon of Immunity. The complete solution of the problem has not yet been arrived at, but much good work has been done, leaving behind a record of real and permanent progress along this whole line of enquiry. The rapid advances made in our knowledge of micro-organisms and the products of their vital activity have rendered possible these investigations, which have led to the opening out of new avenues of thought towards the central mysteries of disease. The successful prosecution of at least two of these lines of enquiry, has led in the one case to the inauguration of a new phase of medical treatment, and in the other to the establishment on a sure and scientific basis of a new method of recognis-

-ing disease. It need hardly be said that the two outstanding discoveries of medical science during the last ten years are here referred to viz:- Serum-therapy and Serum-diagnosis. Both these discoveries are the more immediate result of recent investigations into the properties of the sera of highly immunised animals and of individuals suffering from infective diseases, and in both the sphere of application is therefore somewhat limited, and restricted at present to certain of those diseases consequent upon the introduction into the body of a definite pathogenic organism. The former, SERUM-THERAPY,- based as it is on modern and more accurate conceptions as to the proximate causation of disease- is undoubtedly one of the most rational modes of treatment yet devised, and already has yielded most brilliant results in the treatment of many diseases of microbial origin; whilst the latter, SERUM DIAGNOSIS, may without exaggeration be regarded as one of the most important and practical applications of the science of bacteriology to the practice of medicine, and perhaps the most clinically helpful of recent discoveries in this department of medical science.

It is , in fact, not too much to affirm, that this most valuable clinical method has placed the art of diagnosis on a pinnacle of exactitude, never before attained by a science dealing with such complex biological factors as the interaction of the human body with the micro-organisms of disease.

The principle upon which is based this method of recognising certain infective diseases by means of the reaction presented by their sera depends upon the fact that, when a drop of an emulsion of a motile pathogenic organism is mixed with a drop of the serum of an animal immunised against this particular bacillus, the micro-organisms collect together in clumps and lose their motility. The term "agglutination" is now commonly employed to designate the series of changes just described which ensue when the above-mentioned conditions are fulfilled.

The value of a serum-diagnosis has already been definitely ascertained with respect to enteric fever and Malta fever. Other investigations are also being made with the object of

ascertaining its value in yellow fever, diphtheria, and tubercular disease, but so far nothing of practical importance has been deduced. As Plague is unquestionably a disease of microbial origin, it seemed not improbable that the principle of serum-diagnosis might also be made applicable to this disease. The following investigations were therefore undertaken with the object of elucidating this point. The main part of my work done in connection with plague was directed to this end, but with the material in hand, various other enquiries were entered into. Whilst these latter have really arisen as side issues to the main research, I feel justified in incorporating the results so obtained as belonging to cognate branches of research. The scope of this paper embraces therefore

(1) The presence of an agglutinative reaction in plague and its value in the serum-diagnosis of this disease.

(2) The behaviour of sera derived from cases of infectious diseases other than plague towards the bacillus pestis.

(3) The presence of agglutinative bodies in the urine of plague patients.

(4) The general bacteriology of plague.

(5) The microscopical examination of the blood of plague patients, embracing qualitative and quantitative estimations of the leucocytes.

(6) The artificial immunisation of the lower animals by means of serum derived from plague patients.

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ON THE AGGLUTINATIVE REACTION AND THE SERUM-DIAGNOSIS OF PLAGUE.

The number of observations which have been made up to this time on the agglutinative power of serum from patients in the course of , or during convalescence from an attack of bubonic plague is comparatively limited. This is all the more remarkable when one considers the numerous opportunities which have occurred during the course of the last five or six years for such observations. The literature also bearing on the subject is not at present of such extent or completeness as to furnish a basis for inferences of practical value. The earliest reference to this part of the subject which I have been able to find is contained in the report of the German Commission sent to Bombay for the investigation of Plague. In their abstracted report, published in the "Deutsche Medicinische Wochenschrift for 1897, the existence is announced of a definite reaction

between the serum of man and animals infected with plague and an emulsion of the specific bacillus. The reaction consists in the precipitation of the bacilli in the form of small distinct floccules when such serum is added to a test tube containing an emulsion of plague bacilli. The report also states that no such precipitation of other organisms by plague serum occurs, the emulsion of these remaining uniformly turbid. The report of the Commission, however, makes no mention of the value of the reaction either as a diagnostic or prognostic agent in this disease, and suggests merely that the specificity (ie) of the reaction may constitute a reliable method for the identification of the plague bacillus and its differentiation from other organisms closely resembling it. About the same time Paltauf[1] established the existence of the reaction in the blood of animals (guinea-pigs and horses) which had succumbed to inoculations of dead plague cultures. A very slight degree of dilution of the blood, however, was employed(from 1 to 5 or even 1 to 1)- a fact which tends to diminish the value of the observations.

1.Wiener Klinische Wochenschrift, 1897. p.537 ; also La Semaine Medicale, June 2nd.1897.

Considerably greater practical importance attaches to the results obtained by Wyssokowitz and Zabolotny.[2] These authors state that the agglutinative power is not manifest during the earliest and most acute stage of the disease. It first appears in the blood about the seventh day of illness, gradually increases up till the fourth week and declines after this period. In cases fatal during the first week of illness they found it absent.

A short resume of the results obtained independently by Professor Zabolotny during the epidemic at Bombay in 1897 appeared in the Deutsche Medicinische Wochenschrift for the same year[3], and though no details are given, the conclusions to which he has been led substantially agree with those of the last mentioned observers. In a subsequent paper[4] the method employed for demonstrating the reaction is stated, and from a comparatively large number of single observations the probable curve of the agglutination wave is constructed. I desire here

2 Archives Russes de pathologie. May. 1897. Recherches sur la Peste Bubonique, Annales de l'Institut Pasteur, 1897. p. 663.
3 Über agglutinirende Eigenschaften des Menschenblutserums bei der Pest, Deutsche Med. Wochenschrift. 1897, No. 24, p. 392.
4 Recherches sur la pest. Zabolotny. Archives des Sciences Biologiques, April. 1900, T. VIII., No. 1.
Biologique

to acknowledge my indebtedness to Professor Zeholotny, to whom, during his stay in Glasgow, I owed several valuable suggestions in the carrying out of my work on this subject. Dr. Leumann of the Bombay Plague Laboratory has also made a considerable number of single observations on the bactericidal and agglutinative properties of Plague serum, and while the technique adopted was sufficient for the demonstration of the former property and for rough estimations of the latter, it was evidently quite impossible to obtain reliable comparative data by this method. One or two loopfuls of an agar culture were simply suspended in sterile water and serum was added drop by drop till precipitation of the microbes occurred.[5] No attempt was made to estimate the relative proportions of serum and emulsion, and, as will be shown later, the sine qua non of all agglutinative experiments- viz., a perfectly homogenous emulsion- cannot be obtained by this method. A short paper by Dr. Klein[6] completes the list of

5. Bombay Plague Report Vol. II
6. The Lancet, Feb. 16th, 1901, p. 456.
7. Die Pest von Muller und Poch, 1900.

those which I have hitherto been able to consult. In it he mentions the difficulty and emphasises the importance of obtaining a perfectly homogenous distribution of the bacilli in the emulsion to be used. (This is referred to in a later part of the present paper.) The extensive report of the Austrian Commission contains no reference, so far as I am aware, to this aspect of the subject, and the comparatively short paragraph devoted to it in the exhaustive work of the late Dr. Muller of Vienna[7] is indicative of the necessity for extending our observations on the subject.

The recent outbreak of Bubonic Plague in Glasgow provided an opportunity of furnishing an additional contribution on the character and significance of the agglutinative phenomena in this disease. It has been my desire to gauge as accurately as possible the degree of completeness of the reaction at several stages of the disease in the same individual, so that a basis for the estimation of its value at any particular period may be established. The following record contains particulars of 24 cases examined with this specially in view, and considerably over 500 agglutination tests have been performed to

furnish the strictly comparable data. The fact that the entire series of observations was made by myself is mentioned only to indicate that the standard of comparison by naked-eye examination of all the reactions has been practically uniform throughout.

Unfortunately, many of the earlier agglutinative experiments performed with plague serum in this research were rendered of comparatively little value, owing to the fact that a mode of proceedure was adopted similar in all respects to that now in common use for obtaining the agglutinative reaction in enteric fever. The method adopted for the latter purpose becomes inapplicable to plague because the specific bacillus, unlike that of enteric fever, does not produce in peptone bouillon a homogenous turbidity during growth, but tends to cohere either in the form of strands of varying lengths ("stalactites"), or as minute floccules which are aggregated mainly on the sides and bottom of the tube, and to a lesser extent occur throughout the fluid medium. It was found impossible by means of prolonged agitation of such a culture to produce a homogenous suspension of the bacilli. After such treatment, the micro-organisms were found to settle at the

bottom of the tube in the form of a loose powdery deposit, and on microscopical examination clumps of minute size were found throughout the supernatant fluid. The most important condition for the performance of a trustworthy series of experiments in agglutination or sedimentation -viz., an absolutely homogenous emulsion of the young organism - is therefore, in the case of bacillus pestis, unobtainable with peptone bouillon. The cohesion of the bacilli during growth in bouillon, (possibly related to the developement of an adhesive agent connected with the capsules of the organisms by which zoogloea-like masses are formed) is also exhibited during growth on the surface of agar-agar. If the surface of such a culture of 24 hours' incubator growth be touched with a platinum loop its slimy consistence becomes at once apparent, and while the needle is being removed a fine gelatinous thread is drawn out from the growth on the tip of the wire. This becomes a more marked feature in slightly older cultures. The difficulty in obtaining such an emulsion of plague bacilli was one which entailed a considerable amount of experimental work. It

was ultimately found that a homogenous emulsion could be obtained in sterile 0.75 per cent. salt solution. It is noteworthy that Dr Klein, whose paper on the subject has recently appeared, has solved the same difficulty after trial of various expedients in an almost precisely similar manner. This method yields a perfectly homogenous and workable emulsion which remains practically unchanged for 24 hours, showing no signs of clarification in the upper portion of the fluid, and no great tendency to the deposition of micro-organisms at the foot of the tube. A drop of this fluid examined under the microscope shows the bacilli perfectly free and isolated from each other, and, if carefully prepared, ought to be entirely free from any clump-formations.

TECHNIQUE. The following was found the most suitable method of preparing the emulsion, which, in view of the necessity of obtaining reliable comparative results in each case, was required in considerable quantities. Several sloped agar tubes, which had been inoculated from a 24 hours' culture of bacillus pestis, were incubated for from 24 to 36 hours. Cultures which showed the presence of involution forms in any considerable number

or which had been incubated for longer periods than 48 hours, were not (and should not be) used for this purpose. The tubes were then filled with sufficient 0.75 per cent. sterilised salt solution to cover the solid medium. The growth was then as far as possible transferred to the salt solution. This was carried out most efficiently, and without tearing the surface of the agar in the process, by rubbing the growth with the rounded extremity of a sterile Pasteur pipette, the end of which had been previously curved in the form of a shepherds' crook. The various emulsions so prepared were then decanted into a large sterilised test tube which was set aside for a short time to allow any cohering masses which might be present to settle. If the growth had been very luxuriant a more sensitive emulsion was usually obtained by diluting still further with salt solution. The exact degree of dilution with salt solution is undoubtedly a matter of some importance and can only be determined after considerable experience in agglutinative work. As a matter of frequent observation it may be stated that too concentrated emulsions are agglutinated very slowly and often very imperfectly. In the following series of observations the

emulsions were all prepared from the same stock cultures, incubated for approximately the same length of time, and as far as possible grown under exactly similar conditions. It need hardly be added that the cultures used responded to all the recognised tests for the complete identification of the bacillus pestis. Inoculation of animals with these cultures reproduced the disease with its characteristic lesions and after death the specific bacillus was recovered from the blood or organs.

In performing experiments on agglutination two chief methods are recognised: (1) the microscopical and (2) the macroscopical or sedimentation method. The result of a considerable number of comparative trials of both methods indicated that on the whole the latter yielded more reliable data. By means of the microscopical method, however, pronounced and in every way characteristic reactions can be demonstrated in much higher dilutions than by the latter technique, but data so obtained are frequently vitiated at times by the occurrence of puzzling and apparently unaccountable pseudo-reactions. Consequently, though the potency of a given serum was estimated in most instances by both methods, only the sedimentation test was adopted throughout this series of observations as the standard

standard for performing comparative quantitative estimations, and no serum was regarded as yielding a positive result which failed to give a satisfactory reaction by this method. The reactions were carried out with serum separated directly from blood collected in sterilised Pasteur pipettes, care being taken in every possible way to avoid its contamination. By so doing the possible introduction of a fallacious factor in the means used for obtaining serum by a blister was avoided. It may be stated, however, that no visible difference either in the degree of the reaction or in the time within which it made its appearance could be detected in several instances where serum separated from blood and that obtained from a blister were used from the same case and at the same date for purposes of comparison.

Before undertaking a consecutive series of observations it is well to prepare beforehand all necessary apparatus so that no delay may take place at any stage which might interfere with or vitiate the comparative value of individual reactions. Accordingly it was found advisable to have in readiness a supply of ordinary Pasteur pipettes

about 30 centimeters in length and an equal number of a slightly modified pattern. The latter were provided with a capillary portion of a length equal to the distance between the open extremity of the ordinary Pasteur pipette and the commencement of its capillary portion; in addition a constriction or neck was fashioned on the upper end of the tube about three-quarters of an inch below the level of the cotton plug. Blood is most conveniently obtained from puncture of a finger, the skin first being rendered sterile according to recognised methods. All traces of antiseptics having been removed by a final washing with spirit, the finger is carefully dried with aseptic gauze and the pulp punctured with a sterilised bayonet-pointed neddle. The drop of blood which exudes is immediately aspirated into a sterilised Pasteur pipette the sealed extremity being first passed through a flame and then snapped across at a suitable level by means of sterilised forceps. By "massaging" the finger from above downwards and aspirating each drop as soon as it appears, a quantity, sufficient not only for the immediate performance of the reaction, but also for

purposes of comparison subsequently, can usually be obtained. In this way from half to one cubic centimetre of blood may be furnished by a single successful puncture. During aspiration care should be taken to avoid the introduction of any air bubbles, as these interfere to some extent with the formation of a firm coagulum and a resulting clear serum. The fine extremity of the tube is then sealed by drawing it out in a spirit flame, unnecessary heating of the contents being carefully avoided. The pipette is kept in an upright position and set aside in a cool place for several hours pending the separation of the serum. If no air has been introduced a firm coagulum usually forms at once and may be seen floating in the centre of the tube surrounded by a perfectly clear serum. If, however, during the process of aspiration the blood has been more or less churned up with air, then it is better to set the tube aside for 12 to 18 hours. The resulting serum in this case is apt to contain a considerable number of red corpuscles, and though these in no way interfere with the satisfactory performance of the reaction, a clearer serum is obtained if time be allowed for

the sedimentation of the red corpuscles. The separation of the serum from the coagulum is effected by means of the modified pipettes. The cotton-wool plug being removed from the tube containing the blood, the sealed extremity of a modified pipette is first snapped across with sterilised forceps, the capillary portion introduced into the former and carried down to the conical narrowing of the tube. The clear serum is then aspirated into the second tube and the capillary portion sealed at a suitable level by drawing out in a flame. Before doing so, however, the precaution should be observed of aspirating all the fluid into the body of the tube so as to avoid any undue heating of the serum. If the preservation of the serum for any length of time is desired the pipette may easily be converted into a hermetically sealed capsule by drawing out the constricted portion in a Bunsen flame. On account of the difficulty of preparing two emulsions of exactly the same density it is always better to subject at one time as large a number of sera as possible to the influence of a single emulsion. For the same reason it is absolutely necessary when performing quantitative

estimations to compare the agglutinating power of the serum under observation with that obtained at a previous date, the potency of which has already been ascertained by repeated experiment. Only by so doing is it possible to establish an arbitrary standard and so gain an accurate conception as to the quantitative value of individual reactions. When results materially disagreed with previous observations the data so obtained were disregarded and the series of reactions again observed with a fresh emulsion.

The reaction is conveniently performed in test tubes about nine centimetres in length prepared from ordinary soft-glass tubing of about 0.7 centimetre internal diameter. These are thoroughly cleansed and dried, the open extremity being closed with a plug of cotton-wool. After sterilisation at a temperature of 160 degrees C. for an hour, they are ready for use. In all cases the reaction was carried out in at least three dilutions, and in many instances with sera possessing a high degree of potency in five dilutions. The proportions adoped throughout have been: 1 in 10, 1 in 50, 1 in 75, 1 in 25, and 1 in 100. In no case has an undoubted reaction been obtained in a higher dilution than 1 in 75,

and reactions lower than 1 in 10 have not been recorded, as the necessarily large relative dilution of serum with the emulsion in such preparations yields a practically clear fluid in which the determination by the naked eye of any agglutination becomes a matter of extreme difficulty.

The reaction is conveniently performed as follows. A long Pasteur pipette is taken and its capillary portion snapped across at a level corresponding in diameter with that of the capillary portion of the pipette containing the serum under examination. It is thus ensured that drops issuing from the orifices of these two pipettes will have as nearly as possible the same volume, and in this way any required dilution of serum with emulsion can be readily performed. The Pasteur pipette, the wider portion of which is guarded by two cotton-wool stoppers to prevent possible accidents during inspiration, is filled with the bacillary emulsion. A drop of serum having been introduced into each of a series of sedimentation tubes, the emulsion of bacilli is added to each drop by drop to produce the requisite degree of dilution. The serum and emulsion are thoroughly mixed and the cotton-wool stopper is replaced in the sedimentation tube. It is con-

venient to place vertically in a small deep box the requisite number (from three to five) of sedimentation tubes for each serum under investigation. This obviates the necessity of labelling each tube and at the same time prevents confusion. The degree of dilution can then be readily be inferred from the volume of fluid in each tube. When completed the whole series is set aside in a cool chamber for from 18 to 24 hours. As control experiments in each series two or more tubes containing (1) a quantity of the bacillary emulsion and (2) emulsion with normal serum in corresponding degrees of dilution,- were employed. If at the end of 24 hours any degree of sedimentation had taken place in these latter the data obtained from this series were not recorded. Under ordinary circumstances, however, no changes were observed in the control tubes, whilst in the case of those containing serum from a convalescent plague patient a remarkable alteration became apparent after a variable interval of time and according to the potency of the serum. The emulsion was seen to clarify from above downwards till practically the entire column of fluid became absolutely clear. This change was due to the precipitation of the bacilli to the lower part of the tube, where they form a loose

flocculent deposit. On examining this bacillary deposit under the microscope it was observed that the capsules of the organisms had become indistinct. In the case of powerfully agglutinative sera the process may be observed to commence after one or two hours, but as a rule the reaction is rarely completed till 24 hours have elapsed. Consequently the latter has been adopted as the uniform time limit throughout this series of investigations. In high dilutions or when dealing with sera of feeble agglutinative power the reaction, as might be expected, is less complete. Comparison with the control tubes, however, shows that while a certain degree of clarification and precipitation has occurred at the end of 24 hours, the process has stopped short of completion, leaving a slightly opalescent appearance in the tube. In the following tables such reactions are indicated by the sign Xf (feeble) as the precipitated microbes show the same microscopical changes as in the complete reactions. A complete reaction is indicated by the positive sign X whilst the negative sign- indicates that no reaction has taken place. In the case of powerfully agglutinative sera, when clarification of the emulsion occurs soon after performing the

dilution (from one to two hours) the double sign XX has been used.

The following tables represent in a condensed form the result of over 500 separate agglutination tests of the serum taken at different periods of the illness from 26 plague patients . For purposes of classification the cases have been arranged in the following groups:-

(1) Fatal cases.

(2) Severe cases ending in recovery.

(3) Mild cases ending in recovery.

(4) Cases of Pestis Ambulans.

The more important cases are reported with some attention to detail but it has likewise been deemed advisable to add short clinical resumes of those cases of mild plague in which bacteriological confirmation of the diagnosis was not available. Further, in order to facilitate reference to these cases in subsequent sections a number has been appended to each, and at the close of the last section will be found a table indicating the nature of each case and the page at which the report is to be found.

The cases examined were all of the bubonic type, and of these nine received at one time or other varying doses, either

subcutaneously or intravenously, of Yersin's anti-plague serum. The agglutinative reactions in these cases, however, were probably not modified to any considerable extent by the previous administration of the serum for curative purposes. This is a point of considerable importance as it is quite conceivable that cases might arise during the course of an epidemic in which the circumstantial evidence pointed strongly in favour of there being plague and yet where the clinical evidence did not warrant an absolute diagnosis. In such a case it might be deemed advisable to administer the curative serum and it is satisfactory to know that such treatment would not detract from the value of the ultimate serum diagnosis. In support of this it may be here remarked that the blood serum of individuals who had received one or more prophylactic doses of this serum showed no evidence whatever of any agglutinating power. It is therefore highly improbable that the data so obtained have been vitiated in the slightest degree, as even the remedy itself when mixed with a highly sensitive emulsion fails to produce any visible change after the lapse of 26 hours.

