

A NEW SERUM TEST FOR KALA-AZAR.

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NOTE ON THE APPLICATION OF THE TEST TO THE DIAGNOSIS OF
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[Received for publication, November 17, 1921.]

THE technique of the performance of this test¹ is similar to that described by Gató and Papacosta as the 'Formol-gel test for syphilis'² but the results which are obtained if the serum of Kala-azar patients is tested are quite different from anything that is described by these two writers. The specificity of the test is in no way dependent on the gel formation but on the accompanying formation of a white opacity, so that not only is the name 'Formol-gel test for Kala-azar' inappropriate but is likely to be confused with the 'Formol-gel test for syphilis' and consequent misunderstandings are certain to arise. It is therefore suggested that another name be applied to this test. For reasons which follow we have adopted the name 'Aldehyde test.'

The reaction on which the test is dependent was first noted in the following manner. A quantity of serum was required for certain experiments. The serum was obtained from patients under treatment for Kala-azar by withdrawing the blood from the veins after potassium antimonyl tartrate had been injected. The serum was usually preserved by the addition of carbolic acid. On one occasion, however, formalin was used instead of carbolic acid as the latter was not at hand at the time and also because I had always found the former very satisfactory for preserving bacillary emulsions and, if my memory does not betray me,

high-titre sera during the war. Shortly afterwards it was observed that the lower portion of this serum had solidified and was becoming opaque. Some more serum from another Kala-azar patient was taken and formalin added : a marked opacity occurred. It was then found that the addition of dilute carbolic solution intensified the result. Samples of serum from a few patients, including one who had been diagnosed as a case of chronic malaria but who subsequently turned out to be one of Kala-azar, were tested in this way by the addition of a little dilute formalin and carbolic acid : a similar result was obtained in each case. Normal serum remained quite clear after the addition of the same mixture. About this time my attention was called to the paper by Gate and Papacosta, which I had previously overlooked, by Captain W. C. Spackman, I.M.S., who also gave me his experience with the test in a case of Kala-azar. Subsequently a uniform technique was adopted : *i.e.*, one cubic centimetre of serum was separated and one drop of commercial formalin was added. It was found that by this method a more definite result was obtained.

The test.

About 5 c.c. of blood is withdrawn from the patient's arm and allowed to stand for a sufficient time for the serum to separate. 1 c.c. of clear serum is then placed in a small test tube $\frac{1}{2}$ inch diameter, to this one drop of 30 per cent* formaldehyde in the form of commercial formalin is added, the serum immediately well shaken and placed in a test tube rack at laboratory temperature. The results are as follows :—

The serum from an untreated case of Kala-azar.

The serum will immediately become viscid, within a minute or two will have 'set,' so that the tube can be inverted without the serum being spilled, and will begin to become whitish and opalescent. Within from three to twenty minutes, the time varying with different cases, the whole of the serum will have become absolutely solid and opaque like serum coagulated by heat or the 'white' of a hard boiled egg. (Plate LXXV, Fig. IV.) If the serum is hæmoglobin-stained the coagulated serum will have a pink tinge which will turn chocolate brown after 24 hours.

* *Note.*—The percentage of formaldehyde in commercial formalin is said to be 40 per cent but the samples in our laboratory have been found to be about 30 per cent.

The serum from cases of phthisis, leprosy, malaria and certain other conditions.

Usually no change is at first observed but after half an hour, or in some cases less, the serum becomes jellified and after a few hours a certain degree of opacity may be observed but there is no comparison between this and the complete opacity that occurs in the kala-azar cases. (Plate LXXV, Fig. II.)

After 24 hours jellification occurs in the serum from some cases of syphilis This is the reaction that is described by Gaté and Papacosta.

The serum of a healthy person or a patient suffering from some disease not included in the above categories.

The serum will remain quite clear and fluid for a more or less indefinite period. (Plate LXXV, Fig. I.)

Notes on the reading of results.

In the case of the serum of very young children with Kala-azar the opacity, after the addition of formalin, may not be absolutely complete, but up to the present we have found that in the case of very young children not suffering from Kala-azar the serum will remain absolutely clear, so that if there is jellification and any degree of opacity in that of a child of, say, three years, the result can safely be considered to be 'positive.'

