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[illegible]ELECTROPHORETIC STUDIES ON PASTEURELLA PESTIS

III. — The Serum Antibodies of Recovered Plague Patients

Annales de l'Institut Pasteur,
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The study of serum modification in patients recovered from plague has not been the subject of many studies.

As early as 1949, Favarel [3] agglutinated samples of P. pestis with serum from recovered plague patients. Hoyer and Courdurier [6] demonstrated antiplague precipitins in the tissue of dead plague victims. Neal and coll. [11] studied the agglutination, direct conglutination, polysidic and proteinic hemagglutination reactions for plague diagnosis. Payne and coll. [12] studied the serum of subjects vaccinated with the EV strain or subjects recovered from plague, by hemagglutination after sensitizing the red blood cells with the capsular protein. They also investigated the cutaneous reaction of these subjects to Ajl's toxin in the plague epidemic zone.

The present scarcity of plague cases has forced most of the serum work to be on material of animal origin. Warren [14] brings to light an antitoxin in the blood of monkeys which have been vaccinated with strains of vaccines containing the somatic protein of P. pestis. Khundanov and coll. [7] have described an antibody in the γ-globulins which can passively immunize the mouse. Dzhaparidze and coll. [3] have studied the

*Manuscript received 12 July 1963.

electrophoresis of the serum of mice which have been inoculated experimentally with P. pestis. They showed that while the albumin titer decreased and the globulin titer increased, the total amount of serum protein did not change. Eisler and von Metz [4] have described precipitins which are common to several bacteria in the intestinal flora (Shigella, Proteus) and to one serum antifraction IB.

Due to the kindness of the chiefs of services of Health of the Provinces of Tamatave and Tananarive (See note 1) and the doctors under them, we have received 34 sera from patients attacked by and cured of plague.

[Note 1:] We thank Doctors Destribats and Ramamonjy Ratrimo for the shipments of plague patients' sera.

We have studied these sera by electrophoresis and by immunoelectrophoresis. Hemagglutination and complement deviation studies were made later.

MATERIAL AND METHODS

The 34 sera were divided as follows:

10 sera of subjects cured of pulmonary plague;
23 sera of subjects cured of bubonic plague.

They were taken at different times after the clinical verification of the disease. For two patients (1 and 10) we were able to study sera at J + 22 (J = date of diagnosis) and J + 120.

All of the sera could not be used for paper electrophoresis because hemolysis had occurred during their preparation in the field where conditions were often precarious.

We fractioned the serum proteins by paper electrophoresis. The apparatus used was Elphor H with Watman n° 1 paper and Longworth tampon pH 8.6 ionic force 0.05. The migration occurred for 12 to 15 hours. Bromphenol blue was the indicator. The paper was washed with 2 parts per 100 acetic acid, then with aceto-acetic reagent, then dried and exposed to ammonia fumes and read with densitometer (Photovolt Corporation) which gave the absorption curve. The serum was resolved into fractions which were weighed by the Mettler (precision 0.05 mg).

The immunoelectrophoretic analysis (AIE) was performed by the technique described in an earlier work (Dodin [2]). We established the AIE diagram for the serum concerned. Searching for a specific plague bacillus antibody, we placed a mixture of antihuman horse sera "I.P. Paris 223" and "224" in one lateral groove. In the other groove, we placed the antigen, plague bacilli EV Girard and Robic strain cultivated

at 26° C, and lysed by congelation-decongelation. To date, there is no tularemia or pseudotuberculosis in Madagascar which eliminates the possibility of cross reactions [1]. We wanted to determine to what the plague bacillus specific antibody corresponded on the AIE diagram for plague bacillus EV 26° which was established in an earlier work (Fig. 1). For this we had the antigens (strain EV 26°, lysed by congelation-decongelation) migrate. In the lateral grooves, we placed the different sera of patients attacked by plague.

The double diffusion in agar permitted us to identify a certain number of lines characterized by the AIE (Fig. 2).

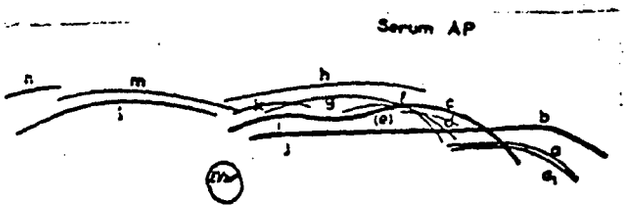

Fig. 1.

