

determined on the present knowledge of the subject; but in the case of animals Bartel<sup>2</sup> has demonstrated that tubercle bacilli may reside in the tissues as long as 104 days without producing any specific changes. He used for his experiment rabbits and guinea-pigs which were fed with human tubercle bacilli, and found that the glands of the infected animals, though normal to the naked eye and microscopic examination, contained tubercle bacilli, as was proved by their potency in producing tuberculosis in animals on inoculation.

Summarising the results obtained by the various workers and myself gives the following table.

TABLE III.

Authors.	Cases examined.	Latent cases.	Latent cases %	Glands in which latent tubercle bacilli were demonstrated.		
				Cervical.	Bronchial.	Mesenteric.
Kæble ... ..	23	2	8·7	—	2	—
Harbitz ... ..	91	18	19·7	16	3	3
Weber u. Baginsky	25	1	3·85	1	—	—
Ipsen ... ..	74	1	1·35	—	—	1
MacFadyean and MacConkey ...	20	2	10·0	—	—	2
Bartel ... ..	68	8	11·75	5 (a)	3	2
Rosenberger ...	14	6	42·85	—	—	6
Goodale ... ..	9	2	22·2	2 (b)	—	—
Own cases ... ..	32	3	9·4	1	1(c)	1
To als ... ..	357	43	12·05	25	9	15

(a) Including 4 tonsils. (b) Tonsils. (c) Bronchial and retroperitoneal glands.

My deep indebtedness is due to Professor James Ritchie for suggesting the line of research, and for his kind supervision and helpful criticism, and to Dr. J. P. McGowan and Dr. James Dawson for help generously given in many directions. To the pathologists, past and present, of the Edinburgh Royal Infirmary and of the Edinburgh Royal Hospital for Sick Children I wish also to record my sincere thanks for courtesy in providing me with material and in allowing me to have access to their post-mortem notes. I have also to thank the Laboratory Committee for all the facilities afforded me in the Royal College of Physicians' Laboratory, Edinburgh, and the Trustees of the Carnegie and the McCunn Research Scholarships, and of the Moray Research Fund for financial assistance.

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**RELIEF WORK IN ARMENIA.**—The chairman of the Armenian Refugees (Lord Mayor's) Fund sends us an account of three months' work in the devastated vilayets of Armenia from an eye-witness, Rev. Harold Buxton, honorary secretary to the fund, who has just returned to England. It is urgently necessary that public opinion should be focussed on the position of these most unfortunate victims of the war. Mr. Buxton confirms the terrible stories of their sufferings told in the House of Lords some months ago by Lord Bryce. Of the estimated 2,000,000 Turkish Armenians, perhaps 1,000,000, the account goes, have been deported and 500,000 massacred. Only some 200,000 escaped into the mountains and so across to Russian soil. These are the people whom the fund is at present relieving, and help must be available during the coming winter. The most urgent matter is now to repatriate them on their own lands. The address of the fund is 96, Victoria-street, S.W.

## THE DIAGNOSIS OF THE ENTERIC FEVERS IN INOCULATED INDIVIDUALS BY THE AGGLUTININ REACTION.

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A REPORT TO THE MEDICAL RESEARCH COMMITTEE.

In addition to material collected at the Department of Pathology in Oxford, we refer in the following communication to details of material already published in outline by Dreyer and Torrens from the — Stationary Hospital, British Expeditionary Force. For permission to make use of this material we are greatly indebted to the officer commanding, Lieutenant-Colonel Evans, D.S.O., R.A.M.C., as well as to the officer then in charge of the bacteriological laboratory of that hospital, Lieutenant-Colonel Grattan, R.A.M.C., and his staff of workers.

The agglutinin reaction constitutes the most valuable guide which we possess for the diagnosis of the enteric fevers (typhoid, paratyphoid A, and paratyphoid B). For while direct cultivation from blood, faeces, and urine is, when successful, final and convincing, the percentage of cases in which positive results are obtained by culture methods does not at a liberal estimate exceed about 50 per cent. Among these cases will, of course, be also included carriers not actually suffering from active disease.