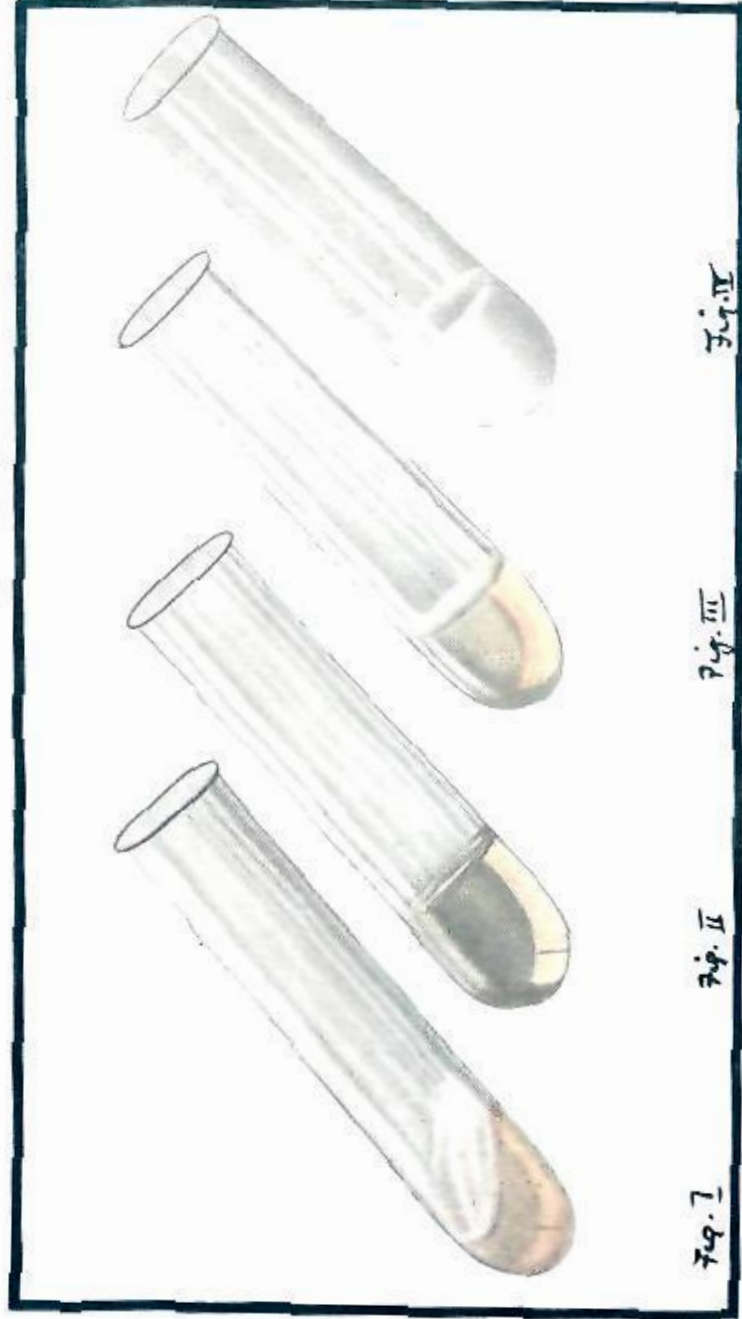
We have observed recently in two cases of malaria, in which there were a large number of parasites actually present in the blood at the time it was withdrawn, that on addition of the formalin the serum became rapidly solid and assumed quite a marked degree of opacity. (Plate LXXV, Fig. III.) On the first occasion that this occurred we immediately assumed that we were dealing with a case of Kala-azar and were surprised not to find Leishman-Donovan bodies in the spleen, but on the second occasion that we came across a reaction of this kind it was immediately recognised as being similar to the previous one and a diagnosis of malaria was made. This diagnosis was subsequently proved to be correct. Even after standing for 24 hours both these sera were distinctly opalescent and had a characteristic greenish tinge which made them easily distinguishable from the Kala-azar serum which is absolutely opaque and, if previously unstained, a dead white. I was disappointed at getting these two somewhat ambiguous results as they

EXPLANATION OF PLATE LXXV.

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Fig. I. Normal serum.	Fluid.	-ive.
" II. Chronic malaria.	Solid but clear.	(-)
" III. Acute malaria.	Solid and slightly clouded.	(-)(-)
" IV. Kala-azar.	Solid and absolutely opaque.	+++.

PLATE LXXV.



tend to show that the test is one of degree rather than an absolute test as I had hoped that it would prove to be, and furthermore they make one afraid to trust any modification of this test in which a smaller quantity of serum is used.

In order to measure the opacity we have made use of a small cylindrical cell, 1.25 cm. in diameter and 0.4 cm. deep, the capacity being about 0.5 c.c. Thoma-Zeiss hæmocytometer white blood counting pipette also has a capacity of 0.5 c.c. and is therefore a useful mixer. One part of 20 per cent formaldehyde and ten parts of serum are taken into the pipette and rapidly expelled into the cell which is immediately covered with a coverslip. The cell is then placed over some standard print and the time noted at which the print becomes unreadable. In the two malaria cases mentioned above the print was still readable at the end of 24 hours, whereas in all cases of untreated Kala-azar it becomes unreadable in from three to twenty minutes or, in extreme cases, two hours. This will be a useful method of measuring the patient's progress under treatment, if the disappearance of this reaction is found to be an indication of the improvement of the patient's condition.

These are the only two cases in which any judgment is required in reading the results of the test.

Signs adopted for recording the results.

(a) If the serum becomes solid and completely opaque within .. 20 minutes ..	+	+	+
(b) 2 hours ..	+	+	
(c) 24 hours ..	+		
(d) If the serum becomes solid and markedly opalescent but never completely opaque ..		(+)	
(e) If the serum becomes solid and slightly opalescent ..		(-)	(-)
(f) If the serum becomes solid but remains quite clear ..		(-)	
(g) If the serum remains fluid and clear for 24 hours ..	-	ive	

In the untreated patient (a), (b) and (c) may be considered 'positive,' (d) should be judged according to the age of the patient, and (e), (f) and (g) should be considered as 'negative.'