With the view of ascertaining the possible effect of a mixed infection on the agglutinative reaction I undertook a complete bacteriological examination of most of the organs in fatal cases of plague. These results are exhibited in tabular form (Table 1.) in the section dealing with the general bacteriology of plague. Reference to the cases in Table 2. shows that the presence of micro-organisms other than the bacillus pestis in the blood or organs of a patient dead of bubonic plague does not prevent the developement of the specific reaction.

The results obtained from six fatal cases are shown in Table 2. and in all of these the presence of the bacillus pestis was verified by reproducing the disease with its characteristic lesions in animals. Cultures of the specific bacillus were obtained during life, either from the bubobor blood of the patient, or post mortem from the various organs. These were either injected subcutaneously or a small quantity of an agar growth placed in the nasal cavity of an animal and the bacillus again recovered from the tissues after its death. In this

group of cases the reaction was also demonstrated with other body fluids -viz., pleural effusion, pericardial effusion, peritoneal fluid, and bile. With respect to the first three of these it will be observed that, in comparison with blood serum, a relatively high degree of agglutination has been noted in the only cases in which it was possible to perform the test with these morbid exudations.

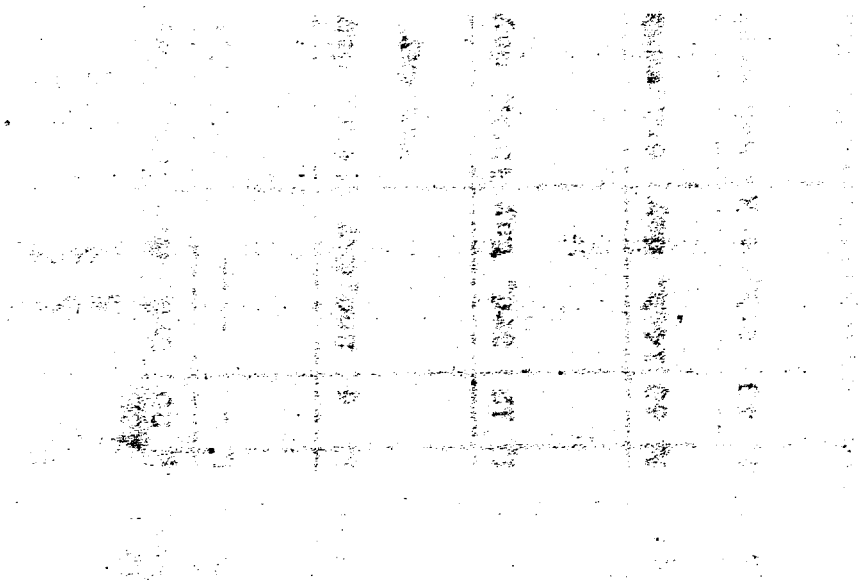


TABLE 2.-Fatal Cases.

NAME	AGE	Date of ill-ness on adm.	Date of Serum Examination.	Amount of Serum (Yersin) injected.	Nature of Fluid examined.	Bacteriological Examination.	Result of animal Inoculation.	Dilution of Serum with Emulsion of Bacillus Pestis in proportions of:- 1-10	1-25	1-50
1) Mrs. M.	20	3rd. day	5th. day	60cc	Blood, P. Mortem.	X	X	-	-	-
2) Baby M.	-	-----	7th. day	-	Post Mortem	X	X			
					Blood			X		-
					Pleural effus.			X	X	-
3) Mary G.	6	2nd. day	4th. day	100cc	Blood Serum	X	X	Xf	-	-
					Post Mortem.					
			7th. day		Blood			X	-	-
					Pleural effus.			X	Xf	-
4) Robt. M.	12	3rd. day	12th. day	80cc	Post Mortem.	X	X			
					Blood			X	-	-
					Pericardial eff.			X	X	-
					Peritoneal eff.			X	X	-
5) Jas. B.	60	14th. day	6th. week	-	Post Mortem.	X	X			
					Blood			XX	X	-
6) Will. W.	48	4th. week	6th. week	40cc	Post Mortem	X	X			
					Blood.			X	Xf	-
					Bile.			X	Xf	-

TABLE 3.- comprises those severe cases terminating in recovery, from all of which the bacillus pestis was obtained by puncturing the bubo. A sterilised hypodermic syringe was inserted into the glandular swelling and from the serous fluid withdrawn the specific organism was demonstrated on direct microscopical examination of smear preparations. Culturally the organism isolated from each case responded to all the recognised tests for the complete identification of the bacillus pestis, and, as in the first group of cases, was further verified by the biological test. In the case of Mrs M.(case No.12) it was thought unnecessary to perform a bacteriological examination of the bubo, as the patient's daughter (Mrs. M.case No.1.), grand-daughter (baby M. case No.2.),and son (Robt.M.case No.4.-see table 2), all died from Bubonic Plague.

TABLE 3.- Severe cases ending in recovery.

NAME	AGE	Date of ill-ness on adm.	Date of Serum Examination.	Amount of Serum (Versia) injected.	Bacteriological Examination.	Result of animal Inoculation.	Dilution of Serum with Emulsion of B. Pestis in proportions of 1-10	1-25	1-50	1-75	1-100
7) Pat. F.	56	2nd. week	7th. week	-	X	X	XX	X	X	-	-
			12th. week				XX	X	-	-	-
8) Mrs. M.	40	6th. day	3rd. week	-	X	X	X	-	-	-	-
			6th. week				XX	XX	XX	Xf	-
			9th. week				X	X	X	-	-
			12th. week				X	Xf	-	—	-
9) Mrs. T.	40	13th. day	3rd. week	-	X	X	XX	X	-	-	-
			8th. week				XX	X	X	Xf	-
			10th. week				X	X	-	-	-
			12th. week				X	X	-	-	-
10) Thos. M.	15	7th. day	4th. week	40cc	X	X	XX	XX	X	-	-
			5th. week				X	X	-	-	-
			6th. week				XX	X	X	Xf	-
			8th. week				XX	XX	XX	Xf	-
			11th. week				XX	XX	X	-	-
			5th. month				X	X	-	-	-
11) Chas. M.	27	2nd. day	16th. day	40cc	X	X	X	X	-	-	-
			4th week				X	X	X	-	-
			6th. week				XX	X	X	Xf	-
			8th. week				XX	X	-	-	-
			4 months				X	X	-	-	-
12) Mrs. M.	41	2nd. day	7th. day	40cc			X	Xf	-	-	-
			18th. day				X	X	X	-	-
			5th. week				X	X	Xf	-	-

PNEUMONIC CASE.

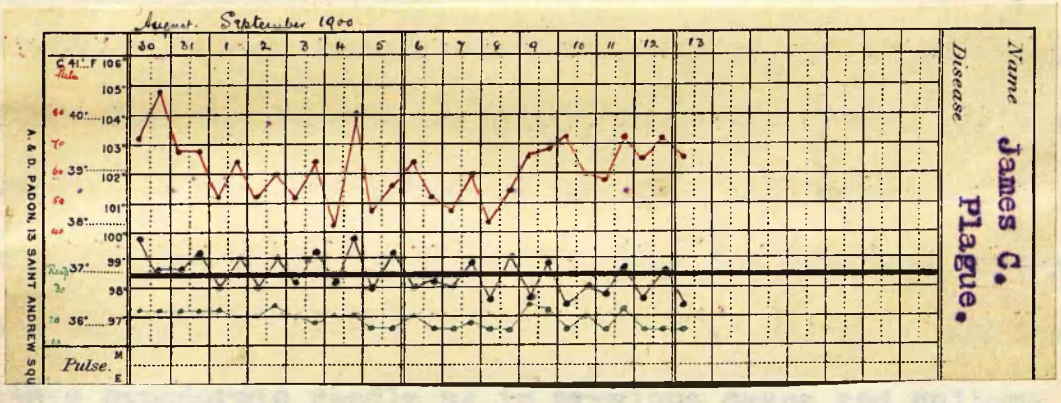
X) Arch. A.	18	2nd. day	9th. day	-	Xf	-	-	-	-	-	-
			3rd. week				X	X	-	-	-
			7th. week				X	X	-	-	-
			10th. week				X	X	-	-	-

TABLE 4.- comprises those comparatively mild cases of plague with characteristic bubo. From the first three of these the specific drganism was isolated and identified as above, but only in the case of Mrs G.(case No.15.) was the proof completed by animal inoculation. In the last three cases- James C.,Rosina M., and Dennis T. (cases No. 16,17, & 18) it was found impossible to cultivate the organism,either from the juice obtained from puncture of the glands, orvat a later stage when the bubo had broken down,from the sero-purulent discharges. The failure to obtain the bacillus in these cases was no doubt due to the comparatively late stage of the illness at which the patients first came under observation. As the disease ran an exactly similar course, however, to those from which the specific organism had been recovered, and as in each case the serum possessed marked agglutinating power, there appears to be every possible reason to regard them as undoubted though mild cases of plague. The following synopsis of the clinical features of these cases reveals their character sufficiently.

Case No.16. James C. aet.24, admitted Aug.30th.1900. This patient slept for a single night on August 13th in a room on the same landing as several of the other plague patients, though his usual place of residence was at a considerable distance from the plague infected area. On August 17th he was seized with an illness, evidently of considerable severity, associated with headache, sickness, and vomiting, and pain in the left inguinal region. The report received from the medical attendant, Dr.G., is as follows:- "Aug.17th. severe headache and vomiting with pain in left inguinal region, where there is some redness and swelling. Temp.103F.; pulse 102. Aug. 21st. Swelling larger and more painful; temp.102.6F., pulse 98. Swelling sausage-shaped and lying along upper border of Poupart's ligament; fairly hard and pitting on pressure."

On admission, patient was evidently convalescent, no sign of illness being present. The temperature was normal. On examination of the left groin, however, a swelling rather larger than a pigeon's egg was discovered immediately above Poupart's ligament and involving the horizontal set of glands. The overlying skin was inflamed and slightly oedematous and

fluctuation could be easily obtained. A hypodermic needle inserted into the glandular swelling withdrew a quantity of dark grumous fluid which, on microscopical examination, showed the presence of numerous degenerated plague-like bacilli, and though a considerable number of agar tubes were inoculated on two separate occasions no growth whatever could be obtained. Convalescence was uninterrupted, the bubo disappearing by absorption.



Case No.18. Rose M. aet.28, admitted 10th Sept.1900. This patient was admitted from the plague-infected area in her third week of illness. At this date all clamant symptoms had subsided, but examination of the inguinal region on each side showed the presence of a bilateral Bubonic condition involving the group of glands situated immediately above Poupart's ligament. Each bubo was about the size of large wal-

-nut, that on the left side being comparatively firm and painless, whilst in the other fluctuation could be easily detected. The history of the illness showed that the onset was sudden and acute and was characterised by high fever, (temp. 104F.) accompanied by rigors and delirium; headache, sickness, and vomiting were also present, and continued for some time after the appearance of the buboes which were discovered by the patient herself on the third day of illness. These were apparently exceedingly painful at this stage. They rapidly increased in size during the succeeding two or three days, after which the tenderness slowly subsided. The right bubo ruptured in the fourth week of illness, and discharged a sero-purulent fluid for about 10 days; the left bubo underwent resolution without rupture. Both buboes were explored with a hypodermic needle as in previous cases and culture tubes inoculated with the aspirated fluid remained sterile. At a later date the subcutaneous injection of a mouse with the discharges from the right bubo was unattended by any serious disturbance in the animal's health.

With respect to Dennis T.(case No.17) no doubt can be entertained as to the nature of the illness,both on account of the association and the clinical appearances. He was admitted on the eighth day of illness with a glandular swelling in the right inguinal region which presented the characteristic appearance of a plague bubo in the stage of resolution. Cultures made as above described proved sterile, though smear preparations showed the presence of numerous degenerated bacilli. The patient's grandfather, however, (James B.case No.5) died from plague, and his mother (Mrs.T.case No.9) passed through a very severe attack of this disease. The identity of the isolated organisms in both these latter cases was verified by animal experimentation.

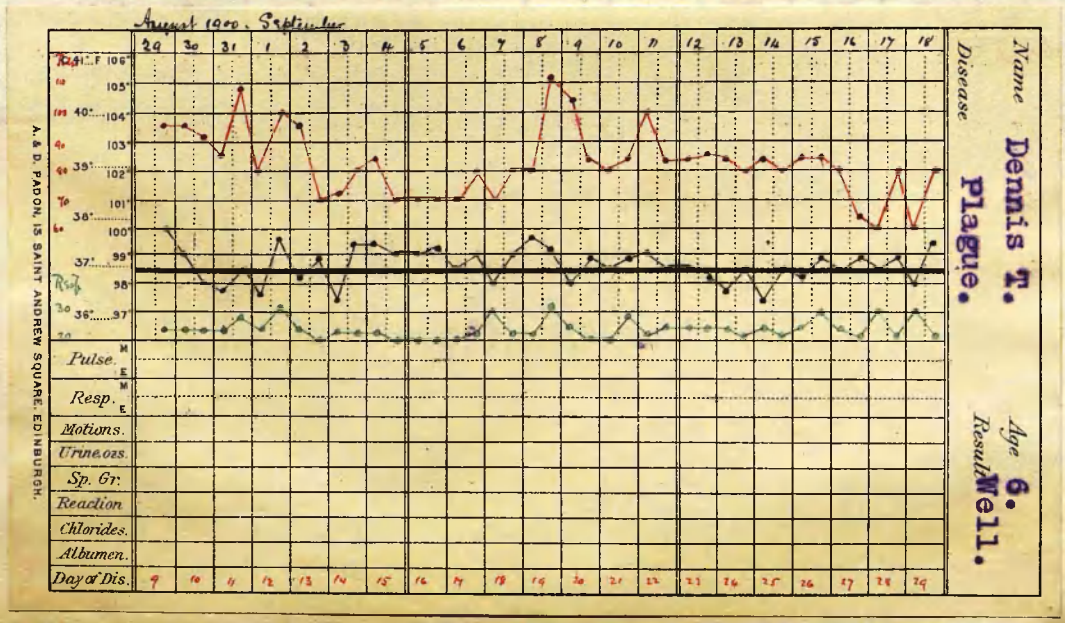


TABLE 4.- MILD CASES.

NAME	AGE	Date of ill--ness on adm.	Date of Serum Examination.	Amount of Serum (Yersin) injected.	Bacteriological Examination.	Result of animal Inoculation.	Dilution of Serum with Emulsion of B.Pestis in proportions of 1-10	1-25	1-50	1-75	1-100
13) Mary M.	14	2nd. day	6th.day	40cc	X	-	X	-	-	-	-
			16th.day				X	X	-	-	-
			3rd.week				XX	X	Xf	-	-
			5th.week				XX	X	-	-	-
14) Mrs. B.	28	2nd.day	9th.day	40cc	X		X	Xf	-	-	-
			18th.day				XX	Xf	-	-	-
			6th.week				X	-	-	-	-
15) Mrs. G.	28	2nd.day	3rd.week	-	X	X	XX	XX	X	-	-
			4th.week				XX	XX	XX	X	-
			6th.week				X	X	X	-	-
			8th.week				X	-	-	-	-
			4½months				X	-	-	-	-
16) Jas. C.	24	3rd.week	3rd.week	-	-	-	X	Xf	-	-	-
			5th.week				X	X	Xf	-	-
			7th.week				X	X	-	-	-
			9th.week				X	X	-	-	-
17) Dan. T.	6	9th. day	5th.week		-	-	XX	XX	X	-	-
18) Rose M.	28	3rd.week	5th.week		-	-	X	X	-	-	-

TABLE 5:- The remaining cases grouped in this table may be regarded both from the etiological and clinical standpoint, as fairly typical examples of "Pestis Ambulans". In all the illness was apparently so slight that, apart from a definite history of contact with infected persons and in the absence of a bacteriological examination, it would have been quite impossible to pronounce upon the exact nature of the glandular affection. It is not proposed here to give a description of the general course and symptomatology of such cases, and it will therefore be sufficient to indicate briefly the salient points of each.

Of these , undoubtedly, the most interesting is the case of a ward-maid (Nellie R. case No 19) who was employed in one of the pavilions set apart for the isolation and treatment of plague patients. Ten days prior to the commencement of her illness she had received a prophylactic injection of 10 cubic centimetres of Yersin's Serum. The illness, however, was of a comparatively trifling nature, the patient only complaining of a moderate degree of indisposition for about three days. Bacteriological

examination, however, demonstrated the presence of the plague bacillus in an exceedingly small cervical bubo. The serum taken during the third week of illness agglutinated an emulsion of plague bacilli in a dilution of 1 in 25, though the constitutional disturbance was so slight that the temperature and pulse throughout the illness never rose above normal.

Agnes R., aet. 3½ years. (case No.20). This patient was a "contact" from the Molloy wake - one of the first discovered foci of infection. The illness, of sudden onset was accompanied by headache, sickness, and vomiting, and pain in left axilla. Examination of this region showed the presence of a small bubo, but an exploratory puncture failed to withdraw any lymphatic gland substance. The temperature rose to 101F. on the first day of illness and subsided to normal two days later. Convalescence was rapid and complete.

The two clinical histories just detailed, may be taken as fairly representative of this type of the disease.

In the remaining cases the sudden appearance of bubo was

associated with symptoms of fairly acute onset, similar in all respects to those already described. In these, however , a bacteriological examination was not undertaken, but the peculiar nature of the illness, taken in conjunction with a very definite history of exposure to infection, seems sufficient to justify these cases also being regarded as examples of the mildest variety of this disease. It may here be added that the serum of patients suffering from various forms of specific disease and taken at various stages of their illness was examined in different degrees of dilution with an emulsion of bacillus pestis. In no case was any reaction observed. The diagnostic value of its appearance, therefore, in cases of slight glandular enlargement is to this extent enhanced.

TABLE 5.-Cases of Pestis Ambulans.

NAME	AGE	Date of ill--ness on adm.	Date of Serum Examination.	Bacteriological Examination.	Amount of Serum (Yersin) injected.	Dilution of Serum with Emulsion of B. Pestis in proportions of 1-10	1-25	1-50	1-75
19) Nellie R.	21	2nd. day	3rd. week	X	10cc	X	X	-	-
20) Agnes R.	3½	1st. day	2nd week	-	-	X	X	-	-
21) Arch. D.	18	7th. day	4th. week	-	-	X	X	-	-
22) Willm. M.	3	5th. day	3rd week	-	-	-	-	-	-
23) Jeanie M.	14	2nd. day	2nd. week	-	-	-	-	-	-
24) Annie McK	12	2nd. day	2nd week	-	-	-	-	-	-
25) Pat. McG	18	2nd day	4th. week	-	-	-	-	-	-

The following deductions appear fully warranted from an examination of the preceding tables.

(1) During the early days of the disease the reaction is not manifested and consequently in rapidly fatal cases is probably never obtained.

(2) Agglutinating properties first appear in the blood towards the close of the first week of illness (dilution of 1 in 10). These gradually increase in intensity up to the sixth week of illness and are sometimes maintained at a high level as late as the eighth week. After this date, however, in the majority of cases, a gradual decline in the agglutinative power of the serum becomes apparent. The rates of increase and decrease of the reaction, generally speaking, are approximately equal; occasionally, however, the reaction wanes and disappears in a shorter period than that occupied between its appearance and point of maximun intensity.

(3) In very severe cases ultimately proving fatal the reaction though present never reaches a high degree of intensity. In cases of almost equal severity, however,

in which an early and rapid convalescence followed, the reaction was of a more marked character.

(4) In the mildest forms of plague a high degree of agglutinating power is probably never attained and in some it appears to be absent. Stricker, in fact, states that the reaction never appears in this class of case, but this is undoubtedly too sweeping an assertion, as an undoubted and characteristic reaction in a dilution of 1 in 25 was obtained in two such cases.

(5) The reaction, as a rule, is most marked in those severe cases characterised by an early and favourable crisis, and in such cases it disappears very slowly, having been shown to be present as late as the fifth month after the primary illness.