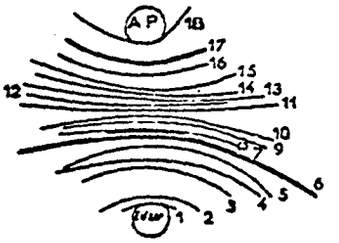

Fig. 2.

Fig. 1 and 2. AIE diagram of P. pestis EV 26° strain antigen. Double diffusion in agar. AP = antiplague serum.

RESULTS

I. Serum of Patients Recovered from Pulmonary Plague.

A. Electrophoresis (Table II).

Our base numbers are the percentages of serum albumin and globulin fractions for different races in Madagascar as given by Meyer [9]. Our patients came from four races: Sihanaka, Merina, Bezanozano, and Tsimihety.

Table I

	Albumins	Globulins		
		α	β	γ
Aver. of 40 subjects	69.6	7.3	7.5	15.6
±	1.3	0.6	0.4	0.9
S	4.1	1.8	1.4	2.8
V	5.6	24.6	18.6	17.9
Aver. of 46 subjects	57.6	9.4	9.5	23.5
±	1.6	0.3	0.61	1.6
S	5.6	2.9	2.1	5.5
V	10	30.8	22	23
Aver. of 41 subjects	64.2	10.9	8.9	16
±	1.3	0.7	0.5	0.6
S	4.3	2.4	1.6	2.1
V	6.7	22	18	13.1
Aver. of 43 subjects	64.9	7.1	7.6	20.4
±	2.2	0.5	0.6	1.9
S	7.5	1.8	2	6.5
V	11.6	25.4	26.3	31.9

± = 1/2 reliability interval
S = error type
V = Variance

The albumins — Compared to Meyer's figures (Table I), the albumin percentages are diminished in important proportions in 7 out of 8 patients. For subjects 2, 4, 8, 12 and 13, this drop is more than two thirds of the average value. The clinical data shows that patients 2, 4 and 13 suffered from severe pulmonary plague with bloody sputum containing Yersin's bacillus and with a temperature of 38.9° C to 39° C. Subjects 8 and 12 had temperatures of 37° C and 37.5° C, and bloody sputum containing Yersin's bacillus.

The drop of the albumin titers are less definite for subjects 6, 7 and 11. These had been treated immediately after contact; their temperatures did not exceed 37.5° C but their sputum was positive. These results confirm that what Dzhaparidze [3] saw in the mouse also occurs in man.

Table II

Number	Sex	Race and age	Serum A sample	Albumin	α_1	α_2	β	γ	AIR	Ouchterlony		Clinical signs			
												Temp.	Sputum Blood	Sputum 1	Pneumonia
2	F	S. 21	J + 21	37.7	2,2	7,8	11,1	41,2	g.a	0	PPC	39	+	+	+
3	F	S. 5	J + 21						g.a	0	PPC	37.5	0	+	+
4	F	S. 3	J + 21	25.5	2,6	22,7	19,1	30,1	g.a	0	PPC	38.9	+	+	+
5	M	S. 60	J + 21						g.a	0	PPC	37	0	+	+
6	F	S. 6	J + 21	47.9	3	12,2	13,6	23,3	g.a	0	PPC	37.5	0	+	+
7	F	S. 15	J + 21	59.3	2,6	4,8	14	20,3	g.a	0	PPC	37.5	+	+	+
8	M	S. 6	J + 21	40.9	2,3	6,9	12,4	37.5	g.a	0	PPC	37.5	0	+	+
11	M	S. 9	J + 21	47,8	1,9	8,7	11,8	29,8	g.a	0	PPC	37.5	+	+	+
12	F	S. 7	J + 21	41,3	4	11,1	17,4	26,2	g.a	0	PPC	37.5	0	+	+
13	F	S. 9	J + 21	32	0,6	20,2	21,7	25.5	g.a	0	PPNC	39.5	+	0	+

J + 21 = Sample taken 21 days after diagnosis.
PPNC = Pulmonary plague not confirmed.
PPC = Pulmonary plague confirmed.
S = Sihanaka.
g.a. = Augmented gamma.

J = days

A = years

Key: 1 - Yersin's bacillus.