There is at present at our disposal only one certain method of the diagnosis of Kala-azar, that is, by the recovery of the causative organism

from the patient. The finding of the Leishman-Donovan bodies in the peripheral blood is an excellent method of making a positive diagnosis but the failure to find them does not constitute a negative one. We have found this method of diagnosis a most unsatisfactory one in Calcutta. On only two occasions have we found Leishman-Donovan bodies in the peripheral blood of patients out of over two hundred definite cases examined. It is true that we were not looking for the parasites in most of the cases but either doing blood counts or looking for malarial parasites. Had the Leishman-Donovan bodies been present in any numbers they would most certainly have been observed. On account of the difficulty experienced in keeping culture medium free from contamination during the rains and hot weather we have been compelled to give up this means (*i.e.*, by culture of the peripheral blood) of diagnosis also and to reserve our energies in this direction for checking the results of treatment. We are therefore left with spleen puncture and the microscopic examination of the films for Leishman-Donovan bodies as the only certain means of diagnosis.

It is obvious that before advocating a new method of diagnosis one must check its accuracy by employing it in conjunction with the most certain of previously used methods. We have therefore tried this test in a number of untreated cases in which a spleen puncture has been done.

Table I gives the result of 150 consecutive untreated cases whose condition simulated Kala-azar and on whom a satisfactory spleen puncture had been done. (This table will be found in the Appendix.)

Analysis of Table I.

	ALDEHYDE TEST RESULT.		
	Total.	Positive.	Negative.
Cases in which L.-D. bodies were found	91	89	2
Cases in which malarial parasites were found	22	0	22
Cases of Leukæmia	2	0	2
Cases in which no parasites were found	35	1	34

It will be seen from the above analysis that the spleen puncture findings and the results of the Aldehyde test correspond in 147 out of 150 cases, that is in 98 per cent of cases.

Case number 38 of this series, the case in which the spleen puncture was negative and the aldehyde test positive, was probably a true case of Kala-azar. She was a woman aged 38 : she had had continuous fever for some months : she had a large spleen, a palpable and tender liver, œdema of the feet and she was anæmic. She has now been under treatment by intravenous antimony for two months and is very considerably improved. She has had no fever for over a month : she has no œdema : her spleen and liver are decreased in size and she is less anæmic.

TABLE I.

No.	Date.	Patient.	Sex.	Age.	Spleen puncture findings.	Aldehyde test.
1	20-6-21	B. C. S No. 89	Male	41	L.-D. bodies	Positive.
2	20-6-21	M. No. 93	Male	21	L.-D. bodies found	Positive.
3	20-6-21	R. No. 95	Male	15	No parasites	Negative.
4	20-6-21	H. N. No. 102	Male	19	L.-D. bodies	Positive.
5	20-6-21	A. C. No. 105	Male	30	L.-D. bodies	Positive.
6	28-6-21	N. No. 105	Male	40	No parasites	Negative.
7	1-7-21	Miss E. No. 118	Female	15	L.-D. bodies	+ +
8	2-7-21	J. K. No. 121	Male	40	L.-D. bodies found	Positive.
9	11-7-21	L. B. No. 126	Male	6	L.-D. bodies	+ + +
10	15-7-21	S. S. No. 131	Male	35	L.-D. bodies	+ + +
11	15-7-21	J. S. No. 133	Male	32	No parasites	(-)
12	18-7-21	L. H. No. 136	Male	15	L.-D. bodies	+ + +
13	18-7-21	R. M. No. 148	Male	36	L.-D. bodies	+ + +
14	21-7-21	J. N. K. No. 149	Male	7	L.-D. bodies	--- ive.
15	21-7-21	A. No. 137	Male	15	L.-D. bodies	+ + +
16	21-7-21	M. K. No. 122	Male	21	L.-D. bodies	+ + +
17	21-7-21	A. No. 147	Male	10	No parasites	- ive.
18	22-7-21	A. G. No. 152	Female	12	L.-D. bodies	+ +
19	22-7-21	T. H. No. 153	Male	8	L.-D. bodies	+ + +
20	22-7-21	P. N. No. 154	Male	27	No parasites	(-)
21	23-7-21	C. Ward No. 53	Male	41	L.-D. bodies	+ + +