OTHER METHODS.- The technique just described is, however, perhaps unnecessarily complicated, as in order to avoid any possible source of fallacy a number of precautions were adopted which later experience showed to be superfluous. The initial difficulty - the preparation of a perfectly homogenous bacillary emulsion -

having been overcome, an almost precisely similar technique to that employed in the ordinary "Widal" reaction may be adopted. As already stated, the dilution of serum with emulsion can be carried to a much higher degree and the time-limit considerably shortened when the microscopical method is employed. By this means reactions in which the bacilli are completely agglutinated in from five to ten minutes are fairly common with potent sera in dilutions of 1 in 10 and 1 in 25. If, however, the dilution be carried further, it is absolutely necessary for trustworthy results to observe the reaction in hanging-drop preparations. In this way well marked reactions have been obtained with a dilution of 1 in 200 and a time-limit of two hours, though preparations made between an ordinary slide and cover-glass showed no evidence whatever of any agglutinative process. The time-limit may be safely extended to 24 hours if the edge of the cover-glass be sealed with vaseline. By this method a reaction can often be demonstrated with sera of feeble agglutinative power or with potent sera which have been highly diluted.

As a control experiment, preparations of (1) emulsion, and (2) emulsion plus normal serum, have been kept for over a week without any signs of undoubted agglutination supervening. Numerous observations of plague sera in progressively increasing dilutions have been made and the time-limit in each case has been carefully noted. The data so obtained may be compared with those got by the sedimentation test at corresponding periods. To illustrate this, the results obtained by this method from two severe cases of plague terminating in recovery (Table 3.) are exhibited in the accompanying table (table6).

Like results may be obtained with similarly prepared emulsions of dead plague bacilli, but in this case the reaction is not so reliable, as the dead organisms are not agglutinated so readily. When working with dead cultures, however, the microscopical is to be preferred to the sedimentation method, since it yields more trustworthy results even in higher degrees of dilution. In the absence of facilities for obtaining a homogenous emulsion of living or dead bacilli Haffkine's prophylactic (which contains the latter) may be

TABLE V.- MICROSCOPICAL SERUM AGGLUTINATIONS.

Thomas H. (case No.10)

Date of Examin^{n}.	DILUTION 1-10	TIME	DILUTION 1-25	TIME	DILUTION 1-50	TIME	DILUTION 1-75	TIME	DILUTION 1-100	TIME	DILUTION 1-150	TIME
5th.week	XX	5mins.	XX	10mins.	XX	15mins.	XX	30mins.	XX	1hour	X	45min
8th.week	XX	5mins.	XX	10mins.	XX	15mins.	XX	45mins.	XX	1hour	X	1hour
11th.week	XX	5mins.	XX	20mins.	XX	30mins.	XX	45mins.	Xf	1hour	X	1hour
4½ month	XX	10min.	XX	30mins.	X	1 hour	XX	...	Xf	2hour	-	18hrs
Charles M. Case No.11)												
4th.week	XX	5mins.	XX	10mins.	X	1 hour	X	2hours	Xf	12hrs	-	18hrs
6th.week	XX	5mins.	XX	10mins.	XX	45mins.	X	2hours	Xf	2hrs	Xf	1hour
8th.week	XX	5mins.	XX	30mins.	Xf	2hours	-	18 hrs	-	...	-	--
4½ month	XX	20min.	XX	30min.	Xf	2hours	-	18 hrs	-	...	-	...

Thomas H. (cont^{d})

8th.week	1-200	TIME	1-300	TIME.
8th.week	Xf	2 hours	1-X?	12 hours
11th.week	X	1 hour	X	1 hour

used to dilute a suspected plague serum. No satisfactory reaction, however, has been got by this means in a higher dilution than 1 in 25, and the sedimentation method is alone applicable owing to Haffkine's prophylactic being a bouillon culture. This last method can only be regarded as a makeshift.

CONCLUDING REMARKS.

From the point of view of hygienic administration the very mild cases of plague are of the utmost importance. The analogy presented by all other infectious diseases indicates that such cases must be regarded as of equal importance with the more severe forms in the possible dissemination of the disease, and constitute therefore a grave source of danger to the community. It is well known that epidemics of measles, scarlet, and diphtheria are frequently preceded by a type of the disease so mild that many cases are allowed to remain untreated, and it is interesting to note that several of the outbreaks of plague in the East have been heralded by the mildest possible form of the disease. Moreover, the difficulties attendant on a successful exploratory puncture of a small

deep-set gland are sufficiently obvious, and any other means of arriving at a definite conclusion as to the nature of an obscure glandular swelling is certainly entitled to the most careful consideration.

In the severer forms of the disease the possibilities of a serum diagnosis are much greater. It has already been shown that during the early stages of the illness the agglutinative properties of the blood are but feebly developed and that unless a careful technique be adopted for its demonstration the reaction is liable to be missed altogether. After the second week of illness, however, and particularly in those cases where the bubo undergoes resolution, the chances of obtaining the bacillus by puncture of the gland diminish rapidly during convalescence. This fact is well illustrated by the last three cases in table 4, in which attempts to cultivate the organism by this method at the end of the first, second, and third weeks of illness respectively, completely failed, though numerous degenerated bacillary forms were seen in smear preparations. Indeed, in the case of Mrs. M. (case No. 8) culture tubes inoculated on the eighth day of illness with fluid obtained from puncture of the bubo proved

sterile, though two days previously an actively growing pure culture had been obtained. On the other hand, the agglutinative power of the serum, insignificant at the commencement of the illness, progressively increases up to the sixth or seventh week of the disease, by which time a comparatively high degree of potency has been obtained especially in the severer forms which recover. Thereafter it begins to decline, but, as already shown, may be present in well marked cases four or five months after the primary illness. It is therefore during and subsequently to the stage of convalescence, when the possibility of a bacteriological diagnosis is more or less remote, that the diagnostic value of the reaction becomes most apparent. In all cases, moreover, associated with the presence of glandular enlargements of dubious nature, the occurrence of which from time to time during the progress of an epidemic of plague may present a diagnostic problem of perplexing character, the application of this reaction cannot fail to prove of signal service.

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SECTION NO. 2.

ON THE BEHAVIOUR OF SERUM DERIVED FROM CASES OF INFECTIOUS DISEASE OTHER THAN PLAGUE TOWARDS AN EMULSION OF BACILLUS PESTIS.

.....

AGGLUTINATION IN OTHER SPECIFIC INFECTIOUS FEVERS.

Having thus determined the presence and constancy of a serum agglutination in plague, it became a point of considerable importance to determine whether the reaction was specific - a conclusion that one might have been tempted, a priori, to assume. With this object in view numerous perfectly parallel experiments were made with sera derived from patients suffering from:-

(1) Enteric fever. - 5 cases.

(2) Typhus fever. - 7 cases.

(3) Scarlet fever. - 6 cases.

(4) Measles. - 3 cases.

(5) Epidemic cerebro-spinal meningitis.- 1 case.

(6) Pneumonia. - 2 cases.

(7) Anthrax. - 2 cases. (verified bacteriologically)

(8) Ague. - 1 case. (parasite in blood)

(9) Purpura haemorrhagica. - 1 case.

In each class of disease the serum was taken at different periods of the illness, both during the acute stage and also during convalescence.

With respect to Enteric fever only those cases were selected which gave a powerfully positive reaction with the specific bacillus, and which presented the characteristic and typical signs of the disease.

Similarly in the case of Typhus fever only those cases were employed in which an absolute diagnosis was assured, not only by the clinical course of the disease, but also by the presence of the characteristic exanthem on the skin.

With regard to Scarlet fever, one or more examples of the main types of this disease were investigated, preference as a rule being given to those cases associated with glandular enlargement.

In the remaining groups of disease investigated and dependant for the most part on the introduction of a definite pathogenic organism into the system, the diagnosis was completed by the isolation of the specific bacillus.

In none of these cases did any reaction occur in a dilution higher than 1 in 10, and in the few instances in which agglutination supervened in low dilutions, the reaction had never the characteristic and well marked appearances which result from the addition of the appropriate serum to an emulsion of the specific bacilli. Whilst therefore from the comparatively limited number of such observations one does not feel justified in making the general statement that agglutination will not occur with plague bacilli where serum from any other disease than plague is employed, yet these perfectly parallel experiments seem to point strongly to the absolute specificity of the reaction. In this connection one feels justified in pointing out the desirability of more extensive investigations being undertaken.

SECTION NO. 3.

AGGLUTINATION IN URINE OF PLAGUE PATIENTS.

AGGLUTINATION IN URINE.

The following observations have been undertaken with the object of determining whether, in Plague as in Enteric Fever, the urine of patients suffering from the former disease gives an agglutinative reaction with the specific bacillus. Eight cases were investigated belonging to the "severe and mild" class, and the observations here recorded were carried out chiefly during the period of convalescence. The urines examined were taken at periods varying from the 17th to the 53rd day of the disease,- i.e. during convalescence, and every endeavour was made to secure the thorough asepsis of the specimens employed. Thus, in the case of females, the urine was withdrawn per catheter. In order to estimate as accurately as possible the quantitative value of the reaction, control observations were made with the serum of each patient, obtained for this special purpose on the same day as the urine experimented with. This was rendered

necessary by the well known fact that the quantitative value of the ordinary "Widal reaction" varies considerably within comparatively short time limits. In each case the urine was carefully examined to determine the presence of the Plague bacillus. In none was the organism found, and in the few instances where a doubtful bacillus was demonstrated, animal inoculations were performed with uniformly negative results. The search for the bacillus was deemed essential, because, according to the researches of Courmont, the addition of specific bacilli to an homologous serum nullifies the reaction. This is apparently due to specific bodies secreted by the bacilli neutralising the agglutinating bodies present in the serum. Thus, if to an homologous serum of previously ascertained agglutinating power, the specific bacilli are added, and after some time removed, such serum will show either no agglutinating effect or a greatly diminished power, if a subsequent addition of bacilli be made. The dilutions employed varied from 5 to 10 to 1 to 75 and from the observations thus performed the following facts are recorded.

(a) In each case the urine yielded positive results with the lower dilutions.(5-10 and 1-10)

(b) In seven,a positive reaction was obtained with a dilution of 1 to 25.

(c) In two cases the reaction was positive with a dilution of 1 to 50 and doubtfully so in three others.

(d) In no case did a urine yield positive results with a greater dilution than 1 to 50, whilst in several a reaction with the corresponding serum could be obtained in a dilution as high as 1 to 75.(

(e) As a control experiment,urine from a healthy individual was taken and similarly tested. In this case a positive reaction was got with 5 to 10 and 2 to 10, but in this instance the reaction was not nearly so marked as with plague urines.

CONCLUSIONS.- As a result of these observations one seems to be justified in drawing the following conclusions:-

(1) An agglutinative reaction is present during convalescence in the urine of patients suffering from Plague.

(2) This reaction can be demonstrated in dilutions varying from 5 to 10 to 1 to 25 and occasionally in a dilution

of 1 to 50.

(3) The agglutinative power of a plague urine seems to show a certain correspondence with that of the patients' serum.

(4) Whilst interesting as a scientific fact, the agglutinating properties of urine from plague patients is apparently of little practical value.

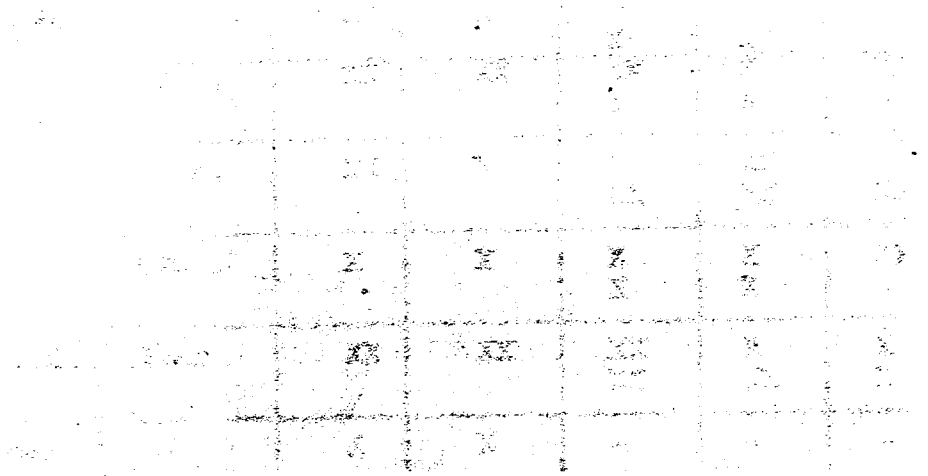


TABLE 7. COMPARATIVE RESULTS OF AGGLUTINATION

IN URINE AND SERUM AT CORRESPONDING DATES

CASE NO	NAME	DAY OF ILLNESS	DILUTIONS OF URINE AND SERUM WITH EMULSION OF BACILLUS PESTIS IN PROPORTIONS OF:- 5-10	2-10	1-10	1-25	1-50	1-75	
8	Mrs. M.	53rd.	XX	X	X	Xf	-	-	URINE
					XX	XX	X	-	SERUM
10	Thos. H.	41st.	XX	XX	XX	XX	X	-	URINE
					XX	XX	X	Xf	SERUM
11	Chas. M.	28th.	XX	XX	XX	X	X?		URINE
					XX	XX	X	Xf	SERUM
12	Mrs. M.	20th.	XX	X	X	-	-	-	URINE
		28th.			X	X	-	-	SERUM
13	Mary M.	28th.	XX	XX	XX	X	Xf	-	URINE
					X	X	-	-	SERUM
15	Mrs. G.	24th.	XX	X	X	Xf	-	-	URINE
					XX	XX	XX	X	SERUM
16	Jas. C.	61st.	X	X	X	X	X?	-	URINE
					X	X	-	-	SERUM
17	Dan. T.	17th.	XX	XX	XX	X	X	-	URINE
					XX	XX	X	-	SERUM
A.	Normal Urine	-	X	X	-	-	-	-	URINE
A.	Normal Serum	-			-	-	--	-	SERUM

BACTERIOLOGY.

SECTION NO. 4.

BACTERIOLOGY.

In the section on serum agglutination, a passing reference was made to the presence of mixed infections in certain cases and the absence of any disturbing effect on the reaction. It is proposed here to give a brief summary of the bacteriological work done by me in connection with the plague cases, to analyse the results so obtained, and to offer some special remarks on the group of mixed infections. These researches were conducted both on the living patient and with material derived post mortem.

Bacteriologically considered, the cases examined naturally fall into three classes:-

(1) Those in which there is a pure plague infection.

(2) Those in which the primary plague infection is associated with a diplococcus or other organism. Clinically, however, this class does not differ materially from the first.

(3) Those in which - generally after the rupture of the bubo - secondary septic infection followed.

The method uniformly adopted was - under aseptic precautions - to puncture the bubo with a hypodermic syringe, and to withdraw some of the glandular juice from its substance. The fluid so obtained was used both for direct microscopical examination and for culture. Simple as this proceedure may appear, the practical difficulties attending its performance are considerable. It is no easy task to puncture a small, possibly deep-set, and tender gland, especially as such are often in close proximity to large vessels. Unless the gland substance be actually entered, there is every likelihood that the fluid withdrawn will yield negative, and at the same time, misleading results. On the other hand, it is satisfactory to know that when proper precautions are taken, a positive result can be obtained even in the case of a very small gland. e.g. Nellie R. (case No.19.) This patient was employed as ward-maid in one of the plague pavilions, and ten days after a prophylactic dose of Yersin's serum, a small tender gland made its appearance in the occipital region. In this instance, the ease with which success-

-ful puncture was accomplished, was no doubt largely due to the situation of the gland which was certainly no larger than a cherry stone.

In all cases examined during the first week of illness, the existence of the bacillus pestis was readily demonstrated in the fluid aspirated from the bubo. It is a rather remarkable fact, however, and quite contrary to what might have been expected, that in all preparations so obtained, the organisms, whilst presenting the characteristic appearances of the bacillus pestis, were present in comparatively small numbers, even in the most severe cases. Whilst this was true of the first week of illness a marked difference was shown at later periods. By the end of the first week the bacilli had generally disappeared from the bubo, especially in the milder cases. As a rule they could not be demonstrated after the second week, although in two cases they were found as late as the eighteenth day of illness. (Pat.F. and Jas.B. cases No.5 and No.7.) In no case was the bacillus recovered after this date.

Whilst, however, culture experiments during this period

remained sterile, the microscope revealed the presence of sundry aberrant and obviously degenerated forms of the bacillus pestis. As a rule, in those cases where the bubo had ruptured spontaneously, the bacillus pestis could not be demonstrated. In one case, however, whilst microscopical examination did not yield undoubted evidence of the presence of the bacillus, yet animal inoculation conclusively proved their existence in a state of undiminished virulence. This exceptionally long period of viability was found in the case of Mrs.G. (No.15.) whose bubo ruptured on the 16th day of illness. Such an occurrence points to the unreliability of microscopical examination, per se, as a means of determining the period at which a patient may be declared non-infective. The accompanying table gives in extenso the bacteriological condition in each of the fatal cases. From this it will be seen that pure plague infection was present in only two of the fatal cases viz., baby M. and Mary G. (cases No.3 and 4.) It will be noted, however, that in the table bacillus coli is mentioned, but as this organism is almost constantly found in

TABLE I.- RESULT OF BACTERIOLOGICAL EXAMINATION OF ORGANS IN FATAL CASES OF PLAGUE & OF ANIMAL INOCULATIONS.

	NAME	Date of sickening	Bact.Exam. during life	BUBO	SPLEEN	BLOOD OF HEART	LUNGS	LIVER	OTHER TISSUES.
1	Mrs. M. Died 16th.Sept.	13th.Sept.	BUBO.-B.Pestis (pure culture) BLOOD.-B.Pestis (pure culture)	B.Pestis (almost pure) B.COli.Comm. (scanty)	B.Pestis (scanty) B.Coli Comm. (abundant)	B.Pestis (pure culture)	B.Pestis. Diplococcus (Friedlander's) B.Catarrhalis	- (Diplococcus	Retro-peritoneal glands B.Pestis. (pure culture)
2	Baby M. Died 27th.Sept.	24th.Sept.	Not undertaken	B.Pestis (almost pure) B.Coli Comm. (scanty)	B.Pestis (abundant) B.Coli Comm. (abundant)	B.Pestis (abundant) B.Coli Comm.	B.Pestis (abundant) B.Coli Comm.	B.Pestis (abundant) B.Coli Comm.	-
3	Mary G. Died 25th.Sept.	18th.Sept.	BUBO.-B.Pestis (pure culture) BLOOD.-Sterile (7 daily Exams)	POST -	- MORTEM	EXAMINATION	REFUSED.	-	-
A	James H. Died 24th.Sept. Admitted 25th.Sept.	10th.Sept.	Not undertaken	B.Pestis abundant Streptococcus (abundant) Large bacilus Diplococcus	B.Pestis (abundant) Streptococcus (abundant) Large Diplo-coccus	B.Pestis (abundant) Streptococcus (abundant)	-	B.Pestis (abundant) Streptococcus (abundant)	KIDNEY B.Pestis Streptococcus Large bacillus
5	James B. Died 24th.Sept.	12th.Aug.	BUBO.-B.Pestis (pure culture) BLOOD.-Sterile	B.Pestis & a small putre-factive bacus	B.Pestis (scanty) Staph.Pyog.Alb (abundant)	-	-	-	-
4	Robert M. Died 28th.Sept.	14th.Sept.	BUBO.-Staph.Py-og.Au.(abunda) (25th.Sept.) BLOOD.-sterile	B.Pestis (scanty) Staph.Pyog.Au. (abundant)	B.Pestis (abundant) Staph.Pyog Au. B.Coli Comm.	B.Pestis (scanty) Staph.Pyog Au. B.Coli Comm.	B.Pestis Staph.Pyog Au. B.Coli Comm.	URINE sterile PERITONEAL FLUID sterile	MEDIASTINAL ABSCESS.B.Pestis Staph.Pyog Au. B.Coli Comm.
6	Willm. W. Died 6th.Oct.	about 24th.Aug.	BUBO.-degenerate Plague bacilli Staph.Pyog.Au. Staph.Pyog.Alb.	Staph.Pyog.Al. Large Diplo-coccus. Streptcoccus	B.Pestis (scanty) Staph.Pyog.Alb Bac.Coli Comm.	-	-	B.Pestis (scanty) B.Coli Comm.	-

any post mortem examination, it may be discounted.

(2) This class comprises four cases, three of which proved fatal. In two of the fatal cases(G.H. and P.M.) the accompanying diplococcus was found in the bubo, and in a third - Mrs.M. (case No.1.) was present in the lungs and also in the retro-peritoneal glands. In the non fatal case Pat.F. (case No.7.) the diplococcus was only present in the bubo.

(3) Of those cases where a primary plague infection was followed after a definite interval - during which the bubo had spontaneously ruptured - by an ordinary septic infection, we have the following three examples; viz., Robt.M., James B., and Will^{m} W. (cases No. 4,5,and 6.)