Unfortunately, there appears to exist a belief that the introduction of protective inoculation against *B. typhosus*, and more recently against the paratyphoid bacilli also, has rendered the agglutinin reaction less reliable or less conclusive diagnostically than was formerly the case. This belief, however, is not justified by the evidence available; though it is undoubtedly the fact that the practice of inoculation has not only enhanced certain difficulties which already existed, but also introduced some new difficulties of its own. As a result of this it is no longer possible to make use with advantage of certain methods largely qualitative in character which before were commonly considered adequate for diagnostic purposes. And it has become necessary to employ some accurately quantitative method. Moreover, since the diagnosis now depends on the results of a series of several (three or more) successive observations, the method used must be one whose results on successive occasions are strictly comparable, *inter se*, and can be expressed in definite and accurate measurements relative to some fixed standard. Such a method has the further very great advantage that wherever and whenever such tests may be performed they will form a part of a homogeneous statistical material. All these requirements are met by the method which has been rendered generally available in naval and military hospitals for the past 12 months by the issue, on behalf of the Medical Research Committee, of the standardised agglutinable cultures prepared in Oxford. But the diagnosis of individual cases could equally well be carried out by any other method of an accurately quantitative character if such method be in use.

The curious and widespread lack of appreciation which still exists with regard to the necessity for employing exact quantitative methods in performing agglutination tests in inoculated individuals has led to most remarkable and contradictory statements. Thus, while C. D. Hamilton,<sup>1</sup> for example, declares that "a positive Widal reaction in any person who has ever been inoculated with the antityphoid vaccine is of no value in diagnosing typhoid fever," H. L. Tidy<sup>2</sup> maintains that "a positive agglutinative reaction to bacillus typhosus after the fifth day of pyrexia is as definite a proof of typhoid fever in an inoculated man as in a non-inoculated one."

The answer to these statements is that the exact measurement of the agglutinin titre of inoculated persons by means of standardised agglutinable cultures shows that they are

equally erroneous. On the one hand, active infection can be diagnosed as well in typhoid-inoculated individuals as in non-inoculated persons; and, on the other hand, the absence of inoculation agglutinins within the first 12 months or more after inoculation is a very rare occurrence in properly inoculated individuals, and is no more frequent in the subjects of pyrexial attacks than in persons who remain in perfect health.

These points have already been dealt with on several occasions by ourselves and others. There is, however, this difference as regards practical bearing between the views of Hamilton and Tidy—namely, that while Hamilton's contention, if accepted, merely leads to the result that about half the cases will go undiagnosed, Tidy's view would entirely vitiate all statistical material bearing on the protective value of antityphoid inoculation. Since, as we have stated before, the vast majority of all febrile attacks occurring in persons inoculated within a year or so would, if his conclusions were accepted, necessarily be diagnosed as typhoid fever. Now that a triple (typhoid, paratyphoid A, and paratyphoid B) vaccine is in general use in the armies at war, the questions at issue will acquire a steadily increasing importance. Accordingly, although we have already endeavoured to explain the fallacy of the contentions put forward by Tidy, his continued efforts to elaborate a defence of his views, his failure to produce evidence in support of them, and the misuse of the data published by ourselves and others into which he has been led, make it necessary for us to seek indulgence while we return once more to the examination of his contentions. We may add that were it not for the urgent practical importance of enteric diagnosis under existing conditions we should not have considered it at all necessary to deal further with statements so obviously at variance with the accumulated evidence of different observers as those put forward by Tidy.

#### H. L. Tidy's Contentions.

We have already quoted one of Tidy's leading statements made at the discussion on paratyphoid fever at the Royal Society of Medicine.<sup>3</sup> We now place in parallel columns the views which he put forward in THE LANCET<sup>2</sup> in the early part of this year and our own replies based on evidence which we presented in the same journal.<sup>4</sup>

##### Tidy's Conclusions.

"1. Inoculation agglutinins are diminished or entirely removed by febrile conditions. It is possible that they are converted into agglutinoids."

"2. In certain cases they may return, but usually do not do so."

"3. A positive agglutination reaction to B. typhosus after the fifth day of pyrexia has the same value in an inoculated as in an uninoculated person."

"4. Immunity conferred by inoculation may be affected at the same time as the inoculation agglutinins."

##### Evidence presented by us to show that:

"1. The febrile condition associated with paratyphoid fever does not cause disappearance of typhoid agglutinins from the blood of persons inoculated against typhoid."

"2. Other febrile conditions also, in so far as they have hitherto presented themselves, do not cause disappearance of typhoid agglutinins from the blood of inoculated persons."

"3. The statement 'opposite' is entirely erroneous. Its acceptance would lead to such deplorable results in diagnosis that we feel compelled to repeat its categorical denial."

