

are frequently made at the same time from one piece of capillary tubing, and are separated by means of a mouth blow-pipe, a constriction being made in the middle of the tube before the introduction of the gas.

It has been urged against the use of emanation tubes that their initial activity, which is often considerable, is rapidly lost, and that their residual activity, which is small in amount but of long duration, cannot be made use of. The answer to the first point is that a treatment rarely exceeds 24 hours, and that an emanation tube can so be made that its average strength during that period shall be the strength required (the loss during 24 hours being only 16 per cent). As regards the second point, when emanation applicators are returned after use to the laboratory they frequently possess considerable residual activity. As a rule the individual tubes are no longer sufficiently powerful to be of any therapeutic value, but an apparatus has been devised whereby the tubes are crushed and the emanation extracted, several tubes of low activity being utilised to make a tube of high activity.

The stock of radium salts available for emanation production has been subdivided for the sake of safety into units each of about 200 mgm. of radium bromide. Each unit has been attached to its own pump and has been mounted separately on the walls of the iron safes in the basement. All the pumps and glasswork have been made by the laboratory staff, considerable time having been spent in their construction. During the year 241 emanation tubes and applicators have been made (as compared with 44 in 1912), and the activity of these applicators was equal to that of over 19 grm. of radium bromide. These applicators were sent by post as required to various places in the British Isles.

Radio-active water.—The output of radio-active water has also been largely increased during the year, over 3600 litres being made in 1913, as compared with 400 litres in 1912. New apparatus has been constructed which permits of 150 bottles being despatched daily by post to patients as directed by their medical attendants. The strength of the water remains the same as for last year—that is to say, approximately 1 millicurie per litre.¹ The emanation used for the production of this large volume of water is equal in activity to about 6.5 grm. of radium bromide.

Radium applicators.—During the year there has been a considerable demand by medical men and by other institutes for the preparation of radium applicators similar to those used in the Institute. Applicators have been prepared for several of the London hospitals as well as for institutes in Germany and Denmark. The number of these amounts to 55 (as compared with 4 made in 1912). In addition 15 specimens of radio-active material were received from various sources for analysis, a branch of the laboratory work which shows signs of increasing since the acquisition of an International standard.

¹ A millicurie of emanation is equivalent to 2,670,000 Maché units.

ROYAL SANITARY INSTITUTE.—The Earl of Plymouth (President) occupied the chair at the dinner of the Royal Sanitary Institute held at the Langham Hotel, London, on May 13th. Amongst those present were Sir Thomas Barlow, President of the Royal College of Physicians of London, Sir Rickman J. Godlee, President of the Royal College of Surgeons of England, Sir Shirley F. Murphy, Professor Sheridan Delépine, Mr. Herbert Jones, Colonel J. Lane Notter, R.A.M.C., Dr. Arthur Newsholme, Surgeon-General Sir Lionel Spencer, Professor H. R. Kenwood, Dr. Louis C. Parkes, Dr. W. J. Howarth, Dr. E. F. Bashford, Professor Bostock Hill, and Mr. A. Wynter Blyth. After the usual loyal toasts had been honoured the toast of "The Royal Sanitary Institute" was proposed by the President, who pointed out that the institute was obtaining support from the most distant parts of the Empire. Sir Henry Tanner, chairman of the council, replied. The health of the visitors was proposed by Mr. H. Percy Boulnois, M.I.C.E., and Sir Thomas Barlow and Principal E. H. Griffiths, F.R.S., of the University College of South Wales and Monmouthshire, responded. The health of the chairman was proposed by Colonel Notter, and Lord Plymouth made suitable acknowledgment.

THE DIAGNOSIS OF PULMONARY TUBERCULOSIS:

THE VALUE OF BESREDKA'S ANTIGEN IN THE COMPLEMENT-FIXATION TEST FOR TUBERCULOUS DISEASE.

BY A. C. INMAN, M.A., M.B. OXON.,
SUPERINTENDENT OF THE LABORATORIES OF THE HOSPITAL FOR
CONSUMPTION AND DISEASES OF THE CHEST, BROMPTON.

IN May, 1913, M. Besredka, of the Pasteur Institute, in Paris, asked me to undertake some observations on patients suffering from pulmonary tuberculosis with a view to determining the diagnostic value of the complement-fixation test of the blood in the presence of a tubercle antigen prepared by himself. For this purpose he kindly supplied me with a quantity of antigen which is prepared by growing tubercle bacilli in a liquid medium consisting of veal broth and the white and yellow of the hen's egg. No peptone, glycerine, or salts are employed. After the growth has proceeded for from three to four weeks the culture is heated to 115° C., filtered free from bacteria, and should contain sufficient tuberculin to kill a tuberculous guinea-pig in a dose of 1.5 c.c. to 2 c.c.