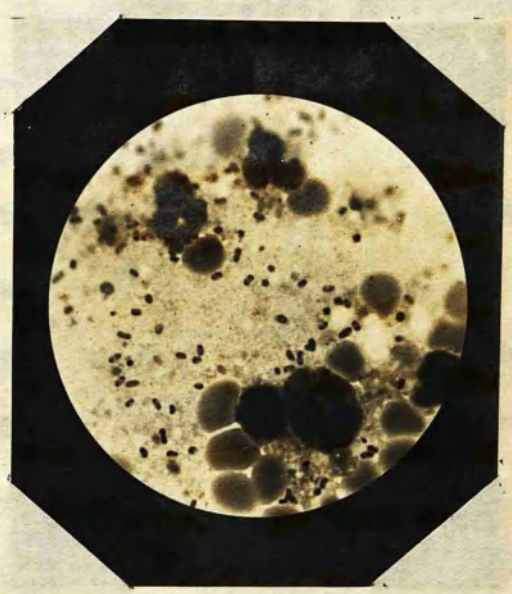
The associated organisms in these cases were; Staphylococcus Pyogenes Aureus and Albus, Streptococcus Pyogenes, bacillus coli communis, and a small putrefactive bacillus. With respect to James B. (case No.5.) it is interesting to observe that, although, the specific organism could not be recovered from the bubo after death yet, it was present in a virulent state in the liver and spleen six weeks after the commencement of the illness.

It is interesting to note here a peculiar state of matters found in the case of Mrs.M. (case No.1.) Puncture of the bubo on the third day of illness demonstrated the characteristic bacillus on direct microscopical examination. at the post mortem examination - the patient having died on the fourth day - typical bacilli were found in practically every organ of the body, and in enormous numbers. On the second day of illness, however, the patient had received an injection of 40 cc of Yersin's serum in the affected thigh, i.e. in the lymphatic drain of the glands forming the bubo. Post mortem examination of the bubo showed a marked change in the characteristics of the bacilli present in this situation. They stained with difficulty and the usual bipolar appearance was lost owing to the stain being absorbed uniformly. Their outline also became indefinite and considerable variety of form was noted. One is therefore driven to the conclusion that this remarkable change was due to the local action of the serum. In this connection, however, it must be remembered that the serum causes no such alteration in vitro.

This local effect of the serum on the bacilli in the inguinal bubo is shown in the accompanying photograph, Fig.1. the coccoid degeneration and indefinite outline of the organisms with total loss of bipolar staining being very apparent. These appearances may be contrasted with those shown in Fig.2, where the characteristic bipolar staining of the bacilli present in the spleen is very evident.

Fig.1. Mrs.M. Degenerated plague bacilli from inguinal bubo. x 1000.

Fig.2. Mrs.M. showing characteristically stained plague bacilli from spleen.



As in some measure tending to confirm these conclusions we may refer to another fatal case of plague in which curative serum was given, both subcutaneously into the drain of the affected glands, and also intravenously. If such altered appearances were in reality due to the local action of the serum on the bacilli, one would expect, à priori, that when serum had been administered intravenously, the bacilli in the spleen and blood would show similar changes. This has actually been found to be the case, and in the accompanying photographs the changes in the morphological characteristics of the bacilli after the subcutaneous and intravenous administration of serum are clearly shown.

Fig. 1. shows the characteristic bipolar-staining bacillus of plague in the bubo prior to the administration of serum.

In fig. 2. the coccoid degeneration and ghost-like appearance of these organisms after administration of serum is exhibited.

Fig. 3. shows similar appearances found in the spleen, but the changes in this instance, as might be expected from the comparatively small quantity of serum introduced into the general circulation are of a less marked character.

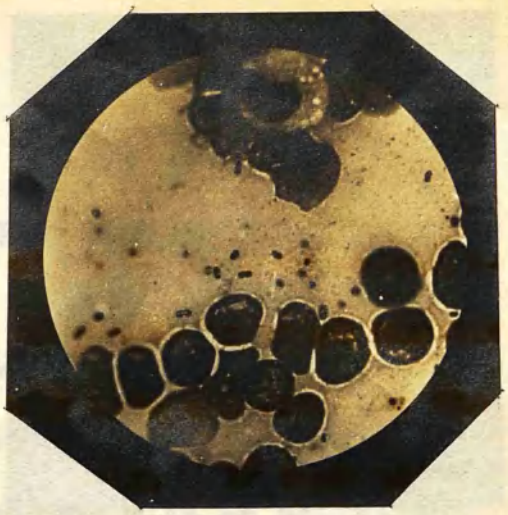


Fig. 1. Bacillus pestis from bubo prior to administration of serum. Shows well-marked polar staining. gent. violet. x1000

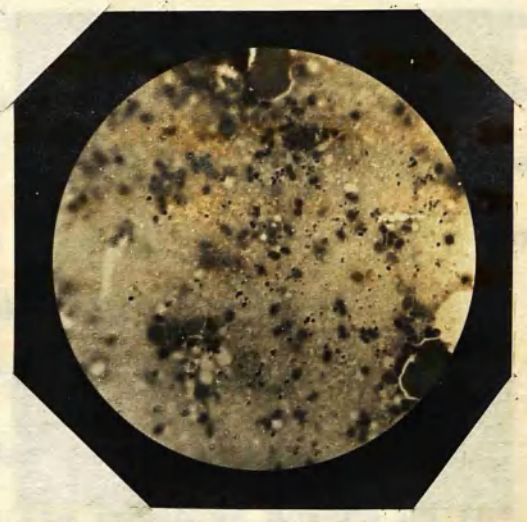


Fig. 2. Bacillus pestis in bubo after administration of serum. Shows coccus-like degeneration of bacilli. Many of the more degenerated, unstained shadowy forms are not visible.

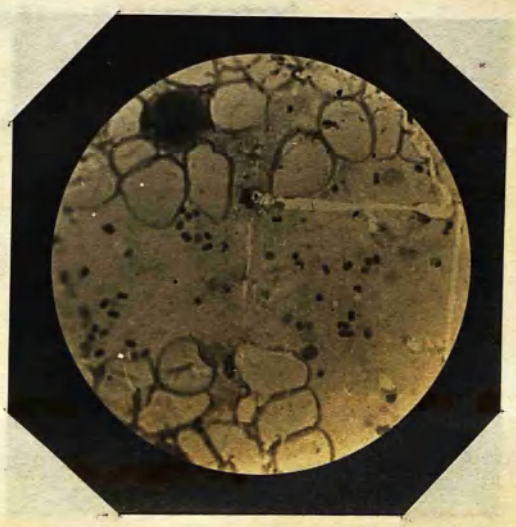


Fig. 3. Bacillus pestis in spleen of same case, 36 hours after intravenous injection of serum. Degenerative changes not so marked and a few normally stained bacilli visible.

With the object of ascertaining whether such a change was the invariable result of serum injection in the animal body, a number of experiments were made upon rabbits, mice, and guinea-pigs. The proceedure was as follows. The animal was inoculated in both thighs with an active and virulent culture of the bacillus pestis, and after the development of inguinal buboes, serum was injected into the lymphatic drain of one thigh. These experiments, however, seemed to show no appreciable local effect of the serum on the bacilli. A possible explanation of this apparently contradictory result is the fact that, the serum so used had deteriorated through long keeping, as shortly afterwards experiments showed that its curative powers on inoculated animals had practically disappeared.

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HAEMATOLOGY

SECTION NO. 8.

HAEMATOLOGY.

Hitherto, the chief object of observers in investigating the morbid anatomy of plague, has apparently been to determine the occurrence of the organisms in the various tissues and secretions. Probably it is owing to the fact of the chief investigations being conducted from the point of view of bacteriology, that a microscopical examination of the blood, apart from the search for the bacillus, has been almost entirely neglected. The earliest reference to this department of research which I have been able to find, is contained in a report to the Russian Government of an epidemic of Plague which occurred in Astrachan in the year 1878-79. The observers, Eichwald and Sommerbrodt, merely mention the fact without further comment, that "eine sichtliche Vermehrung der weissen Blutkorperchen" occurred in this disease.[1] The next observations in point of time are

1. Hirsch & Sommerbrodt: Mittheilungen uber die Pestepidemie im Winter 1878/79 im russichen Gouvernement Astrachan. p.50.

those of the distinguished Japanese bacteriologist, Aoyama, which were carried out at Hong-kong, in the year 1894, and communicated in his report on plague in that city, to the Royal University at Tokio.[2] In four cases investigated, the following startling numbers were obtained:- 20,000 , 110,000; 120,000; 200,000. Aoyama further pointed out that the leucocytosis was due to an increase in the polynuclear cells and that the eosinophiles were greatly diminished. The only other observations which appear to be on record are those made by Koch during the epidemic at Bombay im 1897. This investigator made a number of single isolated counts in several cases, as well as consecutive daily observations in two other cases. The results of these estimations are given in the Report of the Austrian Commission on Plague,[3] and confirm the observations of previous investigators as to the existence of a leucocytosis in this

2. T.Aoyama. Mittheilungen uber die Pestepidemie in Hong-Kong im Jahre 1894. Mittheil.der medid. Facultat der Kaiserl.Japan. Univ. zu Tokio 1896. Vol.3, No.2.
3. Gesammtbericht uber die Beulenpest in Bombay. Albrecht&Gohn. 66.Band der Denkschriften der mathem-naturw. Classe der kaiserl. Akademie der Wissensch.11B. Pat.anat. Untersuch. page 203.

disease. The degree of leucocytosis, according to Poch, varied between 8,900 and 45,000. Aoyama's figures, however, are so much in excess of those obtained by the latter observer, (and also by myself), that one cannot help suspecting the existence of a printer's error. After an exhaustive search through the literature of Bubonic Plague, I have failed to discover any other contribution bearing on this aspect of the disease, and, as will be seen, the somewhat fragmentary observations just quoted, refer mainly, if not wholly, to the presence of a leucocytosis. So far as can be ascertained, no attempt has been made to differentiate the various forms of leucocytes and their relative proportions at different stages of the disease.

The following observations do not pretend to be more than fragmentary, but as an excuse for presenting them here may be urged the fact that no such similar observations have hitherto been placed on record. While only undertaken as a side-issue during my work on agglutination, the results obtained may be at least regarded as suggestive, and may point to a hitherto neglected branch of research.

On account of many unavoidable interruptions, it has been impossible for me to carry out this investigation with any degree of completeness. For a time, the pressure of work in connection with the clinical observations and treatment of cases rendered anything like consecutive and systematic examinations of the blood completely impossible. The cases here observed fall naturally into the three categories already mentioned in the section on agglutination, viz:- Fatal, severe, and mild; so that the observations practically cover all the ordinary types of the disease, and from this point of view they may be regarded as fairly comprehensive. It is to be regretted that opportunities of following out each case in its entirety were not available; I have not hesitated, however, to place on record those cases in which a single and isolated examination of the blood has been made at a definite stage of the illness. Such data fall into line with, and tend to confirm the results obtained at a corresponding stage of the illness from similar cases in which it was impossible to make a

number of consecutive daily observations.

Leucocyte enumerations, quantitative as well as qualitative, were carried out in eleven individuals affected with plague, but only in the case of five of these was it possible - for reasons above mentioned - to perform a number of consecutive daily observations. In seven cases, quantitative estimations of the leucocytes were not carried out, a study of the blood changes being made solely from dried and stained film preparations. It was not originally intended to make a systematic examination of these latter cases, the preparations used for this purpose being obtained during the course of my work on the agglutinative property of the blood in plague. The serum used for the latter purpose was separated directly from blood obtained by puncturing the finger and several blood films were taken at the same time. These were carefully preserved and eventually used to amplify and confirm the qualitative changes of the blood observed at different stages of the disease in the first group of cases.

TECHNIQUE. The enumeration of the white cells was performed with the Thoma-Zeiss apparatus, the blood being diluted with .75% acetic acid tinted with methyl green. All the white cells lying within the entire ruled area of the slide were counted, and the figures submitted were , as a rule, obtained from the average of three such estimations. If, however, any considerable degree of difference occurred in the numbers obtained on consecutive days, one or more independent observations were made, the pipette being filled from a fresh puncture for this purpose.

For the investigation of the qualitative changes in the blood, dried film preparations were employed. Heat was uniformly adopted as the fixing agent, the films being exposed to a temperature of 115 degrees C. for about half an hour. Cell differentiation was obtained by the use of Ehrlich's triacid mixture, which after trial of various other methods, was selected as yielding more uniformly reliable results than those obtained by any other method.

The normal number of leucocytes per cubic millimetre of normal blood is assumed to be about 7000. From the some-

what fluctuating numbers per cent. of the different varieties given by various haematologists, the following figures have been selected as expressing a close approximation to fact viz:-

Polymorpho-nuclear leucocytes	72 %	 5040
Small mono-nuclear leucocytes (lymphocytes)	20 %	 1400
Large mono-nuclear leucocytes	5%	 385
Eosinophile leucocytes	2%	 175
Total	100 %	Total 7000

The cells included in the first second and fourth of the classes mentioned above, conform with Ehrlich's classical descriptions of these respective types. Those included in the third category, however, comprise the following:-

(a) Large hyaline cells with excentrically placed oval or slightly indented nucleus and absolutely homogenous protoplasm.

(b) Cells possessing essentially the same characters but differing from the lymphocytes in possessing slightly greater magnitude and more faintly stained oval nucleus.

(c) Cells with absolutely granule-less protoplasm and a markedly indented, usually horse-shoe-shaped nucleus. Cells of this class may be regarded as "intermediate or transitional forms" between the large hyaline and polymorpho-nuclear forms.

GENERAL LEUCOCYTOSIS.

As far as can be judged from the comparatively small number of cases examined, it would appear that in practically all cases of plague except, perhaps, in the very mildest form of this malady, there is a more or less marked leucocytosis which is apparent from the earliest days of the disease. Not only so, but the degree of leucocytosis was found to vary directly with the degree of severity of the case. Thus:-

FATAL CASES.

The cases which proved fatal within the first week of illness were characterised throughout by a very marked degree of leucocytosis, ranging in those examined from 17,000 to 60,000. When death, however, did not occur till a later period, a fall in the amount of the leucocytosis was observed. Thus in the case of Mary G.(case No 3.) who died on the nineth day of illness, the number of leucocytes per cubic millimetre of blood, abruptly fell from 28,000 on the 5th day to 8,500 and 8,400 on the 6th and 7th days respectively. Similarly in the case of James B.(case No.5.)

TABLE 8.- FATAL CASES.

Case Number.	NAME	AGE	DATE	Day of Illness.	Number of Leucocytes per c.mm.	Percentage Number of:- Large Mono-nuclear cells.	Lymphocytes.	Polynuclear Neutrophiles.	Eosinophiles.	Absolute numbers per c.mm. Number of cells counted.	Large mono-nuclear cells.	Lymphocytes	Polynuclear Neutrophiles.	Eosinophiles.
1	Mrs. M.	20	15th Sept.	2nd.	60,500	3.6%	7.3%	88.8%	0.21%	1381	2180	4432	53753	131
				3rd.	-	4.2%	8.2%	87.4%	0.11%	1843	-	-	-	-
3	Mary G.	6	19th.Sept.	2nd	22,400	4.4%	4.4%	91.1%	0.03%	1168	99?	997	20386	19
			20th. ,,	3rd.	13,500	4.1%	8.6%	87.1%	0.01%	1212	556	1169	11751	22
			21st. ,,	4th.	24,000	7.7%	6.8%	85.3%	0.03%	1175	1858	1634	20486	20
			22nd. ,,	5th.	28,800	4.0%	6.4%	89.4%	-	1062	1166	1871	25762	0
			23rd. ,,	6th.	8,500	7.4%	12.0%	80.0%	0.05%	750	453	1201	6800	45
			24th. ,,	7th.	8,400	4.7%	11.2%	84.0%	-	1071	400	941	7058	0
4	Robt.M.	12	19th.Sept.	6th.	-	5.3%	7.9%	86.0%	-	822	-	-	-	-
5	JamesB.	60	29th.Aug.	15th.	6,700	0.9%	5.7%	93.4%	-	332	60	383	6256	0
			31st. ,,	17th.	4,100	0.7%	2.0%	97.5%	-	323	25	75	3998	0
6	Will.W.	48	2nd.Oct.	35th.	-	1.9%	10.9%	87.1%	0.03%	1159	-	-	-	-
X	Pat. M.	20	27th.Aug.	6th.	17,000	2.9%	9.4%	87.4%	0.1 %	686	495	1756	14807	24

who came under observation during the third week of illness, a leucocyte count of 6,700 was obtained on the 15th day and 4,100 on the 17th day.

SEVERE AND MILD CASES.

It is proposed to consider these classes together as investigations have shown that the changes which take place in the blood in the two clinical types are differences mainly of degree. Unfortunately, however, the majority of the cases which fall into this category were admitted to hospital at a comparatively late stage of the illness, and observations during the early days of the disease were therefore not available. Leucocyte counts were performed in only 7 cases, and the continuity of the observations was, unfortunately, in several instances unavoidably interrupted at a critical period. It is therefore somewhat difficult to form an accurate idea of the entire course of the leucocyte curve on account of the fragmentary nature of the observations, and the impossibility of obtaining such at an early stage in all cases. One feels justified, however,

SEVERE CASES.

TABLE 9.

Case Number.	NAME	AGE	DATE	Day of Illness.	Number of Leucocytes per c.mm.	Percentage Number of:- Large Mono-Nuclear cells.	Lymphocytes.	Polynuclear Neutrophiles.	Eosinophiles.	Absolute numbers per c.mm. Number of cells counted.	Large Mono--nuclear cells.	Lymphocytes.	Polynuclear Neutrophiles.	Eosinophiles.
12	Mrs. M.	41	17th.Sept.	5th.	12200	4.5%	18.2%	76.7%	0.43%	684	552	2229	9364	53
			18th. ,,	6th.	12000	9.2%	12.5%	78.1%	0.09%	1056	1115	1500	9375	11
			19th. ,,	7th.	,, 6700	9.9%	17.7%	72.2%	1.09%	1008	664	1189	4772	111
			20th. ,,	8th.	8500	6.5%	26.3%	65.7%	1.3%	725	559	2236	5592	73
			25th. ,,	13th.	10200	4.0%	32.4%	51.3%	2.1 %	1255	414	3307	6248	218
			2nd.Oct.	20th.	8700	3.2%	43.9%	51.3%	2.4 %	682	280	3737	4464	216
10	Thos.H.	15	29th.Aug.	7th.	11600	2.3%	5. 0%	92.6%	-	1086	267	587	10708	0
			30th. ,,	8th.	23500	2.6%	9.5%	87.8%	-	683	619	2236	20644	0
			31st. ,,	9th.	8000	6 %	10.5%	82.1%	0.13%	749	245	1185	6558	10
			29th.Sept.	38th.	-	4.1%	32.9%	61.8%	o.7 %					
			16th.Oct.	55th.	-	5.1%	28.8%	61.4%	4.5 %					
7	Pat. F.	56	30th.Aug.	16th.	11500	-	-	-	-					
			2nd.Oct.	50th.	-	9.6%	15.5%	74 %	0.74%					
8	Mrs. M.	40	9th.Oct.	56th.	-	10.3%	44.5%	43.8%	1.1 %					
			27th.Oct.	69th.	-	6.6%	43.8%	43.8%	6.1 %					
9	Mrs. T.	40	9th.Oct.	56th.	-	4.0%	32 %	62.6%	1.2 %					
			27th.Oct.	74th.	-	5.4%	42.5%	50 %	2.08%					
11	Chas M.	27	5th.Oct.	28th.	-	5.2%	36.7%	52 %	6.09%					
					-	3 %	26.2%	64.3%	6.5 %					

MILD CASES.

TABLE 10.