*A New Serum Test for Kala-azar.*TABLE I.—*contd.*

No.	Date.	Patient.	Sex.	Age.	Spleen puncture findings.	Aldehyde test.
22	23-7-21	S. J. No. 155	Female	.. 11	No parasites ..	- ive.
23	26-7-21	M. No. 145	Female	.. 48	L.-D. bodies ..	+ + +
24	26-7-21	P.B. B. No. 158	Male	.. 19	L.-D. bodies ..	+ + +
25	26-7-21	M. No. 150	Male	.. 40	L.-D. bodies ..	+ + +
26	26-7-21	S. No. 141	Male	.. 15	Malarial parasites, M. T.	(-)
27	26-7-21	R. No. 157	Male	.. 12	Malarial parasites ..	- ive.
28	26-7-21	P.S. Ward No. 200	Male	.. 16	L.-D. bodies ..	+ + +
29	26-7-21	N. No. 161	Male	.. 32	No parasites ..	- ive.
30	26-7-21	S. No. 159	Male	.. 32	L.-D. bodies ..	+ + +
31	26-7-21	R. No. 160	Male	.. 12	No parasites ..	(-)
32	26-7-21	C. No. 162	Female	.. 16	No parasites ..	(-)
33	26-7-21	S. No. 163	Male	.. 16	L. D. bodies ..	+ + +
34	26-7-21	R. L. No. 161	Male	.. 28	L. D. bodies ..	+ + +
35	26-7-21	C. No. 135	Male	.. 25	L.-D. bodies ..	+ + +
36	26-7-21	N. N. No. 165	Male	.. 19	Malarial parasites, B. T.	(-)
37	27-7-21	S.M.B. No. 166	Male	.. 29	Malarial parasites, M. T.	(-)
38	27-7-21	M. No. 169	Female	.. 36	No parasites found ..	+ + +
39	27-7-21	D. J. No. 168	Male	.. 9	L.-D. bodies ..	+ +
40	27-7-21	M. K. No. 167	Male	.. 27	L.-D. bodies ..	+ +
41	27-7-21	S. No. 138	Male	.. 9	L.-D. bodies ..	+ + +
42	27-7-21	H. No. 156	Male	.. 27	L.-D. bodies ..	+ + +
43	27-7-21	L. R. No. 156	Male	.. 16	L.-D. bodies ..	+ + +
44	27-7-21	U. No. 170	Female	.. 30	Malarial parasites ..	(-)
45	29-7-21	A. R. N. No. 139	Male	.. 14	L.-D. bodies ..	+ + +
46	29-7-21	M. A. No. 172	Male	.. 18	L.-D. bodies ..	+ + +
47	29-7-21	R. Ward No. 244	Male	.. 50	L.-D. bodies ..	+ + +
48	30-7-21	A. Taylor	Male	.. 5	L. D. bodies ..	+ + +
49	4-8-21	S. No. 175	Male	.. 23	Malarial parasites ..	(-)
50	4-8-21	K. B. No. 176	Male	.. 40	L.-D. bodies ..	+ + +

TABLE I.—*contd*

No.	Date.	Patient.	Sex.	Age.	Spleen puncture findings.	Aldehyde test.
51	4-8-21	B. A. No. 177	Male	15	L.-D. bodies	+ + +
52	4-8-21	James	Male	35	L.-D. bodies	+ + +
53	4-8-21	A. A. Ward No. 237	Male	13	L. D. bodies	+ + +
54	5-8-21	M. M. S. Ward No. 236	Male	20	Malarial parasites	- ive.
55	5-8-21	Ward No. 252	Male	40	L.-D. bodies	+ + +
56	5-8-21	N. G. No. 179	Male	39	No parasites	(-)
57	5-8-21	T. G. No. 180	Male	14	L.-D. bodies	+ + +
58	5-8-21	A. K. No. 181	Male	15	No parasites	- ive.
59	10-8-21	Ward No. 221	Male	30	No parasites	(-)
60	10-8-21	Ward No. 210	Male	20	L.-D. bodies	+ + +
61	10-8-21	S. No. 183	Male	20	L.-D. bodies	+ + +
62	10-8-21	L.M.B. No. 184	Male	14	No parasites	- ive.
63	10-8-21	K.C.B. No. 185	Male	30	L.-D. bodies	+ +
64	12-8-21	Ward No. 220	Male	25	L.-D. bodies	+ +
65	12-8-21	Ward No. 217	Male	50	L.-D. bodies	+ + +
66	12-8-21	B. No. 186	Male	6	Malarial parasites	- ive.
67	15-8-21	C. No. 188	Male	35	L.-D. bodies	+ + +
68	15-8-21	H. C. C. No. 190	Male	54	L.-D. bodies	+ + +
69	16-8-21	Ward No. 195	Male	24	L.-D. bodies	+ + +
70	17-8-21	H. No. 191	Male	18	Malarial parasites	(-)
71	17-8-21	I. N. No. 192	Male	20	No parasites	(-)
72	17-8-21	S. No. 194	Male	35	No parasites	- ive.
73	17-8-21	Miss S. No. 195	Female	10	L.-D. bodies	+ + +
74	17-8-21	Miss N. No. 196	Female	17	L.-D. bodies	+ + +
75	17-8-21	M. L. No. 197	Male	17	L.-D. bodies	+ + +
76	17-8-21	S. No. 198	Male	11	L.-D. bodies	+ + +
77	17-8-21	S. No. 199	Male	30	L.-D. bodies	+ + +
78	17-8-21	T. D. No. 124	Female	14	L.-D. bodies	+ + +