The technique employed in the series of experiments referred to in the present communication needs description. In the complement-fixation test the following reagents are required: antigen, control and test human sera, complement, specific hæmolytic amboceptor, the corresponding erythrocytes, and physiological salt solution.

1. *The antigen.*—This was received direct from Paris in hermetically sealed stout-walled test-tubes and placed in the ice safe until required for use. Immediately before each experiment the required amount was decanted into a measuring flask and 1/20 c.c. of a 15 per cent. solution of pure crystallised sodium chloride for every 1 c.c. of antigen was added. The mixture was well shaken and poured into test-tubes. After a series of trials the dose of antigen for each tube was fixed at 0.3 c.c.

2. *The human serum.*—10 c.c. of blood were removed from the median basilic vein by venepuncture. The serum was always inactivated at 55° to 56° C. for 30 minutes. The blood was examined within 48 hours of withdrawal. The dose of serum was fixed at 0.2 c.c. for each tube. In the majority of experiments progressive dilutions of the serum were examined, as will be shown later.

3. *Complement.*—Fresh guinea-pig blood was used. Immediately after withdrawal clotting was allowed to take place in the air incubator at 37° C. for one hour. The clot was then freed from the walls and the tube placed on ice until required for immediate use. A 1 in 30 dilution of the clear serum was used in a dose fixed by titration in the presence of antigen and normal serum. The adequate dose was usually found to be 0.5 c.c. or even 0.4 c.c., rarely 0.6 c.c.

4. *Specific hæmolytic amboceptor.*—A rabbit-sheep hæmolysin was used. This was obtained already standardised from the Sächsisches Serumwerk und Institut für Bakteriotherapie, Dresden, and gave uniformly satisfactory results. The dose used was four to five times the minimal hæmolytic dose.

5. *The test erythrocytes.*—The corpuscles contained in 5 c.c. of fresh whipped sheep's blood were washed at least three times and suspended in 95 c.c. of physiological salt solution. The corpuscles were

sensitised 20 minutes previous to use, and the dose for each tube was fixed at 0.3 c.c.

6. *Physiological salt solution*.—0.85 gramme of pure crystalline sodium chloride was dissolved in 99.15 c.c. freshly distilled water, the solution being newly prepared for each experiment.

The Complement-fixation Test.

A preliminary series of bloods were examined as follows:—The complement 1 in 30 was titrated in the presence of antigen and a known normal serum in doses 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8 c.c. The hæmolytic dose having been established, four tubes were arranged for each blood and received the reagents as shown in Table I.

TABLE I.

Tube number.	Antigen.	Inactivated serum.	Complement (1 in 30).	0.85% NaCl.	Sensitised sheep's corpuscles.
	c.c.	c.c.	c.c.	c.c.	c.c.
1	0.3	0.2	0.5*	0.3	0.3
2	0.3	0.2	0.6	0.2	0.3
3	0.3	0.2	0.7	0.1	0.3
4	0.3	0.2	0.8	—	0.3
Contact for one hour at laboratory temperature and one hour 37° C. in air incubator.					
Contact for half an hour at 37° C. in air incubator.					

* Minimal hæmolytic dose.

The tubes were then placed in the ice safe overnight and the results read off on the following morning. Of 52 sera obtained from patients suffering from pulmonary tuberculosis with tubercle bacilli present in the sputum, 50 cases gave a positive reaction, and 2 cases a negative reaction. At this point I had the advantage of discussing my results with Professor Georges Dreyer, and he advised me to make quantitative examinations of the blood by progressively diluting the serum whilst keeping the other reagents constant. By trial and error the following technique was found to be most suitable for the purpose, and it was thus that the remaining 286 cases referred to in this communication were examined:—

TABLE II.

Tube number.	Antigen.	Inactivated serum.	Complement (1 in 30).	Sensitised sheep's corpuscles.
	c.c.	c.c.	c.c.	c.c.
1	0.3	0.2 undiluted.	0.5*	0.3
2	0.3	0.2 diluted 1 in 2.	0.5	0.3
3	0.3	0.2 „ 1 in 4.	0.5	0.3
4	0.3	0.2 „ 1 in 8.	0.5	0.3
5	0.3	0.2 „ 1 in 16.	0.5	0.3
6	0.3	0.2 „ 1 in 32.	0.5	0.3
7	0.3	0.2 „ 1 in 64.	0.5	0.3
8	0.3	0.2 „ 1 in 128.	0.5	0.3
9	0.3	0.2 „ 1 in 256.	0.5	0.3
Contact for an hour at 37° C. in water bath.				
Contact for half an hour at 37° C. in water bath.				

* Dose determined by titration in presence of antigen and known negative serums.