	NAME	AGE	DATE	Day of Illness.	Number of Leucocytes per c.mm.	Percentage Number of:- Large Mono-nuclear cells.	Lymphocytes.	Polynuclear Neutrophiles.	Eosinophiles.	Absolute numbers per c.mm. Number of cells counted.	Large Mono-nuclear cells.	Lymphocytes.	Polynuclear Neutrophiles.	Eosinophiles.
13	Mary M.	14	16th.Sept.	3rd.	14000	1.9%	31.1%	66.3%	0.05%	1210	277	4362	9290	69
			17th. ,,	4th.	8800	3.2%	34.2%	61.5%	0.7 %	729	289	3017	5432	60
			18th. ,,	5th.	10500	4.6%	31.8%	62.4%	1.05%	663	490	3342	6556	110
			19th. ,,	6th.	12600	2.8%	[illegible].6%	61 %	3.5%	700	360	4032	7758	450
			22nd. ,,	9th.	11600	2.1%	33.2%	59.3%	5.3%	472	245	3858	6881	614
			25th. ,,	12th.	17900	2.2%	27.9%	64.9%	4.9%	308	406	4998	11623	871
			5th.Oct.	22nd.	16000	2.9%	26.9%	62.9%	7 %	635	478	4308	10078	1133
			10th. ,,	27th.	-	2.2%	22.5%	71.1%	4.05%	513	-	-	-	-
			25th. ,,	42nd.	11900	1.9%	23.7%	68.3%	3 %	505	235	3063	8247	353
15	Mrs. G.	28	22nd.Sept.	5th.	7700	4.6%	20.7%	74.5%	-	505	249	1490	5961	0
			25th. ,,	8th.	8300	5.9%	20.3%	70.9%	2.8%	732	471	1707	5887	235
			29th. ,,	10th.	-	7.3%	19.9%	69.8%	2.8 %	859	-	-	-	-
			30th. ,,	13th.	10700	6.2%	21 %	70.9%	3.09%	775	676	2098	7593	331
			16th.Oct.	29th.	-	3.8%	38.5%	50.2%	7.3[illegible]%	597	-	-	-	-
17	Dan. T.	6	29th. Aug.	9th	21000	4.3%	8.5%	85.3%	0.85%	240	909	1727	18225	146
			29th.Sept.	40th.	-	2.3%	48.1%	42.7%	4.7 %	469	-	-	-	-
14	Mrs. B.	28	17th.Sept.	5th.	6500	3 %	16.5%	71 %	-	211	523	1355	4620	0
			2nd. Oct.	20th.	-	4.1%	28.5%	62.7%	4.6%	-	-	-	-	-
16	Jas. C.	24	16th. Oct.	60th.	-	4.3%	28.5%	65.2%	2 %					
18	Rose M.	28	29th.Sept.	35th.	-	4.1%	50 %	44.9%	1.1 %					
			16th. Oct.	52nd.	-	3.7%	27.4%	65.5%	3.0 %					

in concluding that at a very early stage in the disease, there is a more or less marked increase in the number of the leucocytes, depending most probably on the severity of the illness and the powers of resistance of the individual. The increase in the cases observed has been only moderate, and lasting only for the first three or four days of the illness when an abrupt fall to an approximately normal figure occurred about the fourth or fifth day. It is to be regretted that the necessary data for estimating the probable duration of these normal counts are not available, but there seems to be little doubt that they are followed, at a variable but probably short interval, by a secondary leucocytosis which may reach as high a degree as , or even exceed the primary. The observations were not continued for a sufficient length of time to note the decline of the secondary leucocytosis and its return to normal limits, but approximately normal numbers are probably reached during the course of the third or fourth week.

As in some measure tending to confirm the above statement, it is interesting to cite here the results found by the Austrian Plague Commission[1] in the only case in which a complete series of approximately consecutive observations were made. From this it will be seen that there is a gradual fall towards normal on the fifth or sixth day, followed by a subsequent rise.

Name	Day of illness.	No. of Leucocytes. per mm.
C. Desouza.	1st.	19,400
	2nd.	22,600
	3rd.	17,900
	5th.	11,300
	6th.	8 ,900
	8th.	9,400
	9th.	10,900

As illustrating the period of approximately normal counts, the following isolated observations of Poch in four cases are of value.[1]

B. Jankee	4th.	6,400
K. Joti.	3rd.	3,800
S. Baichu.	3rd.	6,200
C. Desouza.	6th.	8,900

1. Gesammtbericht uber die Beulenpest. page 203.

RESULTS OF DIFFERENTIAL ESTIMATIONS OF LEUCOCYTES.

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FATAL CASES.- The leucocytosis which characterises the majority of the acute infectious fevers is polymorphonuclear in character, and fatal cases of plague form no exception to this general rule. In these, the polymorphonuclear cells formed from 84 to 95 per cent. of the total. This abnormally high proportion of polynuclear cells was found to be a constant feature at all stages of the illness in those cases which, either immediately or at a more remote date, proved fatal. Thus in the case of James B. already referred to (case No.5) who died during the sixth week of illness and who gave a subnormal leucocyte count of 4,100 on the 17th day, the Polynuclear cells alone formed 95 per cent. of the total. Reference to table 8 shows that a similar feature was observed in all cases of this class and at all stages of the disease.

It must be added that many of these cells showed signs of

degeneration from a comparatively early period in the disease. These appearances comprised a loss of affinity of the nucleus towards the nuclear dye, as well as occasional vacuolation of protoplasm (about 10% in some cases) and tendency to cellular degeneration.

The increase of the Polynuclear cells takes place at the expense of all the other elements but more particularly at the expense of the lymphocytes. The percentage proportions of this latter class of cell is always much reduced in this type of case, (minimum 2%, maximum 12%) though occasionally their absolute numbers may be slightly in excess of the normal. The percentage proportion of the large mononuclear and transitional cells is usually considerably below the normal, but, as in the former case, the absolute number may be slightly in excess when associated with a very pronounced degree of leucocytosis.

The behaviour of the eosinophiles is especially noteworthy. Both relatively and absolutely they invariably show a considerable diminution, which is accentuated towards the

termination of the case, when they may have practically disappeared. Indeed, in those cases which proved fatal at a comparatively late period, many films were carefully scrutinised without discovering a single example of this variety.

MILD AND SEVERE CASES.

In this class of case, the variations met with in the relative proportions of the different forms of leucocytes are even more interesting than those already shown by the fatal cases. As in the first group of cases, the primary leucocytosis is essentially a polynuclear one, the exact degree of preponderance of this cell depending on the severity of the attack. Their absolute numbers are always considerably in excess of the normal, and in the very severe types of the disease they may form as much as 92 per cent. of the total number. The lymphocytes on the other hand are proportionately diminished, though with a high degree of leucocytosis their absolute numbers may be slightly in excess of the normal. The large hyaline and transitional forms are either approximately normal or perhaps slightly in excess. As in the former group of cases, however, the eosinophiles undergo a much more serious diminution than

any of the other varieties, and this is true even of the mildest forms of the disease. During the acute stage there is a marked fall, and in the severer forms it may be necessary to pass under review many thousand cells to determine their presence. In several instances the search for them proved futile and one is therefore forced to conclude that they are either destroyed at the acme of the fever or that they temporarily disappear from the circulation. It has already been shown that, towards the end of the first week of illness the number of leucocytes falls to approximately normal; but, while the total number is thus diminishing, a marked change in the relative proportions of the various classes is taking place. The percentage of the polynuclear cells is falling whilst that of the other varieties is rising. The striking inequality which was characteristic of the initial leucocytosis is being neutralised, and the various forms are now present in proportions which approximate to those of normal blood. The eosinophiles, however, whilst having risen consider-

-ably, are yet much below their normal proportion. Somewhat later the secondary leucocytosis sets in, and this offers some striking differences when compared with the primary. It has already been shown that the primary leucocytosis is chiefly characterised by the marked preponderance of the polynuclear cells. These, however, not only play no important part in the secondary leucocytosis, but are steadily diminishing. The chief factors in this leucocytosis are the lymphocytes, which it will be remembered, became seriously diminished during the period of primary leucocytosis. This class therefore undergoes an opposite movement in the two periods of leucocytosis, and the same holds good of the other classes of cells, with the exception of the large hyaline and transitional forms which remain practically stationary. During convalescence, this gradual rise in the lymphocytes and eosinophiles is maintained, till at the fifth or sixth week they attain twice their normal proportions. About the same period the polynuclear cells have reached their minimum,

and in some cases their absolute number has been actually below that of the lymphocytes. A reference to the accompanying chart will make these facts clear, as the changes just described in the various types of the disease and at different stages of the illness, are graphically displayed.

CONCLUSIONS. The following conclusions seem to be justified from the observations herein recorded:-

(1) In practically all cases of plague there is a leucocytosis during the first week of illness which is proportional to the severity of the case.

(2) In all cases which survive the first week of illness there is a return to an approximately normal condition about the fourth or fifth day of the disease, and this lasts for about 24 to 48 hours.

(3) About the end of the first week a secondary leucocytosis occurs which persists for a considerable time.

(4) The primary leucocytosis is entirely due to an increase of the polymorpho-nuclear cells, the lymphocytes being greatly diminished and the eosinophiles practically disappearing in the fatal and severe cases.

(5) During the subsidence of the primary leucocytosis the proportion of lymphocytes to eosinophiles is rising whilst that of the polynuclear cells is steadily falling.

(6) The secondary leucocytosis is chiefly due to the rise of the lymphocytes, the polynuclear cells still falling.

(7) These changes continue throughout convalescence till about the 6th - 8th week when they reach their maximum. At this period the lymphocytes and eosinophiles may have attained twice their normal proportions.

(8) In fatal cases the percentage proportion of polynuclear cells is always high, and the eosinophiles practically absent. In cases which recover, however, the reverse condition is found when convalescence has set in.

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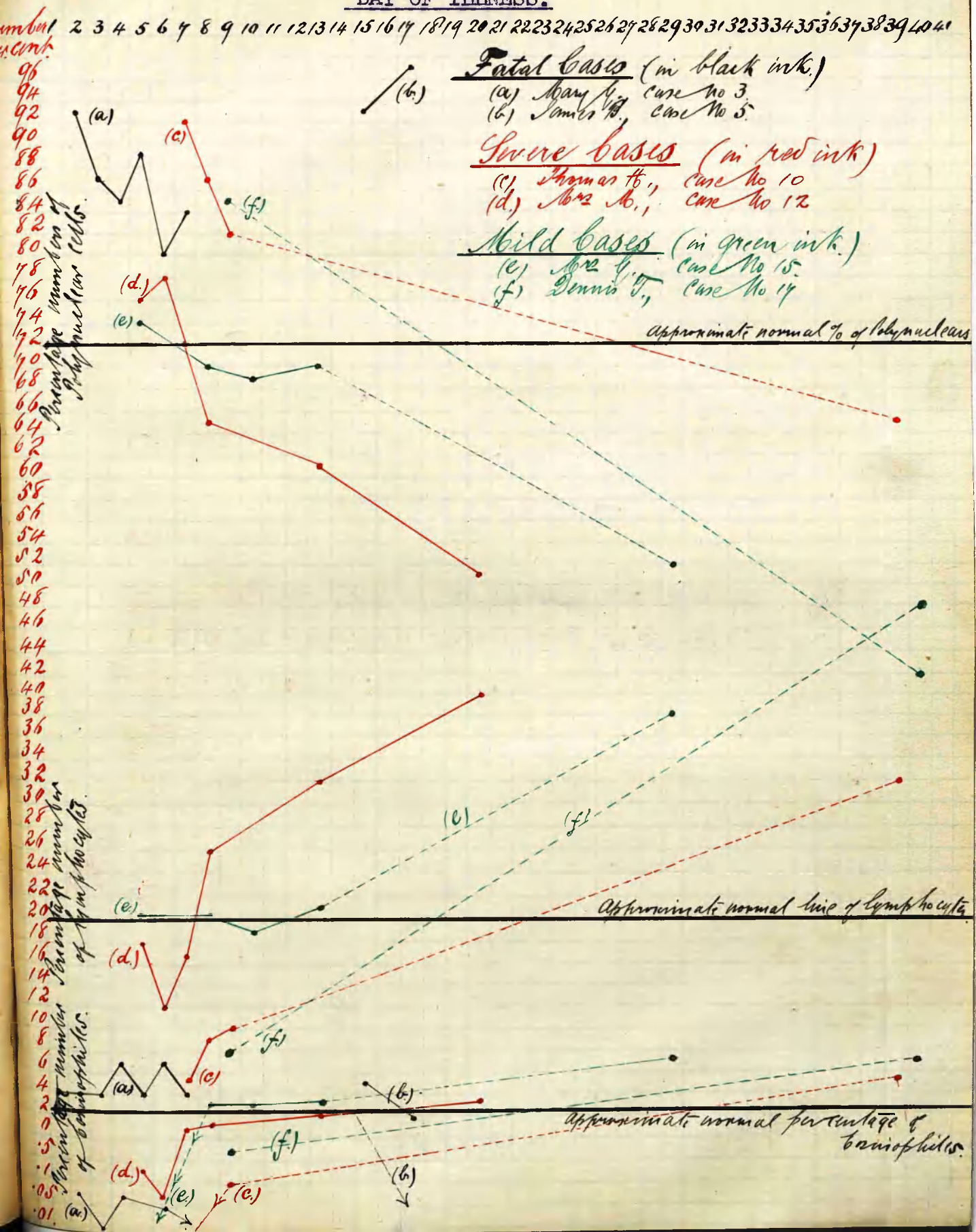
DAY OF ILLNESS.



S E C T I O N N O. 6.

OBSERVATIONS ON THE PROPHYLACTIC PROPERTY OF BLOOD SERUM FROM CONVALESCENT PLAGUE PATIENTS.

OBSERVATIONS ON THE PROPHYLACTIC PROPERTY OF BLOOD SERUM FROM CONVALESCENT PLAGUE PATIENTS.

Experiments by Yersin Calmette and Borrel[1] had shown that animals which had been inoculated with gradually increasing doses of plague bacilli finally became immunised, and that the serum of such animals could be used both prophylactically and therapeutically in the case of other animals inoculated with the same organism.

Arguing by analogy, it was not unreasonable to suppose that a patient who had passed successfully through an attack of plague had acquired immunity, (lasting for a variable period), against the disease; and that the serum of such individuals could be used either in the prophylaxis or cure of experimentally plague infected animals.

1. Yersin, Calmette et Borrel, Annalles de l'Institut Pasteur 1896, p.590.

With this object in view serum was taken at different periods of illness - both during the acute and convalescent stage - from various patients suffering from a severe attack of plague. The serum used for this purpose was obtained by aspirating into a sterilised pipette a quantity of blood obtained by puncturing the finger under aseptic precautions. The tube was then set aside for some time, and after coagulation had taken place, the clear serum was drawn off into a second tube and hermetically sealed.

As an example of the routine followed and the results obtained, we may cite the following experiment in which the serum of Charles M'M.(case No.11) taken during the sixth week of illness was employed. 0.2 cc of this serum was injected subcutaneously into a mouse, and 24 hours later the animal was inoculated with a lethal dose of a virulent culture of the bacillus pestis. A control animal, which, however, had received no serum was similarly inoculated at the same date and from the same culture. The following is the history of the mouse which received serum.

1st day. Mouse injected subcutaneously with 0.2cc of serum of C. M'M.(case No.11) taken in 5th wee week of illness. (Agglutinating potency 1 in 75)

2nd day. Inoculated with bouillon culture of B.Pestis.

3rd day. Animal obviously very ill and refusing food.

4th day. Somewhat better.

5th day. do.

6th day. Looks ill; not eating; "staring coat".

7th day. DIED.

On post mortem examination, the spleen of this animal was found to be slightly enlarged, but otherwise showed no evidence of any pronounced macroscopic change. Microscopical examination of the spleen pulp, however, demonstrated, but only after a prolonged search, the presence of a few free bacilli. In practically all the splenic cells, however, plague bacilli in all stages of digestion and degeneration were found, some of the cells being literally gorged with the disintegrating organisms. As a striking contrast to these appearances were the conditions found in the control animal which died in 18 hours of plague septicaemia.

In this instance the spleen was also slightly swollen, and on microscopical examination was found to be practically swarming with free plague bacilli, none being found englobed by the leucocytes. Whilst therefore this experiment as regards absolute prophylaxis must be looked upon as a failure, still the markedly different appearances found in the two animals seemed to show that curative changes had been in progress when death supervened.

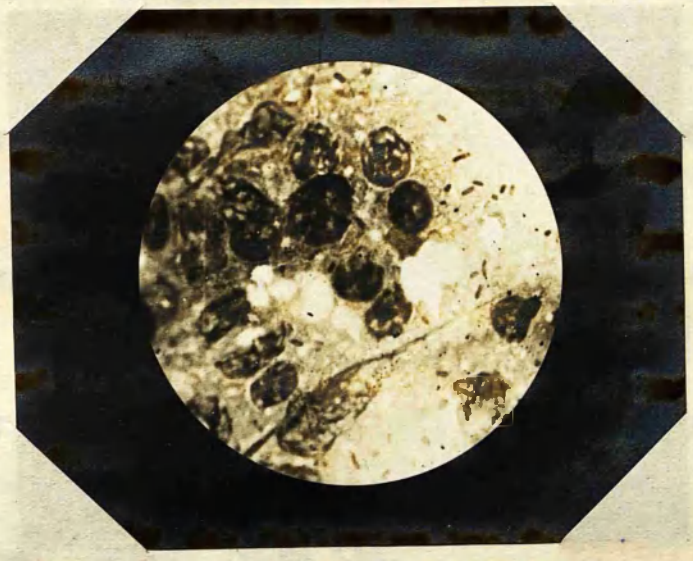
This is evidenced by the following two facts:-

(1) that the bacilli in the animal which had received serum were much more scanty in blood and spleen.

(2) that the bacilli were almost entirely englobed by the splenic cells, very few being found free.

It is greatly to be regretted that, on account of the difficulty in staining degenerated organisms, it has been found impossible to obtain a satisfactory photograph of these bacilli in process of digestion by the splenic cells, the accompanying print giving a very imperfect idea of the usual microscopical appearances.

Fig.6. Plague bacilli englobed by splenic cells. x1000.



It having been apparently demonstrated by this experiment that the serum of a plague patient does possess a certain prophylactic power, it became a point of interest to determine whether there was any relationship between such prophylactic power and the agglutinative potency of the serum. With this object in view the following series of experiments were undertaken. Serum was taken from plague patients at different periods of the disease, and the agglutinative power in each instance carefully noted and estimated. Varying doses of this serum (0.15cc to 0.6cc) were injected

into mice, and 24 hours later the animals received a lethal dose of virulent plague bacilli. The following series of experiments with the serum of Thomas H.(case No.10.) will serve as an illustration of what usually took place.

Mouse A.

1st day. subcutaneous injection of 0.2cc of serum of 8th week of illness. (Potency 1 to 75)

2nd day. Inoculation with bacillus pestis.

3rd to 18th day. Animal apparently well.

19th day. Animal looks ill.

20th day. Animal very ill and refusing food.

21st day. DIED.

Mouse B.

1st day. Subcutaneous injection of 0.15cc of serum of 11th week of illness. (Agglutinating potency 1 in 50.)

2nd day. Inoculation with culture of bacillus pestis.

3rd to 15th day. Animal apparently well.

16th day. DIED without ever exhibiting any symptom of illness.

Mouse C.

1st day. Subcutaneous injection of 0.15cc of serum of 18th week of illness. Potency 1 in 25.

2nd day. Inoculation with lethal dose of bacillus pestis.

This animal never became acutely ill, and three weeks after inoculation gave birth to a healthy family of four individuals.

Mouse D. (control)

This animal was inoculated from the same culture but received no serum and died within 24 hours of plague septicaemia.