*A New Serum Test for Kala-azar.*TABLE I.—*contd.*

No.	Date.	Patient.	Sex.	Age.	Spleen puncture findings.	Aldehyde test.
79	17-8-21	J. No. 200	Female	11	L.-D. bodies	+ + +
80	17-8-21	R. D. No. 202	Male	20	L.-D. bodies	+ + +
81	17-8-21	E. B. No. 203	Male	22	L. D. bodies	+ + +
82	17-8-21	H. H. No. 204	Male	17	Malarial parasites	(-)
83	22-8-21	M. No. 205	Male	18	L.-D. bodies	+ + +
84	22-8-21	R. J. No. 207	Male	30	Malarial parasites	(+)
85	23-8-21	P. No. 208	Male	30	Malarial parasites	(-)
86	23-8-21	S. T. No. 209	Male	35	No parasites	(-)
87	24-8-21	Ward No. 217	Male	30	L.-D. bodies	+ + +
88	24-8-21	H. L. G. No. 211	Male	42	L.-D. bodies	+ + +
89	24-8-21	T. No. 212	Male	18	L.-D. bodies	+ + +
90	25-8-21	P. No. 213	Male	25	L.-D. bodies	+ + +
91	25-8-21	S. G. R. No. 214	Male	23	L.-D. bodies	+ + +
92	25-8-21	Ward No. 229	Male	32	L. D. bodies	+ + +
93	25-8-21	H. B. No. 218	Male	38	L.-D. bodies	+ + +
94	30-8-21	Ward No. 250	Male	29	No parasites	(-)
95	30-8-21	S. No. 219	Male	26	No parasites	(-)
96	31-8-21	A. No. 220	Male	50	L.-D. bodies	+ + +
97	31-8-21	B. K. D. No. 221	Male	30	No parasites	(-)
98	31-8-21	P. P. G. No. 4	Male	17	No parasites	(-)
99	31-8-21	B. N. M. No. 222	Male	22	No parasites	(-)
100	31-8-21	P. D. No. 223	Male	26	Malarial parasites	(-)
101	1-9-21	K. No. 224	Male	10	L.-D. bodies	+ + +
102	1-9-21	A. H. No. 226	Male	15	L.-D. bodies	+ + +
103	1-9-21	Ward No. 54	Male	36	No parasites, leukæmia	(-)
104	2-9-21	S. No. 227	Male	15	No parasites	(-)
105	2-9-21	L. H. No. 228	Male	15	L.-D. bodies	+ +
106	2-9-21	S. No. 229	Male	15	L.-D. bodies	+ + +
107	7-9-21	B. No. 230	Male	22	No parasites	(-)

TABLE I.—*contd.*

No.	Date.	Patient.	Sex.	Age.	Spleen puncture findings.	Aldehyde test.
108	7-9-21	J. G. No. 231	Male	18	L.-D. bodies	+++
109	7-9-21	N. No. 232	Male	16	L.-D. bodies	- ive.
110	7-9-21	R. G. No. 233	Male	32	L.-D. bodies	+++
111	7-9-21	B. No. 235	Male	13	No parasites	- ive.
112	10-9-21	Ward No. 225	Male	17	L.-D. bodies	+++
113	10-9-21	H. No. 239	Male	16	Malarial parasites	(-)
114	10-9-21	H. L. M. No. 240	Male	25	No parasites	- ive.
115	19-9-21	S. No. 245	Male	28	No parasites	- ive.
116	19-9-21	G. M. No. 247	Male	12	L.-D. bodies	+++
117	19-9-21	S. A. No. 248	Male	20	L.-D. bodies	+++
118	21-9-21	K. C. No. 249	Male	20	L.-D. bodies	+++
119	21-9-21	K. M. No. 250	Male	29	L.-D. bodies	+++
120	21-9-21	H. G. B. No. 251	Male	26	No parasites	- ive.
121	21-9-21	R. No. 252	Male	16	Malarial parasites	(-)
122	21-9-21	W. I. B. No. 253	Male	27	L.-D. bodies	+++
123	21-9-21	New No. 254	Male	9	Malarial parasites, B. T.	- ive.
124	21-9-21	N. No. 256	Male	40	No parasites, leukæmia.	- ive.
125	22-9-21	K. No. 257	Male	30	L.-D. bodies.	+++
126	22-9-21	M. No. 258	Male	20	Malarial parasites	(-)
127	26-9-21	Ward No. 215	Male	45	No parasites	(-)
128	26-9-21	Ashu No. 261	Male	23	L.-D. bodies	+++
129	26-9-21	I. No. 263	Female	16	L.-D. bodies	+++
130	26-9-21	N. No. 264	Female	25	L.-D. bodies	+++
131	26-9-21	G. No. 266	Male	14	L. D. bodies	+++
132	28-9-21	H. No. 267	Male	30	Malarial parasites	(+)
133	28-9-21	A. M. No. 269	Male	11	Malarial parasites, B. T.	(-)
134	30-9-21	O. M. No. 270	Male	32	L.-D. bodies	+++
135	30-9-21	H. No. 271	Male	22	No parasites	(-)
136	30-9-21	T. No. 273	Male	35	No parasites	(-)