Table III

Number	Sex	Race & age	Serum A sample	Albumin	α_1	α_2	β	γ	A T E	Specific Gamma	Ouchterlony	Observations
1	F	M. 28	J + 22	30,5	1,1	9,5	21,6	37,3	c (e) i j k	+	5,6 + 7	B.C.
2	»	»	J + 120	41,1	3,1	5,8	16,2	20,9	c i	+	6 + 7	B.C.
10	M	M. 24	J + 37	32,3	2,1	8,3	21,4	35,4	c i j	+	5,6 + 7	B.C.
10	»	»	J + 120	39	4,9	9,2	15,9	31	c i	+	6 + 7	B.C
33	M	M. 23	J + 20	30,8	2	8,5	19,5	39,2	c (e) i j	+	5,6 + 7	B.C.
9	M	S. 7	J + 29	38,4	2,7	12	14,8	32,1	c i j	+	6 + 7	B.C.
14	M	S. 8	J + 21						o	o	o	B.N.C.
15	M	S. 18	J + 29						c (e) i j	+	5,6 + 7	B.C.
16	F	S. 10	J + 27	45,2	2	12	17,2	23,5	b c (e) i j	+	4,5,6+7	B.C.
17	M	S. 5	J + 14	38,1	0,9	5,5	32,6	22,9	o	o	o	B.N.C.
18	M	S. 12	J + 24	26,6	2,6	22,7	17	31,1	b c (e) i j	+	4,5,6 + 7	B.C
19	F	S. 8	J + 26						c i	+	6 + 7 weak	B.C.
20	F	S. 14	J + 20						o	o	o	B.C.
23	F	S. 75	J + 23						o	o	o	B.C.
26	M	S. 10	J + 31						c (e) i j	+	5weak6+7	B.C.
27	M	S. 13	J + 60	37	5,9	13,6	12,3	31,2	c i j	+	5,6 + 7	B.C.
21	M	B. 8	J + 3	33,7	3,5	11,5	15,7	35,6	c i	+	6 + 7	B.N.C
22	M	B. 22	J + 7	40,7	3,8	6	10,5	39	i	+	6 + 7 weak	B.C.
24	M	B. 22	A + 7	68,2	3,5	4	10	14,8	o	o	o	B.C.
25	F	B. 18	A + 7	67,9	3	5,2	12	11,9	o	o	o	B.C.
28	M	T. 6	A + 4						o	o	o	B.C.
29	F	T. 16	A + 8	49	4,5	10,5	18,1	29,9	o	o	o	B.C.
30	M	T. 20	A + 1,5						c i	+	5,6 + 7	B.C.
31	M	T. 42	A + 8	32	3,5	4,2	19	15,3	o	o	o	B.C.
32	F	T. 28	A + 3						o	o	o	B.C.

M = Marina. B = Bonnegesso. S = Sihanaka. T = Talmihety.

B.C. = Bubonic plague confirmed.

b.N.C. = Bubonic plague not confirmed.

The globulins -- The α_1 α_2 group is little changed except in subjects 4 and 13 where α_2 has doubled quantitatively. All of the subjects in Table II except n° 13 had confirmed pulmonary plague. The strain was isolated by cultural methods and by mouse inoculation. For these ten patients, the serum sample was taken 21 days after the start of the disease.

The β-globulins increased in our 8 patients. This rise is parallel to that of the α_2-globulins. The γ-globulins are increased for each subject, but more definitely in n°s 2, 4, 8 and 11.

B. Immunoelectrophoresis.

Immunoelectrophoretic analysis showed no specific precipitation line of plague bacillus with the γ-globulin. No precipitation line from the plague bacillus EV 26° diagram appeared in AIE or in double diffusion in agar.

II. Serum of Patients Recovered from Bubonic Plague.

A. Electrophoresis (Table III).

1. Immediate Modifications -- We performed 15 electrophoretic analyses with the sera of subjects recovered from bubonic plague. In comparison to the average figures given for each race, the sera of subjects recently afflicted by plague all show a definite albumin titer drop. For subjects of the Merina race, this drop is about 50 p. 100 (subjects 1, 10 and 33). For the other races, this drop is also important. The α_1 α_2 globulins are little modified except for a definite α_2 increase for subjects 18 and 27.

The β-globulins are definitely increased in all the races. The largest rise is in the γ-globulins which are, in all cases, more than two times larger than the average.

2. Modification in Time -- Sera 21 and 22 were taken eight and seven days respectively after the clinical verification of the disease. These are subjects seen during the epidemic and it is logical to assume that the disease was just beginning. The modifications noted in the preceding paragraph are already established.