In the case of the first two animals (A and B) which ultimately died, the post mortem appearances are of considerable interest and importance. They were alike in both and exhibited the bacilli, for the most part englobed by the leucocytes, and showing marked degenerative changes. Such appearances must be regarded as evidences of partial cure, as not only were they not found in any animal which had not previously received serum, but they were in every respect similar to

those found in buboes undergoing spontaneous resolution. (cure)

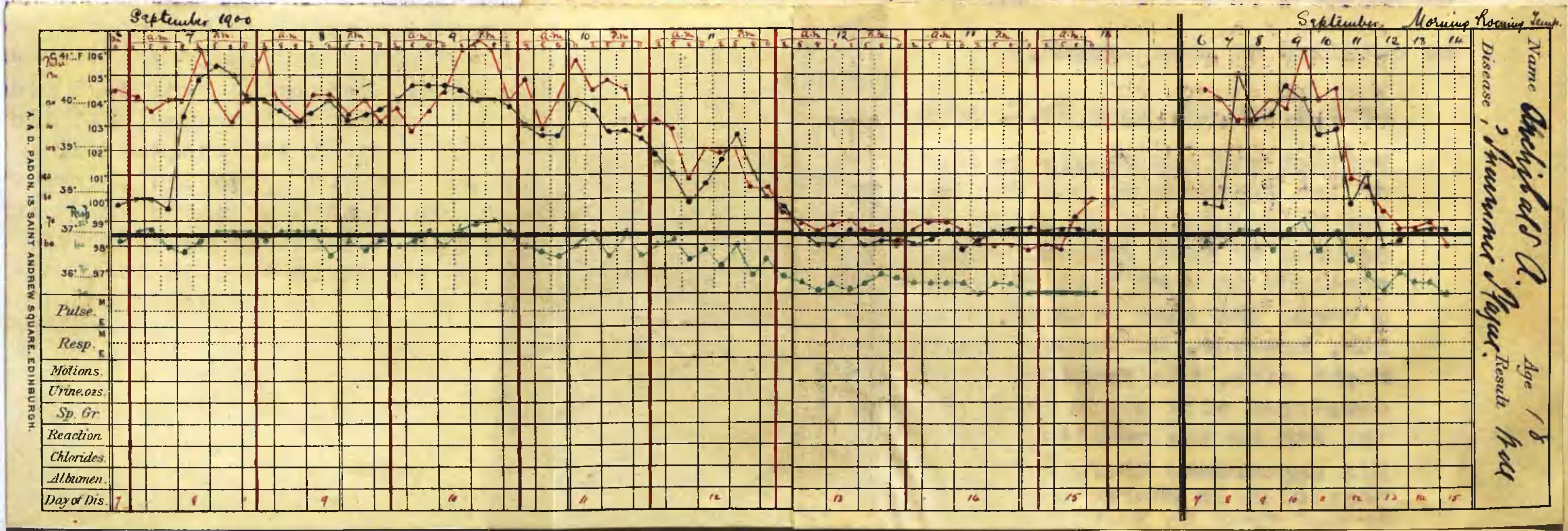
This series of experiments seems to indicate that, the serum of a convalescent plague patient possesses increasing prophylactic powers according to the time which has elapsed since the acute illness. Thus 0.2cc of serum at the 8th week kept the animal alive for 21 days; a smaller dose 0.15cc protected another for 16 days. Whilst the sera taken at the 8th and 11th weeks may be regarded as practically equivalent in prophylactic value, the serum at a much later date - 18 weeks - and in the smaller dose, afforded the animal complete immunity. It is apparent therefore that in advanced convalescence the protective value of the serum reaches its maximum. In the section on agglutination attention was drawn to the fact that the agglutinative power of serum reached its maximum about the sixth or eighth week and thereafter rapidly declined. On the other hand, the experiments just detailed have shown that the prophylactic powers of the serum slowly rise during convalescence and only long after it has been completed do they reach their

maximum. There seems to be therefore no constant relationship between these two properties of the serum.

The occurrence, during advanced convalescence, of undoubted prophylactic qualities in the serum of plagueb patients , is a fact of considerable value, as it affords an additional means of determining the nature of suspected cases of plague which may have been missed in their earlier stages. Thus if the serum of a convalescent case of suspected plague be taken and utilised as in the foregoing experiment, the true na nature of the illness may be elucidated by its prophylactic action being demonstrated.

As an instance of this the following interesting case may be detailed at some length.

> Arch.A.aet.18, case No.X. admitted Sept.6th 1900.
>
> Patient was apparently in full health on the morning of 1st Septtember, but during the course of the evening he complained of slight headache. From this point onwards he felt somewhat out of sorts, bub was able to continue at work till the 4th and was even present at a dance the same evening. Early on the morning of the 5th, however, he became acutely ill with pain i n the right side. The symptoms of illness appear to have developed with great rapidity, and on the evening of the 6th he was admitted to the Western Infirmary. His appearance there being regarded with suspicion, the physician in charge deemed it advisable to have him transferred to Belvidere the same evening. On ad-


September 1900
Pulse
Resp.
Motions
Urine ozs.
Sp. Gr.
Reaction
Chlorides
Albumen
Day of Dis.
A. & D. PADON, 13 SAINT ANDREW SQUARE, EDINBURGH.
September. Morning Temp.
Name
Age 18
Disease
Result Well

mission he was acutely ill. The temperature was 104F. the pulse 124 and the respirations 42. There was no evidence of respiratory distress. The face was of an ashy-grey colour, with a dusky flush in the malar regions. The conjunctivae were infected and the pupils dilated. The skin showed, especially on the trunk a general erythematous mottling, resembling that already noted in plague. This was also present on the extensor surfaces of the forearms. Examination of the lymphatic glandular system revealed slight enlargement of the glands in the right axilla, and also of those in the left groin. Physical examination of the lungs revealed slight relative dulness to percussion over the left back from the spine of the scapula downwards. The breath sounds over this area were distinctly tubular, and the vocal resonance and vocal fremitus were both exaggerated. Next day patients condition was much the same, the evening temperature being 105F. He was drowsy, however, and had some slight muttering delirium. The expectoration, which was very scanty and viscous, was slightly tinged with blood. On direct microscopical examination of the sputum, large numbers of diplococci were seen, and there was also present in very considerable numbers a bi-polar staining bacillus, which was decolourised by Gram's method, and morphologically was indistinguishable from that of plague. In many parts of the slide this bacillus appeared to be present in almost pure culture. The temperature reached normal on 12th Sept., and terminated, not by a true crisis, but by a somewhat rapid lysis extending over 48 hours. This lysis resembled the lysis of plague rather than that of pneumonia, and was unaccompanied by sweating, the skin remaining dry to the touch. Convalescence was rapid and uninterrupted.

Unfortunately the sputum from this case was not inoculated directly into an animal, and on account of an accident to the regulator of the incubator, the only culture of the above-mentioned plague-resembling bacillus was destroyed at a time when it was no longer possible to obtain another. From this point of view, therefore, the definite diagnosis of the case remained to a certain extent doubtful. During convalescence, however, a very pronounced agglutinative reaction was obtained in a dilution of 1 to 25.

In order to confirm the diagnosis of plague the prophylactic properties of the serum were investigated and the following experiments performed.

Two white mice, X and Y, of approximately equal weight were taken, and each inoculated with an lethal dose of an active and virulent culture of the bacillus pestis.

In the case of Y, however, ½cc. of blood serum, obtained from the patient during the nineth week of illness was injected into the subcutaneous tissues of the back 24 hours before performing the inoculation. The following results were observed:- Control animal X became obviously very ill

12 hours after inoculation, and died at the end of 24 hours. Post mortem examination showed the spleen to be slightly enlarged and gorged with free bacilli(pestis) none of which were enclosed by the splenic cells. The organisms were also present in the blood in very large numbers. Animal Y never appeared to be particularly ill at any time, but died suddenly on the 12th day after injection. Post mortem examination showed the spleen to be slightly enlarged, and on examining smear preparations of splenic juice only a few free bacilli could be found after a prolonged search. The splenic cells, however, contained plague bacilli in all stages of digestion.

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S E C T I O N N O. 7.

CLINICAL HISTORIES AND POST MORTEM EXAMINATIONS.

CLINICAL HISTORIES AND POST MORTEM EXAMINATIONS.

It is not proposed here to deal clinically with the symptomatology of Plague, but only to give brief clinical histories of the cases, accompanied by the result of post mortem examinations. These are subjoined, purely with the object of furnishing a handy reference to each case as it calls for remark under the various sections.

Case No. 1.- Mrs M. aet.28, admitted Sept.15th 1900. Duration of illness doubtful but certainly under 48 hours. Onset severe and sudden, with headache, acute pain in back, diarrhoea, and great prostration. Face pallid, tongue dry, and lips covered with sordes. Pulse soft and rapid; respirations hurried and laboured. Patient 8½ months pregnant and labour in progress on admission. In left inguinal region, a large indurated and tender bubo, from which, as also from the blood, a pure culture of the bacillus pestis was obtained on evening of admission. On this date 20 cc of Yersin's Serum were injected subcutaneously and a like amount into area below left groin. During the evening patient's condition became extremely grave, and as the os was fully dilated about 4 a.m., instrumental delivery was at once effected. Child born asphyxiated and recussitated with difficulty. No gross lesion of placenta. On 16th Sept., 20 additional cc. of serum were injected, but patient's condition gradually became worse, and death occurred the same evening.

C ase No. 1. Mrs. M. (cont.)

POST MORTEM Examination by Dr.R.M.Buchanan.
SUMMARY. Right inguinal bubo,involving vertical set of glands and retroperitoneal gland just above Poupart's Ligament,with periglandular oedema. Glands intensely hyperaemic and finely mottled. Similar changes observed in lymphatic glands generally,including prevertebral glands and those of mesentery. Heart firm in consistence,with subpericardial ecchymoses at apex of left ventricle,and some cloudy swelling of myocardium. Hypostatic congestion of lungs. Liver,spleen,and kidneys somewhat hyperaemic. Uterus characteristic of recent delivery.

BACTERIOLOGICAL EXAMINATION.
Bacillus Pestis in bubo, blood, spleen, lungs, liver,and retroperitoneal glands.
Pneumococcus in lungs and retroperitoneal gland.

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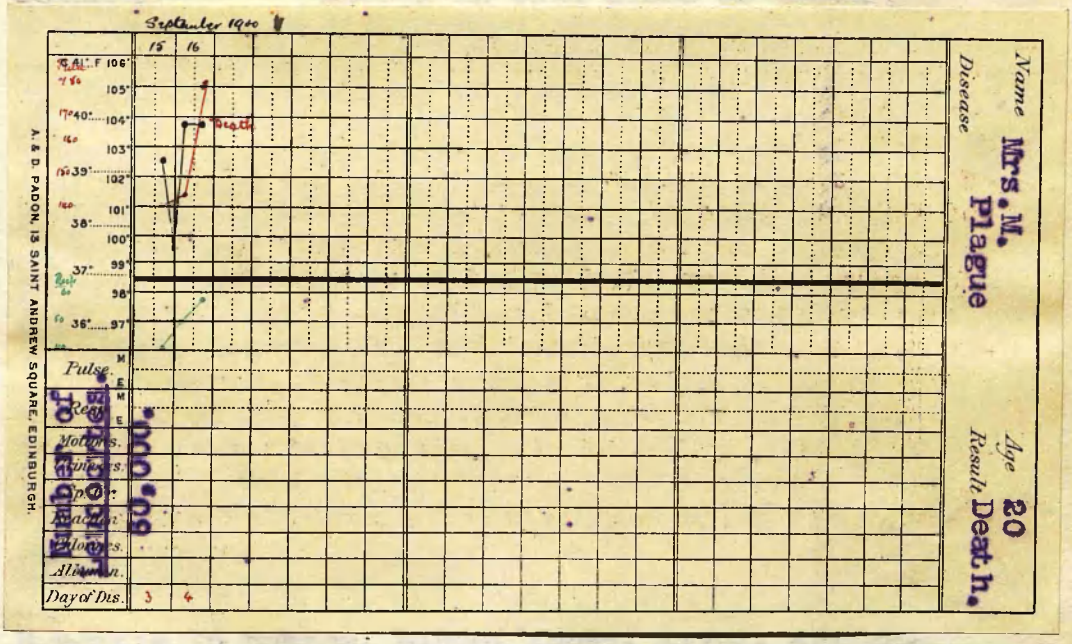

Case No. 2.- Baby M. born in hospital, 16th Sept.

Although the mother had a blood infection of B.pestis (v.s.) the placenta showed no macroscopic lesion. For the first eight days after birth the child seemed perfectly well, but on the ninth day the neck was observed to be rigid, and on examination some induration was discovered on left side of neck, high up, at the posterior border of the sterno-mastoid. Next day a few isolated glands could be felt, about the size of a pea, in the above mentioned situation, and by this time the group of glands lying in the anterior triangle of the neck were also affected. Glands on right side of neck also slightly enlarged. Temperature previously normal now rose to 101 F. Next day child vomited frequently and refused its food. It rapidly sank and died the following afternoon.

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POST MORTEM EXAM. by Dr. R.M.Buchanan.

SUMMARY.- Buboes on both sides of neck with general enlargement of axillary, bronchial, mesenteric, inguinal, and prevertebral glands. Haemorrhage and oedema of subcutaneous tissues of neck. Parenchymatous degeneration of heart, liver, and kidneys. Numerous small haemorrhagic condensations in lungs, many of which show a yellowish necrotic centre. Fibrinous exudation on surface of both lungs. Opaque yellow fluid in pleural cavities. Spleen much enlarged and of firm, liver-like consistence. Liver enlarged and intensely hyperaemic, with marked cloudy swelling at parts. A small number of minute yellow necrotic points throughout hepatic tissue. Kidneys very hyperaemic, and revealing on section minute haemorrhagic foci, each with a yellowish centre disposed chiefly in the pyramids.

BACTERIOLOGICAL EXAMINATION.

B.pestis in buboes, blood, lungs, liver, spleen, kidneys, and suprarenals.

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Case No.3.- Mary G.aet.6, admitted Sept.19th 1900.

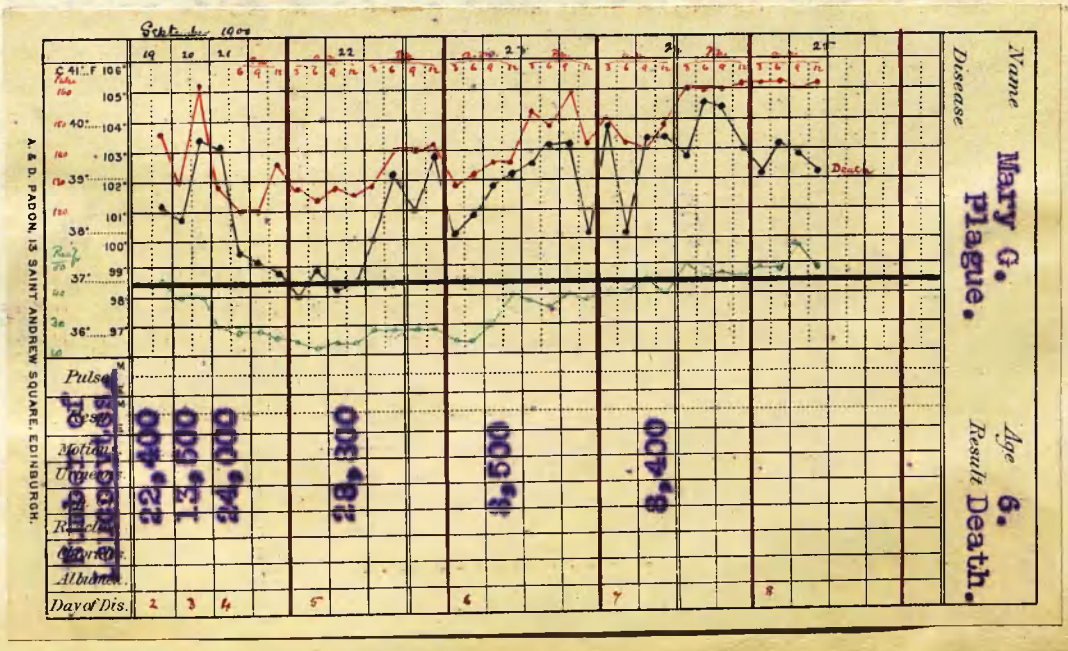
This patient who is the daughter of Mrs G.and niece of Charles M'M.(cases No.15 and No.11)became ill on day prior to admission with severe prostration and headache. On admission she was semi-comatose and unable to recognise her mother who lay in the next bed. Temp.101.2F., pulse 146; respirations 44. Face pale and cyanotic;lips dry and livid. In right inguinal region a large and excessively tender bubo about the size of a hen's egg with much perigland-ular infiltration. 100 cc of serum in divided doses were injected subcutaneously between the evening of admission and the 21st inst. Considerable im-provement observed on the latter date and child now conscious for first time. Improvement, however, not maintained, and on 22nd the temperature again rose, the pulse became feeble,and from this point on-wards she sank steadily. Death was associated with marked hypostatic congestion of the lungs and was directly due to heart failure.

Post Mortem examination refused.

Bacteriological Examination.

Bacillus pestis in bubo.

Blood sterile (seven consecutive daily examinations)



Case No.4.-Robert M.aet.12,admitted 16th Sept.1900.

Patient became ill on evening of 14th Sept. with slight headache. Next morning he was considerably prostrated and complained of acute pain in left axilla.No history of rigor, sickness, or vomiting. Patient evidently acutely ill on admission,with characteristic plague"facies",and slight mental obscuration. Lips dry and fissured; tongue dry and brown. The slightest movement caused most acute pain referred to the region of the left axilla,and palpation in this situation discovered a small but [illegible] tender bubo. Physical examination of the lungs revealed several localised patches of crepitant rale. Heart's action very feeble and apex impulse widely diffused. Pulse rapid,full and bounding,but of poor tension. Spleen not enlarged. Urine contained no albumen.
On admission 20 cc serum were injected subcutaneously and a like amount intravenously. A slight temporary improvement which occurred on the 17th was not maintained,and on the 18th and 19th,20cc were again given. At this date condition very grave;respirations more frequent,and physical signs in lungs advancing. There was,however, no cough,and no sputum could be obtained. Subsequent course of temperatures approximated to the septicaemic type.An indefinite swelling which had been forming for some days at the outer part of the left pectoral region,and which was now frankly fluctuant,was freely incised on the 25th inst.,and a large quantity of sero-purulent fluid evacuated. Cultivation yielded a pure culture of Staphylococcus Pyogenes Aureus,but no plague bacilli could be isolated. The abscess was found to be situated below the pectoralis minor. From this time patient's condition became rapidly worse,and he died on Sept.28th. Throughout,the urine remained free from albumen except on the 22nd and 23rd September, when the specimens obtained showed a faint trace.

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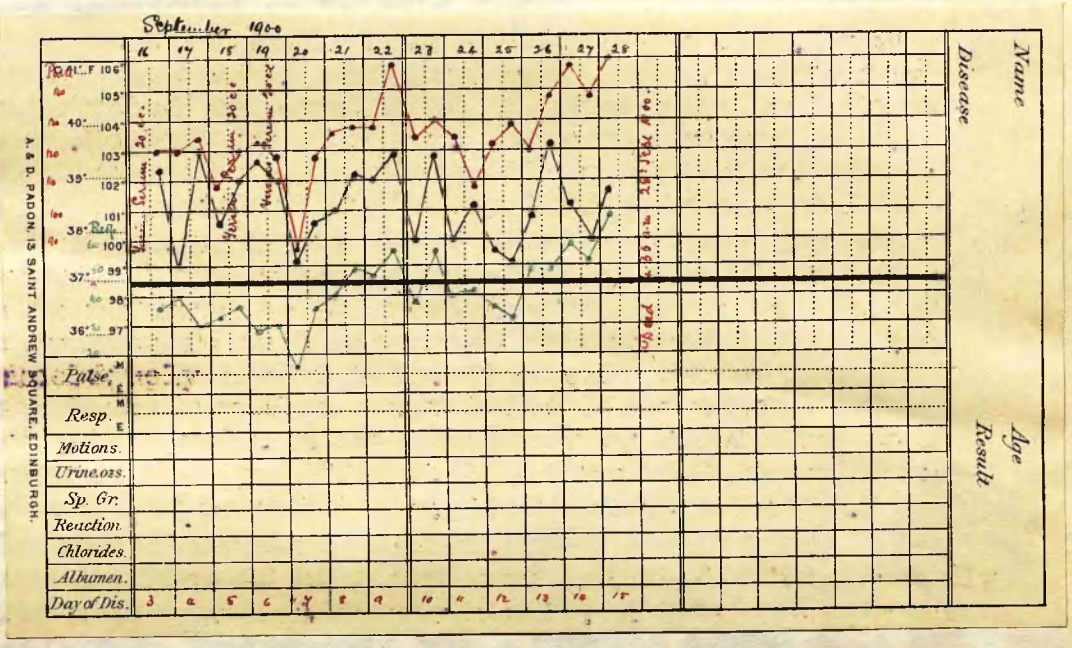
Case No.4.- Robert M.-(cont^d)



POST MORTEM EXAMINATION by Prof. Pertik, Buda-Pesth.
SUMMARY:- Purulent axillary and subclavicular buboes. A mass of purulent tissue, consisting of enlarged lymphatic glands and softened connective tissue, extending from axilla under pect. minor, the glands being partly softened and yellowish grey, partly firm and granular, with dull areas of coagulation necrosis. Abscess in thymus gland, and purulent infiltration of anterior mediastinum. Polyadenitis. Lobar and lobular pneumonia, with numerous well defined reddish grey necrotic areas about the size of a hazel nut, in both lungs; fibrinous exudation on both pleural surfacec. Myocardium pale, friable, and opaque from cloudy swelling. Spleen enlarged, cut surface smooth; pulp firm and malpighian bodies dark brown. Kidneys of medium size; cortex friable and opaque. Liver of medium size pale yellowish brown and friable from cloudy swelling. Punctiform haemorrhages in stomach.

BACTERIOLOGICAL EXAMINATION-
B. Pestis in bubo, blood, spleen, lungs, and mediastinal abscess. Staph. Pyog. Au. in bubo, blood, spleen, lungs, and mediastinal abscess. B. Coli Com. do.