TABLE I.—*concl'd.*

No.	Date.	Patient.	Sex.	Age.	Spleen puncture findings.	Aldehyde test.
137	30-9-21	D. N. No. 274	Male ..	16	L.-D. bodies ..	+ + +
138	3-10-21	A. No. 275	Female ..	16	Malarial parasites, B. T.	(-)
139	3-10-21	D. No. 277	Male ..	10	L.-D. bodies ..	+ + +
140	3-10-21	R. H. No. 278	Male ..	30	No parasites ..	(-)
141	3-10-21	M. No. 279	Male ..	22	L.-D. bodies ..	+ + +
142	5-10-21	M. O. No. 280	Male ..	34	No parasites ..	(-)
143	5-10-21	M. No. 282	Male ..	30	No parasites ..	(+)
144	6-10-21	G. N. No. 283	Male ..	20	L.-D. bodies ..	+ + +
145	13-10-21	R. F. No. 285	Male ..	13	L.-D. bodies ..	+ + +
146	13-10-21	K. No. 286	Male ..	11	L.-D. bodies ..	+ + +
147	13-10-21	A. No. 287	Male ..	30	Malarial parasites ..	- ive.
148	13-10-21	G. K. No. 288	Male ..	32	No parasites ..	(-)
149	13-10-21	A. G. No. 289	Male ..	28	Malarial parasites, B. T.	(-)
150	13-10-21	M. N. No. 291	Male ..	40	No parasites ..	(+)

Numbers 14 and 109, the two cases in which the aldehyde test was negative but in whose spleens the parasites of Leishmaniasis were found, were clinically very much alike. They gave a history of a year and a month, respectively. They denied having lost weight and both looked well nourished. They gave no history of bleeding from the gums or nose: were only slightly if at all anæmic: their pulses were slow and their spleens not markedly enlarged. The clinical diagnosis in each case, prior to spleen puncture, had been chronic malaria. The former patient attended for treatment without much change in his condition for about a month and then discontinued of his own accord. The latter did not attend for treatment.

The aldehyde test and the Wassermann reaction.

As the formol-gel test, the father of this test, was put forward as a test for syphilis it was felt that it would be interesting to compare the results of the aldehyde test with those of the Wassermann reaction. The

Wassermann reaction was done in 86 of the 150 cases reported in table I. This work was carried out by the Imperial Serologist. Table II gives these results.

TABLE II.

	Total.	WASSERMANN REACTION.		
		Positive.		Negative.
		Strongly.	Partially.	
Cases in which the aldehyde test was 'positive'	60	12	7	41
Cases in which the aldehyde test was 'negative'	26	4	5	17

The figures seem to suggest that there is very little, if any, relationship between the two reactions.

Further controls.

Although the test has been done on nearly 60 non-kala-azar cases amongst the spleen-puncture series, it was thought advisable to do it on a few more samples from patients suffering from other diseases. Fifty cases were chosen in whom a definite diagnosis of some other disease had been made and their serum was subjected to the aldehyde test. Table III gives these results. The patients were all adults. It was only in the

TABLE III.

Controls.—Cases with a definite diagnosis of some condition other than Kala-azar.

No.	Patient.	Diagnosis.	Aldehyde test.
1	No. 3 Ward ..	Enteric	— ive.
2	No. 45 O. P. ..	Filariasis	— ive.
3	Leper 'A' ..	Leprosy, advanced	(—) (—)
4	Leper 'B' ..	Leprosy, moderately severe	(—)
5	Leper 'C' ..	" " " "	(—)
6	Leper 'D' ..	" " " "	— ive.
7	Leper 'E' ..	" " " "	(—)