The results obtained may be conveniently given in tabular form. Table III. summarises the findings gained from single examinations in 100 cases of pulmonary tuberculosis, having tubercle bacilli

present in the sputum at the time of the blood examination, and chosen at random from the in-patients at the hospital.

TABLE III.

1. Hæmolytic with serum undiluted...	5 = 5 per cent. negative.
2. „ „ „ diluted 1/2 ...	5
3. „ „ „ „ 1/4 ...	7
4. „ „ „ „ 1/8 ...	16
5. „ „ „ „ 1/16 ...	19
6. „ „ „ „ 1/32 ...	22
7. „ „ „ „ 1/64 ...	14
8. „ „ „ „ 1/128 ...	10
9. „ „ „ „ 1/256 ...	2
100	

Of the five negative cases three were "resting febrile," one "ambulant febrile," and one "ambulant afebrile." In all five cases the duration of the disease was under one year. Subsequent re-examination of these cases gave a positive reaction in four, whilst one of them remained negative.

Table IV. deals with the strength of the reaction

TABLE IV.

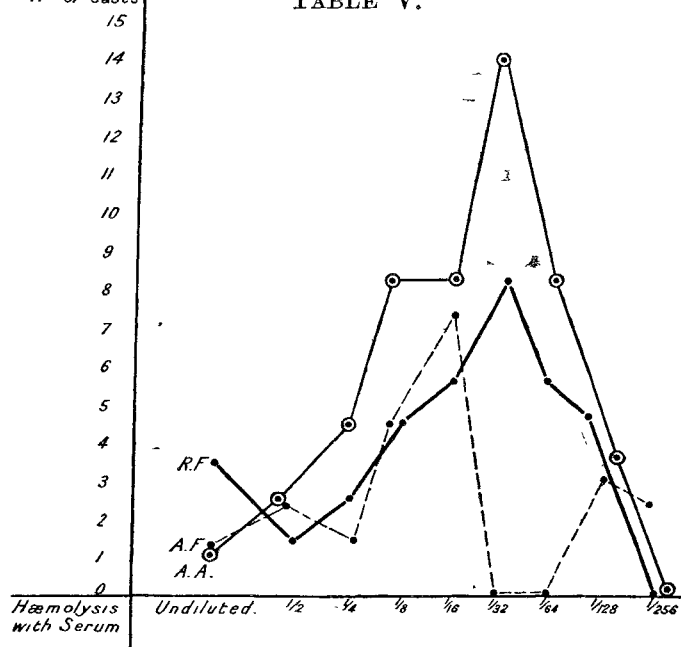
Hæmolytic with serum—	Duration of disease.			
	1 month—12 months.	1 year—3 years.	3 years—5 years.	Over 5 years.
Undiluted ...	5	0	0	0
Diluted 1 in 2 ...	5	0	0	1
„ 1 in 4 ...	5	0	1	0
„ 1 in 8 ...	6	8	0	2
„ 1 in 16 ...	11	5	2	2
„ 1 in 32 ...	12	6	1	2
„ 1 in 64 ...	9	4	0	0
„ 1 in 128 ...	5	4	2	0
„ 1 in 256 ...	1	1	0	0
Total ...	59	28	6	7

in relation to the duration of the disease, and is self-explanatory.

Table V. graphically represents the frequency of

N° of Cases

TABLE V.



R.F., Resting febrile (32 cases). A.F., Ambulant febrile (20 cases). A.A., Ambulant afebrile (48 cases).

strong and weak reactions in febrile and afebrile cases. It also is self-explanatory.

For controls reliance had to be placed on bloods obtained from patients admitted to general hospitals in London suffering from diseases presumably other than tuberculosis. Manifest sources of error creep in here. Firstly, cases admitted for the treatment of traumatic, infective, or neoplastic diseases, or of diseases anatomically defective in origin, are not minutely examined for evidence of tuberculous infection. Secondly, in many of the cases here dealt with a special examination with a view to determining the presence or absence of active tuberculous infection was impossible. Thirdly, tuberculous infection, at all events of a transient nature and of such a degree of activity as only to be detected with difficulty, is common amongst the hospital classes of the population of the country. Such evidence as is to hand tends to show that the number of positive reactions would be considerably diminished were a series of healthy adults drawn from the same social status to act as controls.

TABLE VI.

1. Hæmolysis with serum undiluted	...	76	= 76% negative.
2. " " " diluted 1/2	...	7	
3. " " " " 1/4	...	8	
4. " " " " 1/8	...	5	
5. " " " " 1/16	...	4	24% positive.
6. " " " " 1/32	...	0	
7. " " " " 1/64	...	0	
8. " " " " 1/128	...	0	0% positive.
		100	

Table VI. gives the results obtained, and is valuable in that it shows that no positive reactions were obtained with a 32-fold dilution of the serum, whilst reference to Table III. shows that nearly one-half of the certainly active cases of pulmonary tuberculosis give a positive reaction with the serum thus diluted.