We were able to study sera 1 and 10 (Fig. 3 and 4) with samples taken on the 22nd and 120th days and on the 37th and 120th days respectively. The early sera show the general irregularities and the late sera already show a tendency towards the established averages. This return to normal occurred for sera 24 and 25 which were sampled seven years after recovery and for sera 29 and 31 which were sampled eight and three years respectively after recovery.

Table III

Number	Sex	Race & age	Serum A sample	Albumin	α_1	α_2	β	γ	A I E	Specific Cerum	Occip. Lony	Observations
1	F	M. 28	J + 22	30.5	1.1	9.5	21.6	37.3	c (e) i , k	+	5.6 + 7	B.C.
2	»	»	J + 120	41.1	3.1	5.8	16.2	20.9	c i	+	6 + 7	B.C.
10	M	M. 24	J + 37	32.8	2.1	8.3	21.4	35.4	c i j	+	5.6 + 7	B.C.
10	»	»	J + 120	39	4.9	9.2	15.9	31	c i	+	6 + 7	B.C
33	M	M. 23	J + 20	30.8	2	8.5	19.5	39.2	c (e) i j	+	5.6 + 7	B.C.
9	M	S. 7	J + 29	38.4	2.7	12	14.8	32.1	c i j	+	6 + 7	B.C.
14	M	S. 8	J + 21						o	o	o	B.N.C.
15	M	S. 18	J + 29						c (e) i j	+	5.6 + 7	B.C.
16	F	S. 10	J + 27	45.2	2	12	17.2	23.5	b c (e) i j	+	4.5.6 + 7	B.C
17	M	S. 5	J + 14	38.1	0.9	5.5	32.6	22.9	o	o	o	B.N.C.
18	M	S. 12	J + 24	26.6	2.6	22.7	17	31.1	b c (e) i j	+	4.5.6 + 7	B.C
19	F	S. 8	J + 26						c i	+	6 + 7 weak	B.C.
20	F	S. 14	J + 20						o	o	o	B.C.
23	F	S. 75	J + 23						o	o	o	B.C.
26	M	S. 10	J + 31						c (e) i j	+	5weak6+7	B.C.
27	M	S. 13	J + 60	37	5.9	13.6	12.3	31.2	c i j	+	5.6 + 7	B.C.
21	M	B. 8	J + 3	33.7	3.5	11.5	15.7	35.6	c i	+	6 + 7	B.N.C
22	M	B. 22	J + 7	40.7	3.8	6	10.5	30	i	+	6 + 7 weak	B.C.
24	M	B. 22	A + 7	68.2	3.5	4	10	14.8	o	o	o	B.C.
25	F	B. 18	A + 7	67.9	3	5.2	12	11.9	o	o	o	B.C.
28	M	T. 6	A + 4						o	o	o	B.C.
29	F	T. 16	A + 8	49	4.5	10,5	18,1	29.9	o	o	o	B.C.
30	M	T. 20	A + 1.5						c i	+	5.6 + 7	B.C.
31	M	T. 41	A + 3	52	3.5	4.2	19	15.3	o	o	o	B.C.
32	F	T. 29	A + 3						o	o	o	B.C.

M = Merina. B = Betsileo. S = Sihanaka. T = Tsimihety.

B.C. = Bubonic plague confirmed.

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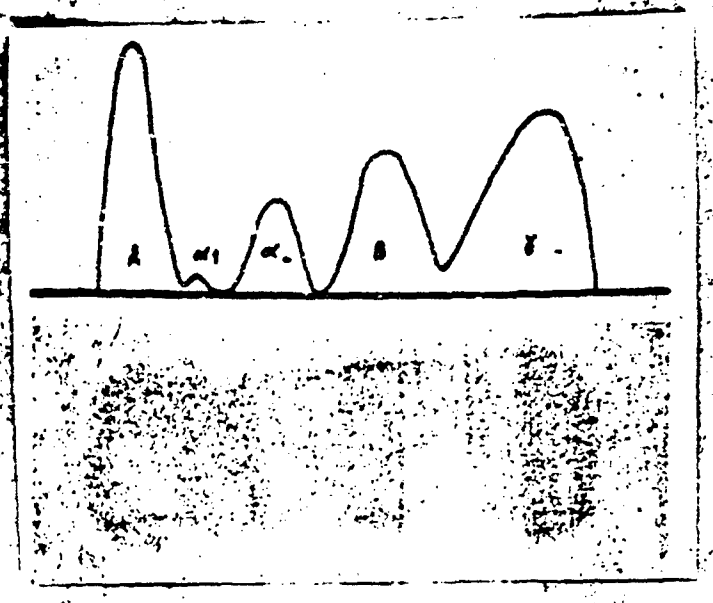

Fig. 3. N° 1, 22 days after diagnosis. Albumin = 30.5 p. 100. Globulins α_1 = 1.1 p. 100. Globulins α_2 = 9.5 p. 100. Globulins β = 21.6 p. 100. Globulins γ = 37.3 p. 100.