TABLE III.—*contd.*

No.	Patient.	Diagnosis.	Aldehyde test.
8	Leper 'F' ..	Leprosy, moderately severe ..	- ive.
9	Leper 'G' ..	" " " ..	(-)
10	Leper 'H' ..	" " " ..	(-)
11	C. F. G. ..	Dysentery ..	- ive.
12	No. 182 O. P. ..	Syphilis (Wassermann positive) ..	- ive.
13	T. B. No. 1 ..	Advanced pulmonary tuberculosis ..	(-)
14	T. B. No. 3 ..	" " " ..	(-) (-)
15	T. B. No. 7 ..	" " " ..	(-)
16	T. B. No. 17 ..	" " " ..	(-)
17	T. B. No. 19 ..	" " " ..	(-)
18	T. B., O. P. a. ..	Pulmonary tuberculosis ..	(-)
19	T. B., O. P. b. ..	" " ..	- ive.
20	T. B., O. P. c. ..	" " ..	(-)
21	T. B., O. P. d. ..	" " ..	(-)
22	T. B., O. P. e. ..	" " ..	(-) (-)
23	No. 248 Ward ..	Malignant disease ..	(-)
24	No. 206 ..	Pelvic inflammation ..	- ive.
25	Jew ..	Appendicitis ..	- ive.
26	No. 160 ..	Advanced T. B. lungs ..	(+)
27	No. 167 ..	Leukæmia, spleno-medullary ..	- ive.
28	No. 261 ..	Early tuberculosis, lungs ..	- ive.
29	No. 272 ..	B. T. malaria ..	- ive.
30	No. 232 ..	M. T. malaria ..	()
31	Ward No. 228 ..	Gastric ulcer ..	- ive.
32	Ward No. 230 ..	Malaria ..	- ive.
33	Ward No. 232 ..	Morbus Cordis ..	- ive.
34	Ward No. 239 ..	Nephritis ..	- ive.
35	Ward No. 243 ..	" ..	- ive.
36	Ward No. 244 ..	Hemiplegia ..	- ive.
37	Ward No. 209 ..	Gonorrhœa ..	(-)
38	Ward No. 214 ..	M. C. Mitral ..	- ive.
39	Ward No. 216 ..	" ..	- ive.
40	Ward No. 219 ..	Dysentery ..	(-)
41	Ward No. 221 ..	Morbus Cordis ..	(-)
42	Ward No. 200 ..	Malaria ..	- ive.
43	Ward No. 217 ..	Beri-Beri ..	- ive.
44	Ward No. 206 ..	Malaria ..	- ive.
45	M.'s case ..	Tuberculosis ..	(-)
46	McV.'s case, a. ..	Ankylostomiasis ..	- ive.
47	McV.'s case, b. ..	" ..	- ive.
48	No. 198 ..	Broncho-pneumonia ..	- ive.
49	No. 234 ..	Lobar pneumonia ..	- ive.
50	No. 231 ..	Malaria, M. T. ..	(-)

tuberculosis and leprosy cases that any clouding of the serum was observed. It was not considered necessary to do the test with the serum from normal individuals and from cases of uncomplicated syphilis as this has already been done by the originators of the Formol-gel reaction and other observers with apparently uniformly '- ive' or '(-)' results.

*The test in Kala-azar cases under treatment.*³

The results which are obtained if the serum of patients under antimony treatment be tested vary between strongly positive and absolutely negative. There is a decided tendency for the reaction to disappear as the treatment progresses but we are not yet in a position to say to what extent we can rely on this test as an indication of the progress of the patient towards recovery. If it is found that the progress of the patient can be measured in this way an accurate method of measuring the opacity of the serum will have to be introduced. Possibly some method on the lines of the one that I have already suggested might be used. Tables IV and V give the results of the test in treated Kala-azar cases.

TABLE IV.

Cases under treatment.

No.	Date.	Patient.	Age and sex.	Mgms. of antimony salt given.	Result.
1	15-7-21	No. 59	30 male ..	2,000	(+)
2	15-7-21	No. 54	12 male ..	1,500	- ive.
3	21-7-21	No. 109	27 female ..	100	(-) (-)
4	21-7-21	No. 45	13 male ..	1,400	(+)
5	21-7-21	No. 123	20 male ..	100	+
6	22-7-21	No. 225 Ward ..	28 male ..	2,000	+
7	22-7-21	No. 226 Ward ..	18 male ..	100	(+) no gel
8	22-7-21	No. 70	8 female ..	1,000	- ive.
9	23-7-21	No. 63	17 male ..	800	- ive.
10	23-7-21	No. 12	35 male ..	2,000	+ +
11	23-7-21	No. 211 Ward ..	11 male ..	2,200	- ive.
12	23-7-21	No. 223 Ward ..	12 male ..	1,800	(+)
13	23-7-21	No. 52	25 male ..	700	+ +
14	23-7-21	No. 35	26 male ..	2,000	- ive.
15	23-7-21	No. 99	43 male ..	600	(+)
16	29-7-21	No. 10 Ward ..	12 female ..	500	(+)
17	29-7-21	No. 96	9 male ..	650	(+)
18	4-8-21	No. 78	21 male ..	1,000	+ + +
19	4-8-21	No. 48	11 male ..	1,500	(+)
20	4-8-21	No. 32	7 female ..	1,000	- ive.
21	4-8-21	No. 13	5 male ..	800	(+)
22	4-8-21	No. 28	9 female ..	1,200	- ive.
23	4-8-21	No. 107 Ward ..	15 female ..	1,000	+ +
24	4-8-21	No. 121	32 male ..	500	+
25	4-8-21	No. 59	30 male ..	2,500	(-)
26	12-8-21	No. 106	40 male ..	400	+ + +
27	12-8-21	Ezra	28 male ..	100	+ +
28	16-8-21	Ranao	14 male ..	1,000	(+)
29	16-8-21	E. B.	12 male ..	2,000	- ive.

TABLE V.

Cases in which the aldehyde test was done prior to treatment and again after treatment was considered complete.