Case No.5.- James B.aet.60,admitted 28th Aug 1900. and certified as Enteric fever.
The onset of illness,which dates from 15th Aug.was sudden and severe,and characterised by shivering, nausea,and vomiting,associated with severe headache and slight abdominal pain. From 25thAug.he suffered from diarrhoea,and for the 24 hours preceding admission he had been delirious. The characteristic pale and anxious "facies"waspresent on admission. Pupils dilated and conjunctivae injected. Tongue coated with a thick grey fur in centre;edges moist and clean. A finely mottled rash,resembling in many respects the macular rash of Typhus,was present on abdomen. In right groin a very tense red and oedematous swelling of considerable size occupying the situation of the vertical set of glands. This bubo was exquisitely tender and surrounded by densely infiltrated tissues. Lymphatic glands above Poupart's Ligament enlarged and tender,and skin over right iliac region oedematous. Examination of heart and lungs negative. Spleen not enlarged. Temperature ran a febrile course till 2nd Sept. when crisis occurred with a fall to 97.6F. This was associa iated with symptoms of general improvement. The redness and oedema surrounding the bubo continued, however, to spread,till practically the entire upper half of thigh became involved, whilst,at the same time,oedema without redness spread from right iliac region till nearly the whole abdominal wall pitted on pressure. The inflammatory process reached its height on SeptThe lst,and after that date slow but steady disappearance of redness and oedema round bubo. Oedema of abdominal also receded but there appeared coincident with this considerable oedema of left foot. On Sept.6th a second ary fever commenced, due to the suppurating bubo,and the temperature ranged continuously between 100and102F. The discharge from the bubo was copious and serous,and there was never any attempt at separation of slough. Patient gradually became more feeble,remained constantly delirious and died on September 24th.

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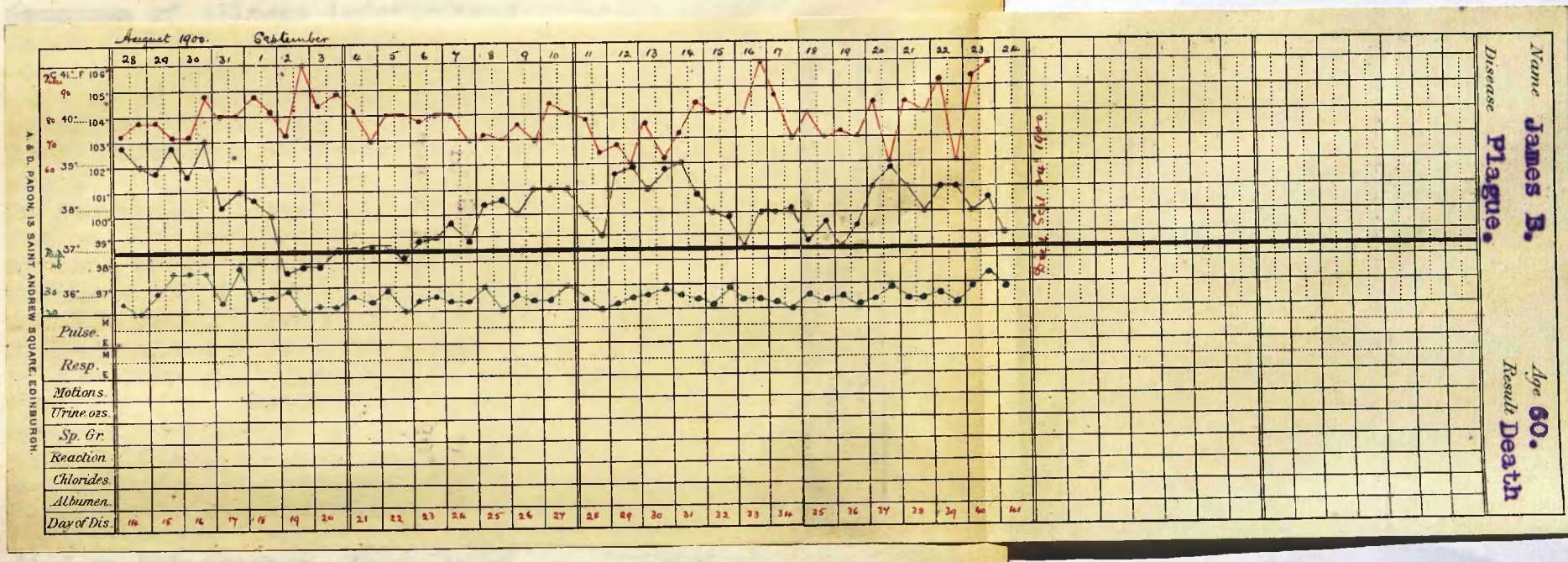
Case No.5.- James B. (cont^{d})



POST MORTEM EXAMINATION by Dr.R.M.Buchanan.
SUMMARY.- Right inguinal bubo,with indolent sloughing ulcer in skin. Under floor of ulcer a dense mass of yellowish necrotic substance,like inspissated pus,extend ing upwards in the course of the vessels under Poupart's ligament,and continuous with a chain of partitioned ab- scesses partly filling the right iliac fossa,the right side of pelvis,and impinging on the right wall of bladder. purulent infiltration of psoas muscle upwards to lumbar vertebrae. Purulent infiltration of the subcutaneous and deeper tissues half-way down the thigh. Glands in other regions appear normal. Lungs hyperaemic and oedematous. Heart muscle pale and cloudy; fibrinous thickening of aortic and mitral curtains. Liver hyperaemic. Spleen enlarged and of firm consistence. In both kidneys there are numerous small yellow points like tubercles visible i in the cortex.

BACTERIOLOGICAL EXAMINATION.
B.Pestis in bubo and spleen.
Staph.Pyog.Au. in bubo and Staph.Py.Alb. in spleen.
Small putrefactive bacillus in bubo.

Case No.6.- Will^{m} W. admitted 20th Sept 1900.

Symptoms of illness indefinite-commencing about four weeks prior to admission and coincident with the appearance of a large and tender bubo in left groin. This was incised in the first, and again in the third week of illness without any pus being found. On the fourth, a purulent discharge from the incision commenced, and patient was sent to the Western Infirmary and thence to Belvidere. On admission he was evidently very ill. His intelligence was clouded, and he was unable to give any satisfactory account of his illness. Face livid and congested, eyes suffused and wandering. Tongue dry and heavily coated. In left groin a large livid-red swelling, bisected by a long vertical incision, from which large quantities of ichorous fluid exuded. In this fluid, on direct microscopical examination, numerous degenerated bacillary forms were observed free in the fluid and also contained in the leucocytes. No definitely typical forms of bacillus pestis, however, were seen. Temperature ran a continuously hectic course throughout. Patient was delirious and frequently violent. He gradually sank and died on 6th October. It is to be noted, however, that the bacillus pestis was recovered post mortem.

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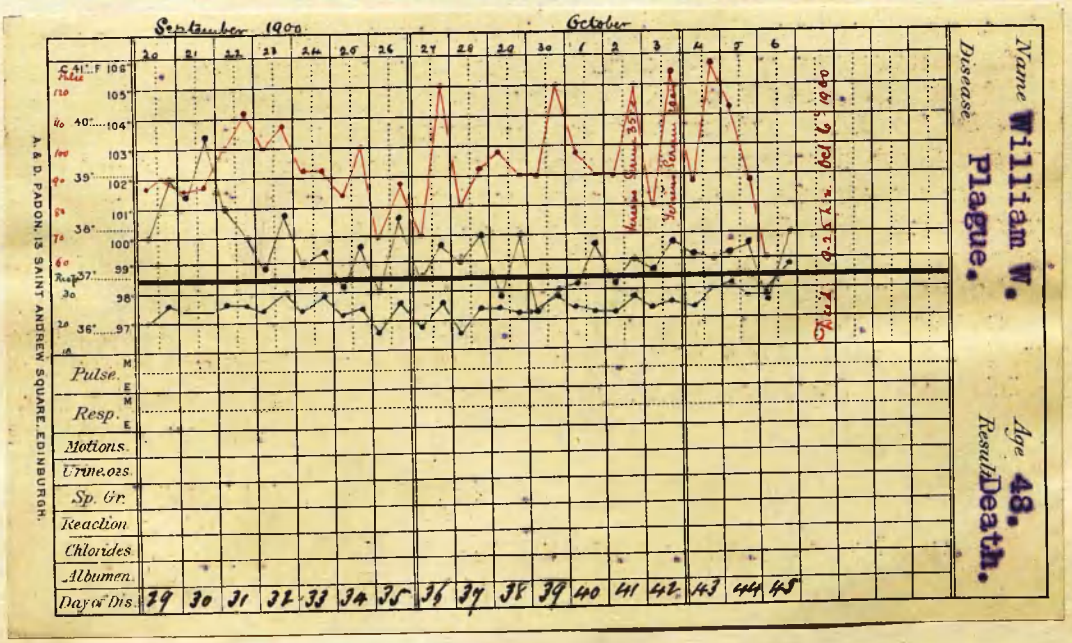
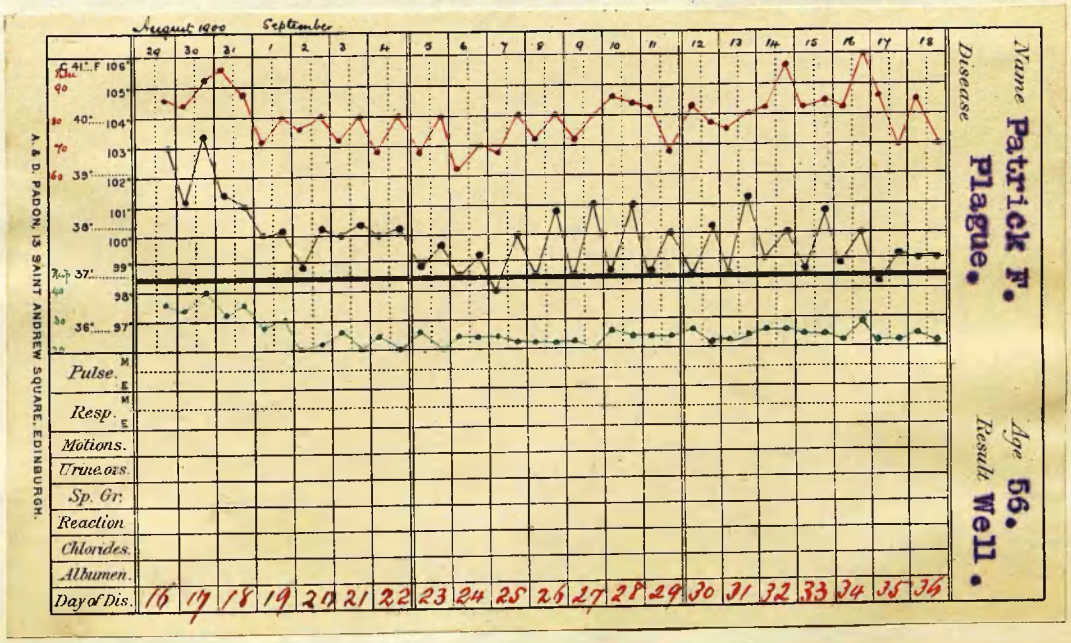


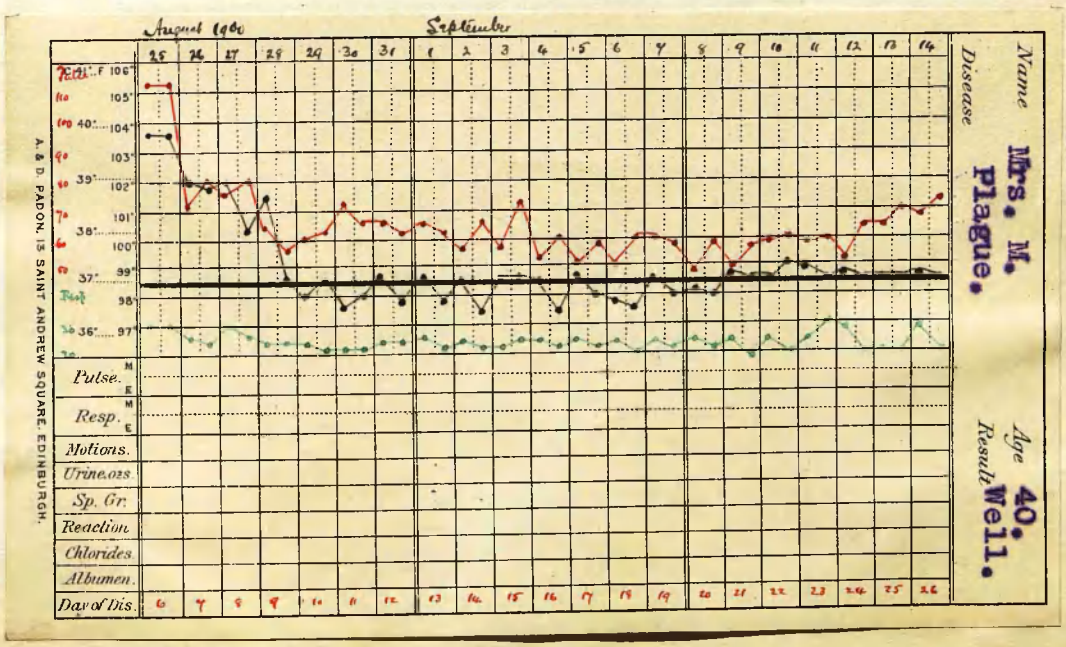
Case No.7.- Pat. F. aet.56. admitted 29th Aug. 1900.

Patient became ill on 14th Aug. with headache, sickness and vomiting, diarrhoea, and extreme malaise. Two days later he discovered a painful swelling in the left groin which continued to increase till admission. He was then acutely ill and frequently delirious. The eyes were suffused and the pupils dilated. Tongue dry and coated; pulse small and feeble. In left inguinal region, filling up the whole of the groin, was a large indurated swelling, acutely tender to the touch and covered by highly inflamed and oedematous skin. Puncture of this swelling shortly after admission, demonstrated the characteristic bacillus of plague on direct microscopical examination. The glands above Poupart's ligament were apparently not enlarged. Spontaneous rupture of the bubo, accompanied by the separation of large sloughs of necrotic tissue, occurred on 6th September, after which the temperature, which up to this time had been pursuing a hectic course, fell to normal. By 6th October the wound had closed, but patient was left much enfeebled by the illness.



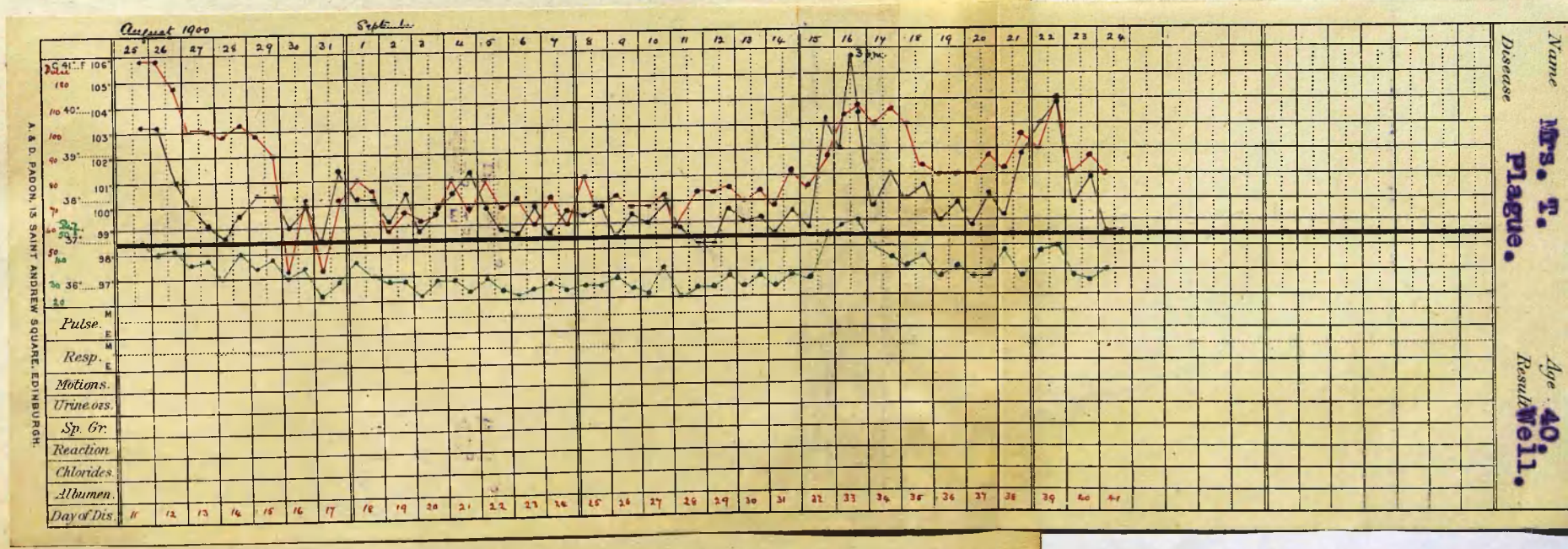
Case No.8.- Mrs. M. aet 40. admitted 25th Aug.1900.
certified as Enteric (?).

Patient took ill on 20th August, and ten days prior to the commencement of her illness she attended the wake of a woman (wife of Jas.B. case No.5) who died presumably from plague. The initial symptoms were, shivering, headache, vomiting, diarrhoea, and abdominal pain. Next day she discovered a small tender swelling in the right groin which rapidly increased in size, whilst the general symptoms also became more urgent. On admission the right groin was filled with a mass of enlarged and acutely tender lymphatic glands, surrounded by highly inflamed and oedematous tissues. The deep lymphatic glands on the same side were also enlarged and tender, but the glands accessible to palpation in all other regions were apparently normal. bPuncture of the bubo placed the diagnosis beyond any doubt, the specific bacillus being present in relatively large numbers. The temperature, which since admission had been continuously febrile, fell by crisis to normal on the 9th day of illness. Thereafter the bubo gradually subsided, ruptured spontaneously somewhat later and discharged a thin serous fluid for some weeks. Patient dismissed well 8th October.



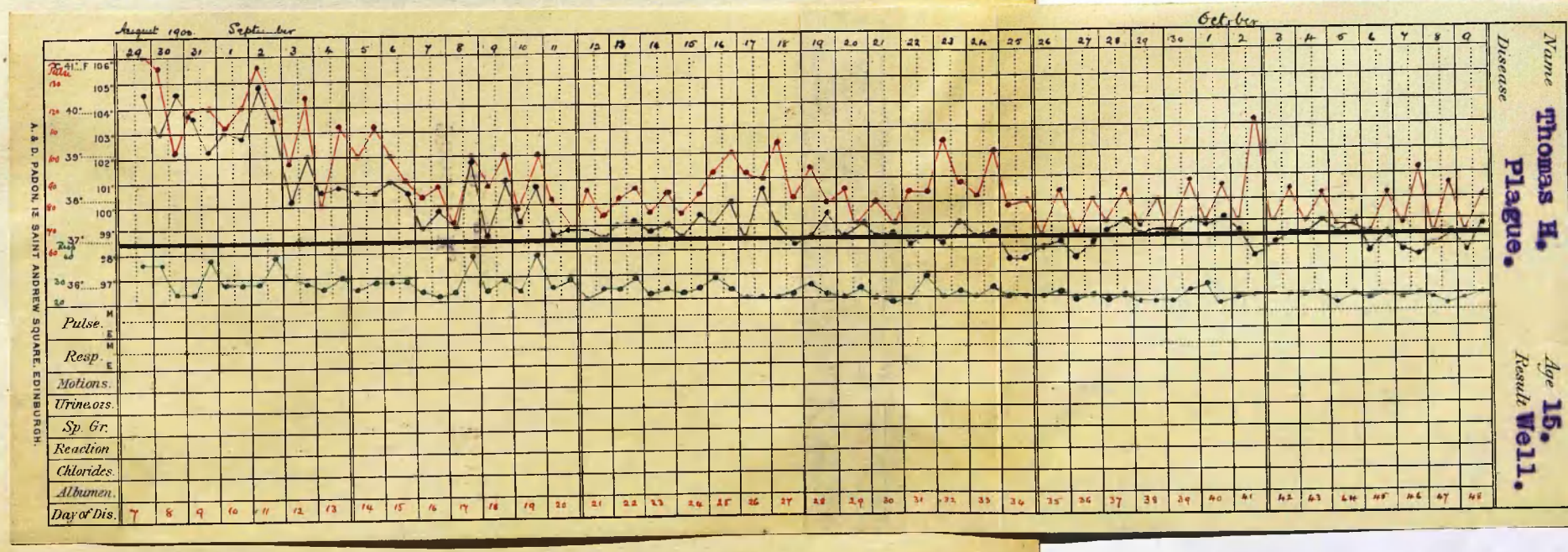
Case No.9. Mrs.T. aet.40. admitted 25th Aug.1900.
certified "Typhus fever".