The composition of the antigen suggested the possibility of a positive reaction being obtained in cases of syphilitic infection. Indeed, this proved to be the case. Samples of serum examined by independent observers at several London hospitals were investigated according to the technique described above, and the following results were obtained:—

TABLE VII.

15 Wassermann negative sera.	
1 ...	{ Complement-fixation test negative in 13
"	" " positive in 2*
21 Wassermann positive sera.	
2 ...	{ Complement-fixation test negative in 0
"	" " positive in 21

* The clinical diagnosis was not inquired into in these cases.

It remained, therefore, to determine in how far this necessitates the simultaneous determination of the Wassermann reaction if the test is to be used for diagnostic purposes.

In a series of 158 cases of pulmonary tuberculosis at the Brompton Hospital, excluding those cases with clinical manifestations or a history of syphilis, Captain A. N. Dickson, I.M.S., and myself have found that 8 cases (5 per cent.) gave a positive Wassermann reaction. Strictly speaking, then, the possibility of a positive Wassermann reaction interfering with the correct interpretation of the test must be excluded if the test be applied for the diagnosis of tuberculous infection. In practice, however, it can hardly be gainsaid that such a precaution, at all events in reasonably selected cases, need not be seriously considered so long as the contingency is borne in mind.

Fifty cases were examined because the diagnosis of active tuberculosis was uncertain. It must be understood that the positive diagnosis in these cases may have been probable or improbable; the sole reason for their investigation was the absence of tubercle bacilli from the sputum. Table VIII. summarises the results obtained.

TABLE VIII.

1. Hæmolysis with serum undiluted...	...	20	= 40% negative.
2. " " " diluted 1/2	...	4	
3. " " " " 1/4	...	10	
4. " " " " 1/8	...	7	
5. " " " " 1/16	...	3	60% positive.
6. " " " " 1/32	...	1	
7. " " " " 1/64	...	3	
8. " " " " 1/128	...	2	

It is suggestive that these figures almost exactly coincide with those obtained in a similar class of case investigated by means of the tuberculo-opsonic index test.

Exercise produces no immediate quantitative effect upon the antihæmolytic power of tuberculous serum as the following experiment shows:—

TABLE IX.

Case.	Hæmolysis with inactivated serum.								Tuberculo-opsonic index.	
	Un-diluted.	1/2	1/4	1/8	1/16	1/32	1/64	1/128	Active serum.	Heated serum.
1.—At rest ...	0	0	+	C	C	C	C	C	0.78	0.16
After 1 1/2 miles' walk	0	0	+	C	C	C	C	C	1.00	0.47
2.—At rest ...	0	0	0	0	0	+	C	C	0.88	0.32
After 3 miles' walk	0	0	0	0	0	+	C	C	0.68	0.28
3.—At rest ...	0	0	+	C	C	C	C	C	1.04	0.12
After 1 mile walk	0	0	+	C	C	C	C	C	1.00	0.06

0 = no hæmolysis. + = partial hæmolysis. C = complete hæmolysis.

Conclusions.

- 1. The antihæmolytic power of tuberculous serum can be made manifest in a hæmolytic experiment by introducing Besredka's antigen into the mixture.
- 2. Repeated positive reactions, especially with a 32-fold serum dilution, in the absence of a positive Wassermann reaction, indicate the presence of an active tuberculous lesion.
- 3. Repeated negative reactions indicate the absence of an active tuberculous lesion, if cases of under 12 months' duration be excepted.

A word of thanks is due to those who have helped in the accumulation of material for this research. To those physicians at Brompton Hospital who so unreservedly placed cases under their charge at my disposal, to Dr. Arthur Latham, Dr. J. W. Linnell, Dr. D. Embleton, Dr. A. Paine, Dr. A. Leitch, Mr. E. A. Tozer, Dr. H. A. Treadgold, and Dr. W. P. Johnston I express my gratitude. To Professor Georges Dreyer I owe an especial debt of gratitude for friendly criticism and advice. To M. Besredka I extend my thanks with the wish that his labours in the interest of suffering mankind may meet with the success they deserve.

Bibliography.—A. Besredka: Annales de l'Institut Pasteur, November, 1913. A. Besredka et J. Manoukhim: Comptes Rendus de la Société de Biologie, vol. lxxvi., p. 130. A. Besredka et F. Jupille: Ibid., p. 197. E. Debains et F. Jupille: Ibid., p. 199. Kuss, Leredde, et Rubinstein: Ibid., p. 244. A. C. Inman: Ibid., p. 251. A. Besredka: Comptes Rendus de l'Académie des Sciences, vol. clvi., p. 1633. Brompton Hospital, S.W.