Patient.	Sex and age	Aldehyde test prior to treatment.	Nature of treatment adopted.	Aldehyde test after treatment.
No. 227 Ward. ..	Male 15 ..	+ + +	Intra-muscular S.A.T.	(-)
No. 40 Ward ..	Male 5 ..	+ +	do. do.	(-)
No. 118 ..	Female 15 ..	+ +	Intravenous Pot. A. T.	(-)
No. 53 Ward ..	Male 41 ..	+ + +	Intra-muscular. S.A.T.	(+)
No. 126 ..	Male 6 ..	+ + +	do. do.	- ive.

Suggested modifications.

As it stands the test can be performed by any medical practitioner who is capable of withdrawing blood from a patient's veins. The veins of small children are sometimes difficult to puncture and there are still some practitioners who hesitate, or are unable, to perform this simple operation even on an adult. One is therefore anxious to find some method which will give equally accurate results without the necessity of venipuncture. This, I think, will be difficult to find.

If blood is drawn from the finger into a Wright's capsule and allowed to clot the test can be performed with the drop of serum thus obtained in a number of ways.

(a) One part of 15 per cent formaldehyde and ten parts of serum can be drawn into a fine Wright's pipette, mixed and sealed. The opacity can easily be observed by holding it against a black background and the consistency can be noted by breaking the pipette. (b) The serum and formaldehyde can be more accurately measured by means of a hæmocytometer pipette and mixed on a slide where the opacity and the consistency can easily be observed.

(c) A drop of serum can be placed on the inside wall of a test tube, held at an angle so that the drop does not run down, in which a few grains of para-formaldehyde have been placed and the test tube heated gently. Formaldehyde is liberated which will solidify a 'positive' serum in the same way that the solution does. All these modifications

tend to decrease the accuracy of the test. The modification that I have found most useful is the following :

Four drops are taken directly from the patient's finger into a small test tube containing 0.5 c.c. of citrate saline (Sodium chloride 0.85 grammes, sodium 0.5 grammes to 100 c.c. of water) in which there is one drop of formalin. If this is left overnight the red blood cells will settle and the supernatant fluid will be milky in the Kala-azar case, clear in the other.

But even with this modification the difference between a '++' result and a '(+)' result is not sufficiently distinct to make the method really reliable.

Notes on the chemistry of the reaction.

At first we used commercial formalin, the formaldehyde content of which was 30 per cent, but latterly Dr. Sudhamoy Ghosh has kindly prepared a solution of pure formaldehyde in distilled water which we have found gives much clearer results. A 10 per cent solution was used and a drop was added to 1 c.c. of serum. The question of the optimum proportions is one that will have to be investigated. I have found that one part of formaldehyde, by weight, to 200 parts of serum gives a rapid and complete result. If the amount of formaldehyde be increased any extent the reaction will tend to be less marked and if excess of formaldehyde be added the reaction does not occur.

The reaction seems to be essentially an aldehyde one as the next higher aldehyde, acet-aldehyde, also gives the reaction but less completely and more slowly. Six sera were tested with acet-aldehyde, a 40 per cent solution in distilled water being used.

The results were as follows :—

Serum.			Formaldehyde (usual signs used.)	Acet-aldehyde. (Readings after 24 hours.)
a.	+ + +	Solid and quite opaque.
b.	+ + +	Solid and almost opaque.
c.	+ +	Solid and opalescent.
d.	(--)	Solid but clear.
e.	(--)	Solid but clear.
f.	- ive.	Fluid.

The explanation of the reaction is not obvious and an attempt to seek it would, I fear, carry me into the realms of physical chemistry where I should very soon be completely lost. A few facts however have been noted.

The portion of the serum which is actually responsible for the formation of the specific opacity seems to be grouped amongst the eu-globulins. After 33 per cent saturation with ammonium sulphate and subsequent removal of the ammonium sulphate by dialysation from the filtrate, the latter will not give the reaction, whereas the precipitate dissolved in physiological saline and also freed from ammonium sulphate does give it. Again, after dialysation of the serum against 50 times its volume of distilled water, and consequent precipitation of most of the globulins, the speed of the reaction is very markedly increased, in fact on a drop of formalin being added the serum coagulates almost instantaneously, but if dialysation is continued in running distilled water further precipitation of the globulins occurs and the remaining serum fails to react in the characteristic manner with formaldehyde. The precipitated globulins when dissolved in saline give the reaction.

It seems possible that the reaction is in some way dependent on the hydrogen-ion concentration of the serum. The PH is much increased in the serum of Kala-azar patients. Alteration of the PH in either direction will prevent the aldehyde reaction occurring in a 'positive' serum and very light increase in the PH of some negative sera will, in combination with the addition of formaldehyde, cause coagulation to occur. Further investigation is at present being carried out on this point.

My thanks are due to Captain W. C. Spackman, I.M.S., for first drawing my attention to Gaté and Papacostas' paper and thereby considerably stimulating my interest in the subject.

My thanks are also due to Major R. B. Lloyd, I.M.S., Imperial Serologist, for doing the Wasserman reactions, to Dr. Sudhamoy Ghosh, Professor of Chemistry at the Calcutta School of Tropical Medicine, for preparing and estimating the strength of pure formaldehyde solutions and for his valuable advice, and to my assistant Dr. P. Murugesan.