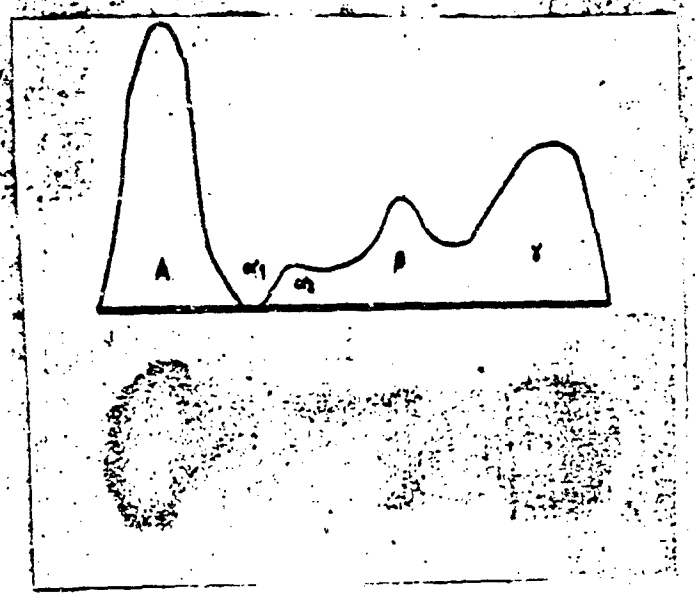

Fig. 4. N° 1, 120 days after diagnosis. Albumin = 41 p. 100. Globulins α_1 = 3.1 p. 100. Globulins α_2 = 5.8 p. 100. Globulins β = 16.2 p. 100. Globulins γ = 20.9 p. 100.

The plague bacillus thus seems to provoke a brutal electrophoretic modification of the serum proteins. Is this imbalance specific for P. pestis? This is what we will show by immunoelectrophoretic analysis.

B. Immunoelectrophoresis.

a) Immunoelectrophoretic analysis of serum from patients recovered from bubonic plague — The immunoelectrophoresis of serum from patients recovered from bubonic plague produces a specific precipitation line with the EV 26° antigen, which migrates with the γ-globulins. This line persists, attenuated, after saturation of the serum of past plague patients by Pasteurella Pseudotuberculosis.

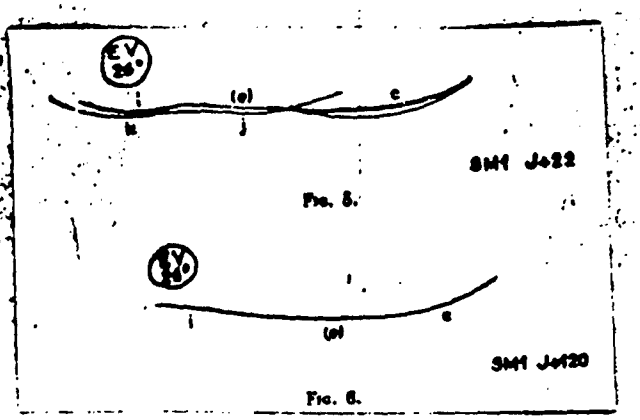

Fig. 5 and 6. In the central well: Cryolysat of P. pestis EV 26° strain. In the grooves: AP = antiplague serum. SMI J + 22 = Serum of patient n° 1 22 days after diagnosis. SMI J + 120 = Serum of patient n° 1 120 days after diagnosis.

Of 13 patients who had confirmed bubonic plague less than 18 months earlier, 11 showed a specific γ-globulin for the plague bacillus. Two (n°s 10 and 23), did not show this specific antibody. Of the three non confirmed bubonic plague cases, one (n° 21) showed a precipitation line with the plague antigen on the level of the γ-globulins.