Ten days prior to admission, patient took suddenly ill with severe headache, sickness, and vomiting. She was then three months pregnant and aborted four days before admission. On adm. patient was acutely ill and her mental condition much obscured. The face was pale and heavy, the eyes suffused, the tongue dry. In left groin, above Poupart's ligament, there was a large tender swelling about 4 inches in length and 2 inches in breadth, and covered by highly inflamed and oedematous skin. From puncture of this bubo on 29th Aug. a pure culture of the bacillus pestis was obtained. The temp.fell by a rapid lysis and touched normal on the 14th day of illness. The bubo ruptured spontaneously on 5th Sept. and discharged a sero-purulent fluid for some time. On account of secondary infection occurring, the temp. ran a remittent course till 16th Sept., when a sharp attack of left-sided pleurisy was ushered in by a severe rigor and a temp. of 105.6F. This quickly subsided, but on the 22nd inst. the right side became similarly affected the temp. remaining elevated for three days and then falling to normal. On 25th Sept. a discharge of pus from the left ear occurred which on cultivation yielded B. pyocyaneus and diplococci but no B. pestis. The ear ceased discharging on Oct. 2nd., convalescence thereafter being good.



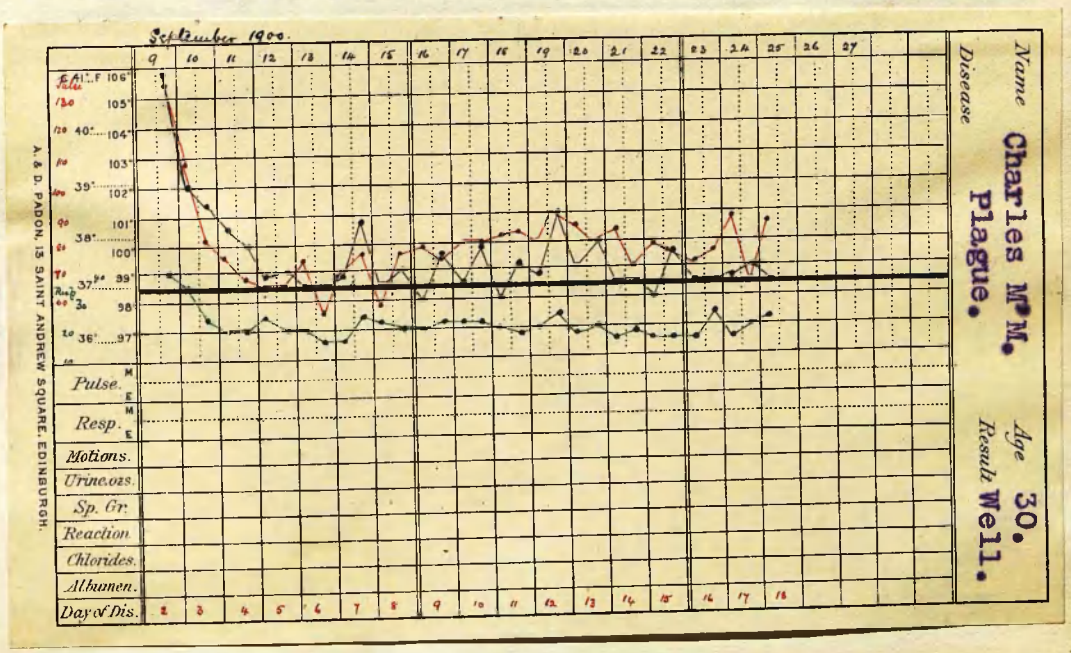
Case No. 10.- Thos. H. aet 14, admitted 29th Aug.1900.
certified as Enteric(?).

Patient became suddenly ill on 23rd.August with headache, sickness,vomiting, and pain in back and right axilla. The last symptom rapidly became worse and was followed by pain in left side of neck and in right groin. During this period he was highly fevered and frequently delirious. On admission the face was flushed and the expression though dull and heavy yet apprehensive. Lips dry and tongue heavily coated in centre. Skin hot and dry, with a faint mottling,most marked on back,flanks,and buttocks. There was general enlarg ment of the glands in both groins,axillae,and neck; those in right axilla and groin being the largest and most tender and covered by slightly inflamed skin. From the former,a pure culture of the bacillus pestis was obtained. The temp.was 104.6F; pulse soft and rapid- 140; respirations 26. Examination of lungs revealed a few subcrepitant rales in left lateral region. The spleen was slightly enlarged. A consignment of Yersin's serum arrived on 2nd Sept. and as the patient was still acutely ill and frequently delirious,(temp. 103-105F.)15cc.were injected intravenously and 25 cc. subcutaneously. After this he slept soundly, perspired freely for the first time since adm., whilst the acute tenderness of the bubos had almost entirely disappeared. From this point temp. fell gradually, convalescence being fairly rapid.



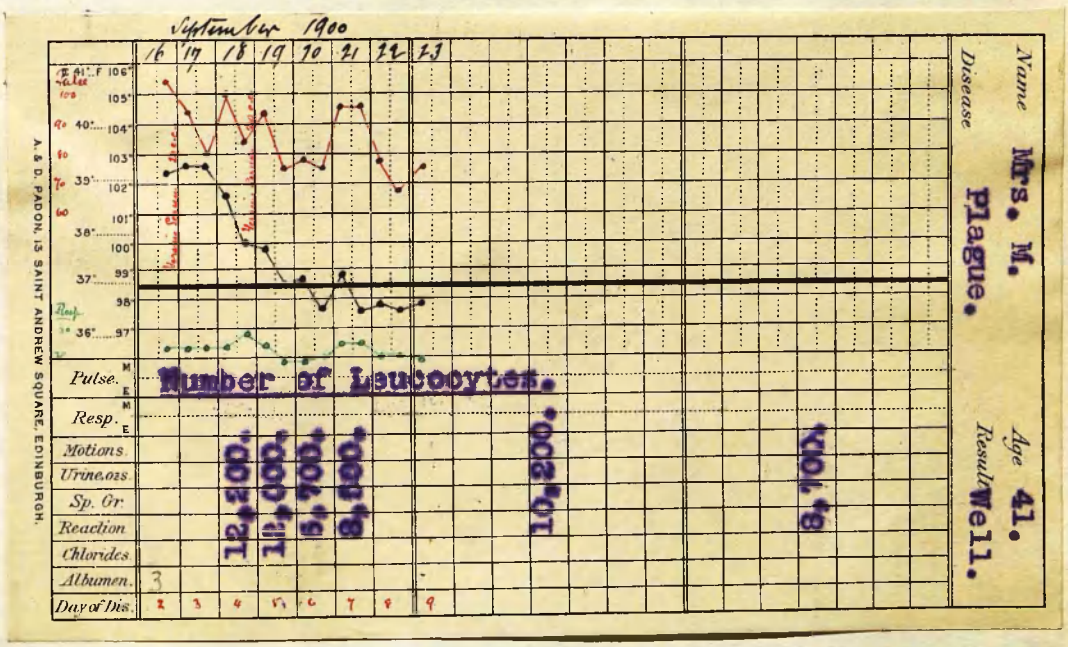
Case No.11. Chas.McM. aet.30, admitted Sept.9th 1900.

A week before adm. patient suffered from moderately severe headache and slight malaise, but continued at his work till 8th Sept., when a rigor with increased severity of headache, and sickness, compelled him to take to bed. By noon he was unconscious, and during the night he was delirious and very violent. On adm. he was acutely ill and quite unconscious. The temp. was 105.8F.; pulse 134; respirations 40. Face was pale and conjunctivae injected. The glands in left axilla were considerably enlarged and acutely tender, so much so in fact, that when palpation was attempted he made violent efforts of resistance, though still quite unconscious. From this bubo, a pure culture of bacillus pestis was recovered. At midnight 20 cc of serum were injected intravenously, and a like amount subcutaneously. Six hours later the temp. had fallen four degrees, the pain in axilla was greatly lessened and the glands could now be palpated without undue resistance On the 5th day the temp. fell to 98.6F., and patient felt so well that he desired to get up. From this point, with the exception of a slight rise of temp. on the 12th day associated with pain in the left knee and thigh and due in all probability to the serum injection, convalescence was uninterrupted.

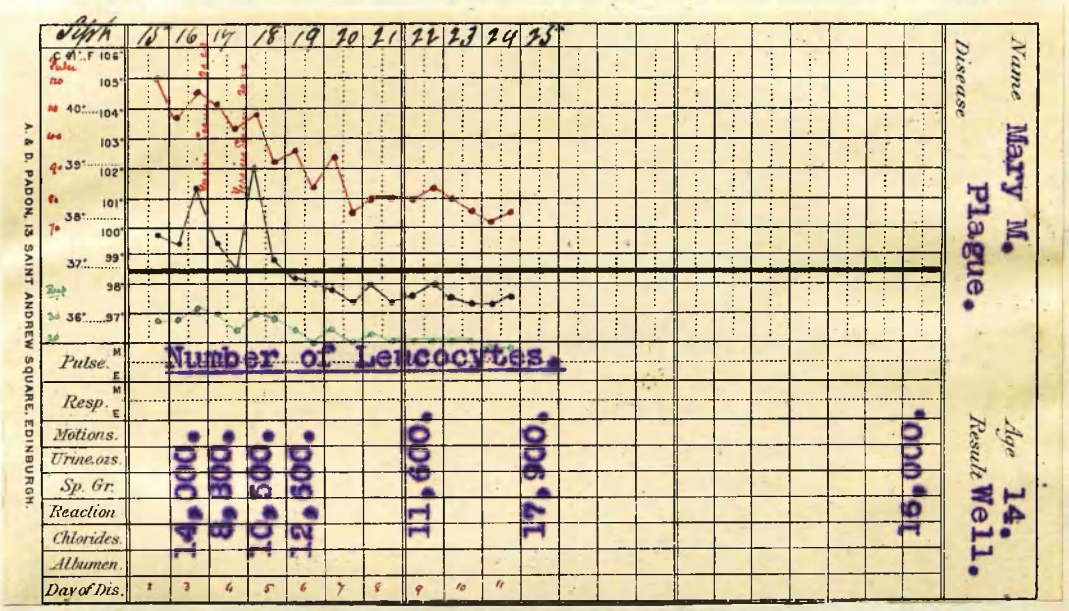


Case No.12, Mrs.M.aet.41, admitted Sept.16th 1900.

Duration of illness prior to admission somewhat indefinite but probably commencing two days before with severe headache and general malaise. Next day she discovered a small tender swelling in the right groin. On admission patient complained of severe headache and was obviously very ill. The temp. was 102.4F.; the pulse 104; and the respirations 24. The face was very anxious looking and the tongue dry. A small and exceedingly tender bubo, about the size of a pigeons' egg, was present in the right groin. The surrounding tissues were much indurated but the skin over it was freely moveable and not inflamed. On evening of admission, 20 cc of serum were injected into the abdominal wall. No improvement having taken place by next day, 20 cc of serum were given intravenously. This was followed by a striking amelioration of the general symptoms and by a pronounced lessening of the tenderness in the bubo, which could now be manipulated without pain. The headache disappeared, the temperature fell to normal and from this point convalescence was interrupted.

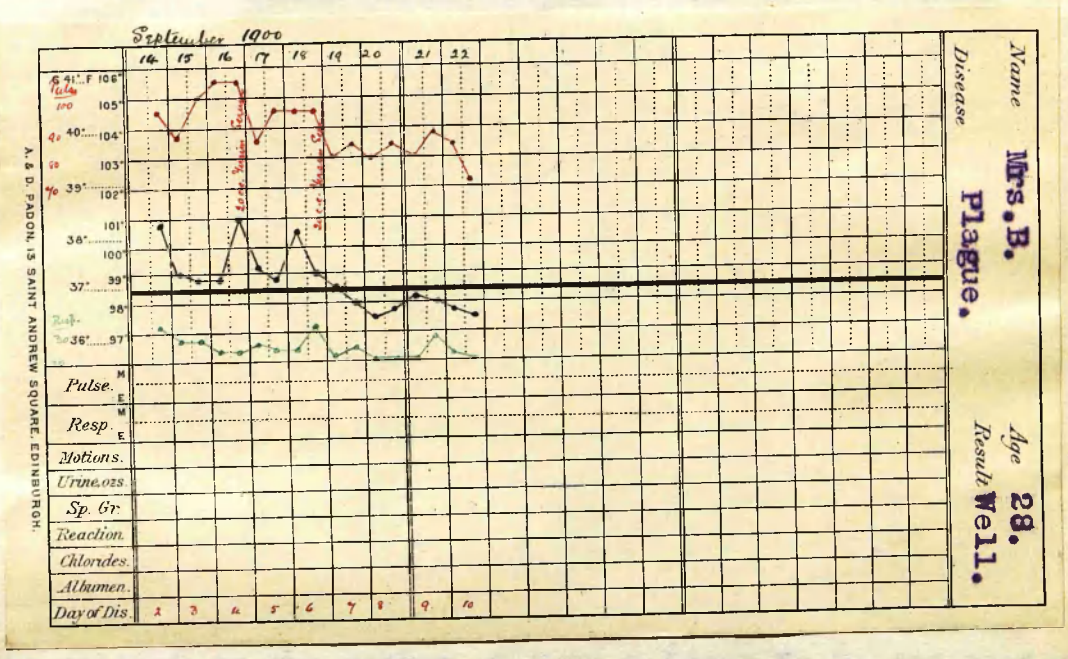


Case No.13. Mary M. aet.14, admitted Sept.15th 1900.



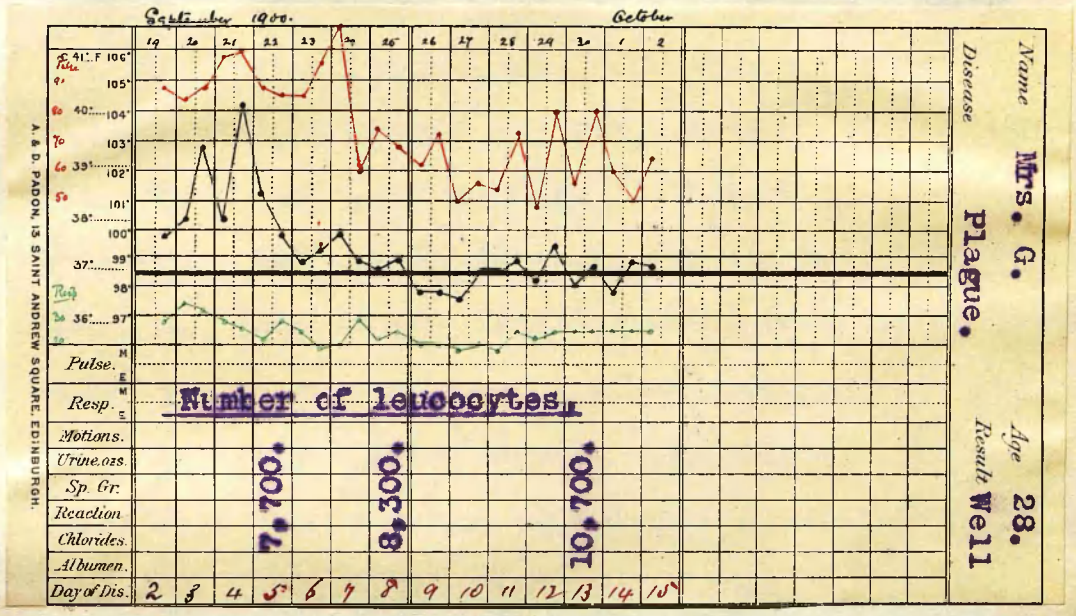
This patient is the daughter of Mrs.M.(case No.12)and sister of Mrs.M.(case No.1.)and of Robt.M.(case No.4). She took ill on night prior to admission with severe frontal headache, sickness and vomiting. On admission she complained of a tender swelling in left axilla, and on examination a single, small, freely moveable, and tender gland was discovered four inches from base of axilla, and behind, at border of latissimus dorsi, another gland but less tender. Next evening, a small pustule, surrounded by a ring of minute pustules, and also by an inflammatory zone of skin, was found on back at level of eighth dorsal vertebra. The fluid from the pustule yielded the typical organisms of plague, both culturally and on direct microscopical examination. 20 cc of serum were injected subcutaneously at this date, the temperature having risen to 101.4F. On the 18th patient seemed to be much worse, and a fresh area of congestion with a yellowish centre appeared on left flank. 30 cc were again injected, but intravenously, and the same evening patient spontaneously expressed herself much better; the temperature fell to normal, no subsequent rise occurring. The slough in the centre of the pustule now separated quickly leavind a healthy granulating surface, and the second erythematous area in the flank had almost completely disappeared. The buboes also subsided rapidly and from this point convalescence was uninterrupted

Case No.14. Mrs.B. aet.29, admitted Sept.14th 1900.



Illness began on day prior to admission with severe frontal headache and rigors, and pains in back and limbs. Next day the right groin became painful, and on examining this region she discovered a small tender swelling. On admission patient did not look particularly ill, but the face was flushed and the eyes congested. Heart and lungs normal to physical exam. An exceedingly tender bubo, involving chiefly the horizontal chain of glands below Poupart's ligament, was present in the right groin. The vertical chain was only slightly involved, but there was considerable oedema of the surrounding tissues and reddening of the skin. On the 16th. 20 cc of serum were given subcutaneously as the bubo was now larger and more inflamed. This produced a temporary improvement, but as the local condition had again advanced (with extension to the deep inguinal glands) and as the constitutional symptoms had again become more severe, 20 cc of serum were given intravenously. This was followed by a marked improvement in the general condition, the temperature falling to normal and remaining so till dismissal. The bubo softened and ruptured spontaneously on 23rd Sept. A pure culture of the bacillus pestis was obtained from the bubo on admission. After rupture the discharge proved sterile, but contained many degenerate forms of bacilli.

Case No.15. Mrs.G.aet24. admitted Sept.19th 1900.



This patient is the mother of Mary G.(case No.3) and sister of Charles M'M.(case No.11). She became ill on day prior to admission with headache and general malaise, and, coincident with these symptoms, she discovered a small tender swelling in the right groin. The initial symptoms were comparatively mild, due perhaps to a prophylactic injection of 10 cc of Yersin's serum seven days previously. On examination, a bubo about the size of a walnut, and involving the vertical chain of glands, was found in the right groin. The glands were more or less adherent to one other, but the skin was freely moveable and not inflamed. The illness had interrupted the suckling of a three-months'-old child and the breasts were therefore enlarged and tender. The course of the illness was short lasting only six days. The fever, however, continued high for 2-3 days ranging between 100 and104F.,and might be regarded as due to three factors. (1)The attack of plague-probably the most important;(2)the condition of the breasts;(3)serum fever, the maximum temperature occurring at the period when the pyrexia due to serum,usually made its appearance. On 4th October spontaneous rupture of the bubo took place, and from the discharge virulent plague bacilli were recovered. From this point onwards, convalescence was rapid and complete.

CASE NO.	NAME	PAGE	SITE OF BUBO AND NATURE OF ATTACK.
5	James B.	112	Right inguinal, septicaemic, DEATH.
14	Mrs. B.	122	Right inguinal. Mild.
16	James C.	32	Double inguinal. Mild.
7	Pat. F.	115	Left inguinal. Severe.
3	Mary G.	1[illegible]	Right inguinal, septicaemic, DEATH.
15	Mrs. G.	123	Right inguinal, serum previously, Mild.
10	Thos. H.	118	Right axillary and inguinal, Severe.
1	Mrs. M.	106	Left inguinal, septicaemic, DEATH.
2	Baby M.	108	Cervical ultimately septicaemic, DEATH.
12	Mrs. M.	120	Right inguinal, Severe.
13	Mary M.	121	Right axillary, pustular plague, Mild.
4	Robt. M.	110	Left axillary, septicaemic, DEATH.
8	Mrs. M.	116	Right inguinal, Severe.
18	Rosa M.	33	Double inguinal, Mild.
11	Chas. M'M	119	Left axillary , Severe.
19	NellieR.	37	Post. Cervical, Pestis ambulans.
20	Agnes R	38	Right axillary, Pestis ambulans.
9	Mrs. T.	117	Left inguinal, Severe.
17	Dan. T.	35	Right inguinal, Mild.
6	Will. W.	114	Left inguinal, septicaemic, DEATH.
X	Arch. A.	101	Pneumonic case.