Over 18 months after the disease, this precipitation line was not found in any of the tested sera (from three to eight years). As a matter of fact, the γ-globulin concerned is composed of different antibody fractions which we have identified according to the plague bacillus EV 26° AIE diagram and by double diffusion in agar (Fig. 5, 6, 7).

b) AIE of the plague bacillus with the help of patients' sera — The 15 sera having shown antiplague globulin, developed one or more precipitation lines with the plague antigen. The lines seen most often are c (14/15), i (14/15), j (9/15) and (e) which provides the c-i junction and

shows in certain conditions (cf. Dodin [2]), (6/15). The line b appeared only twice (nos 16 and 18) and k once.

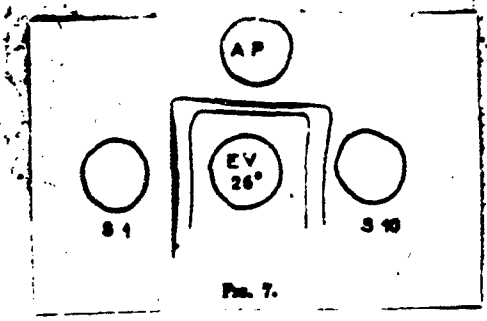

Fig. 7.

Each time the c (e) i or c-i lines have appeared in AIE, the lines 6 and 7 have appeared in double diffusion in agar. For each j line (or j + k), a corresponding line 5 appeared and for each of the two b lines, a corresponding line 4 appeared on the Ouchterlony plates.

c) Appearance of these lines as a function of time — At J + 7 (n° 22) there was only the i line. On J + 8 (n° 21) the c-i lines were produced. In the majority of the cases the lines c, i, j appeared in that order. The appearance of further lines seems to be in inverse proportion to the severity of the disease. Subjects nos 16 and 18 were in excellent general health with no fever and their antiplague globulins corresponded to the b, c, (e), i, j lines. On the other hand subject n° 1, whose general condition was bad with dyspnea, coughing, sputum, right inguinal adenophlegmon and obnubilation, showed lines c, (e), i, j, and k. Subjects 9, 10, 15, 19, 26 and 27 all showed a marked infectious condition with temperature around 40° C and adenophlegmon.

We were able to follow the modification of these lines in time with the sera nos 1 and 10. These were seen on J + 22 and J + 120, J + 37 and J + 120 respectively. After 100 days these lines (e), j, k for serum n° 1 and j for n° 10 had disappeared. After 18 months, serum 30 showed lines c, i and after three years, we found no trace of the plague infection by our AIE method.

DISCUSSION

Indeed we saturated our sera with Pasteurella pseudotuberculosis, but we did not do it with extracts of Shigella and E. coli or Proteus. Eisler and von Metz [4] demonstrated the presence of precipitins against P. pestis in the serum of rabbits immunized against the FI fraction, in new mammals and in human serum. The fact that the sera of our pulmonary plague patients and recovered plague subjects produced no precipitation lines is in favor of a real specificity of the γ-globulins thus put in evidence.

We have established that the lines c, (e), i were formed by the antigens of the plague bacillus type. The line c was in fact the sum of four lines of glycoprotein, cI, cII, cIII and cIV. The antigen which causes the appearance of cI is found in the sera of plague infected rabbits where the disease develops. Thus, the line c of our patients sera likely corresponds to the glycoprotein vaccinate and to another type c antigen.

The antigen corresponding to the b precipitation line is equally present in the plague infected rabbit serum and was refound in the serum of subjects who had a mild disease. It seems that, in general, the lines j, k are associated with the serious toxic bubonic plague.

Further studies will show if this is the antitoxic fraction. In Meyer's [10] hands, serum n° 1 showed four precipitation lines. Three were identified as F_1, the toxin and the E antigen. The third was not identified.

SUMMARY

Immunoelectrophoretic Studies on Pasteurella pestis. Serum Antibodies in Patients After Recovery.

The author has studied the serum of 11 patients suffering from pulmonary and the serum of 25 patients suffering from bubonic plague, after recovery. From the onset of the disease, a significant decrease of albumin and an increase of globulins, particularly γ-globulins, has been demonstrated.

Three months after recovery, the electrophoretic pattern returns to the normal.

In sera of patients recovered from bubonic plague, the immunoelectrophoretic analysis shows the presence of a globulin migrating with the specific anti-plague γ-globulin. This globulin is constituted, according to the moment of the disease, of a certain number of antibodies, corresponding to antigens determined on the P. pestis immunoelectrophoretic diagram. The lines i, c (e) seem to appear first; then the lines b, j, k, then or according to the severity of the disease, the line b (benign disease), j, k (toxic infection).

These lines disappear in an inverse order of their appearance, and all of them disappear 18 months after the end of the disease.

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