These three statements in reply to Tidy's conclusions were proved quite fully by the figures which we published. But shortly after the appearance of our contradiction Tidy, ignoring altogether the fact that this demonstration of the futility of his original contentions had been afforded, but at the same time tacitly abandoning all further effort to defend them, proceeded in a letter to the Editor of THE LANCET<sup>5</sup> to re-shuffle his cards. He then took up an entirely new position, but with such adroitness as to convey to the casual reader the impression that what he now put forward was actually the view that we had attacked. And he took us severely to task for having failed to offer evidence adequate to disprove this new theory which had not been before us when we wrote. Finally, by making considerable play with a totally inaccurate interpretation of the figures which we submitted he purported to show that they were really favourable to his view. Upon this letter a brief comment was written by one of us (E. W. A. W.), pointing out that Tidy had been entirely misled in his use of our figures. Tidy then replied in a new letter,<sup>7</sup> which showed still more clearly his

inability or unwillingness to appreciate the meaning of numerically expressed scientific observations, and castigated us again, because, as he stated, we had failed to submit figures in disproof of his revised but equally fallacious views which, as a matter of fact, he has not himself as yet supported by any solid or significant evidence.\*

Having thus briefly, but as we believe correctly, indicated the general character of Tidy's dialectic, we shall now try to deal as concisely as possible with the details of his arguments, and to show how completely he has misinterpreted the evidence.

On two different occasions Tidy applied two different processes to our figures. On the first occasion he compared the arithmetical mean of the numbers of standard agglutinin units found in normal inoculated individuals (Dreyer and Inman<sup>6</sup>) with the means of the numbers of the units found in inoculated persons suffering from paratyphoid fever or other febrile conditions. This procedure was fallacious for the following reasons.

1. The relatively limited number of observations given in each of our series showed an enormous range of individual variation, as, for instance, among 74 normal inoculated individuals a range from 30 to 1390 standard agglutinin units; in the case of 75 individuals suffering from paratyphoid fevers a range from 6 to 875 units; and in 33 cases of other febrile conditions a range from 6 to 438 units. They therefore constituted a material to which the method of taking means was quite inapplicable.

2. The normal inoculated individuals were not comparable as regards inoculation agglutinins with the febrile cases, since the former were examined within a comparatively short time of their inoculation, while the latter were only observed after the lapse of a much longer interval of time. This point is naturally fundamental, since, as is well known, the inoculation agglutinins steadily diminish from the period, within a few weeks of the date of inoculation, when their maximum is reached.

On the second occasion Tidy instituted a comparison between the values of the middle terms of the different series. This method, however, was equally unsuitable, and for the same reasons. Moreover, the fact that the figures which he arrived at by the method of means were widely divergent from those which he obtained by his second method of taking middle terms should have afforded him a mathematical demonstration of the fact that both these methods of comparison were inapplicable to the figures in question.

To make this point clear we place in parallel columns the results obtained by taking arithmetical means and by taking middle terms as done by Tidy.

TABLE I.

Series.	Averages arrived at by	
	Arithmetical means.	Middle terms.
Normal typhoid inoculated persons ... ..	238.9†	139.0†
Paratyphoid A occurring in typhoid inoculated persons ... ..	37.2	32.5
Paratyphoid B occurring in typhoid inoculated persons ... ..	150.3	75.0
Other febrile conditions occurring in typhoid inoculated persons ... ..	77.5	44.0

† Tidy gives these figures incorrectly as 225 and 138 respectively.

Now it is well known that unless the figure obtained by taking the arithmetical mean of a series of observations is approximately the same as the value of the middle term of the series, the material concerned cannot properly be used for the purpose of arriving at mean values. In the table above it is seen that only in one of the series (paratyphoid A cases) do these figures at all approach each other in value. In the paratyphoid B series the figure for the arithmetical mean is double that of the middle term of the series. And in the other two series the difference is enormous, though not quite so great. It follows that the arguments which Tidy bases on the mean values obtained from our figures by these two methods necessarily fall to the ground.

\* Since the present communication was written it has come to the knowledge of one of us that Dr. Tidy prepared his material for publication some eight months ago. Its non-appearance hitherto has been due to circumstances beyond his control.

That the inoculation agglutinins do not disappear as a result of febrile attacks we had already proved. And from what has just been said it is evident that our material cannot be made use of to support the view that they are diminished.

Tidy further states that if he applies a method of comparison which we ourselves made use of—namely, to take the presence of as much as 25 Standard Agglutinin Units per c.c. of serum as an arbitrary standard of comparison—to the normal inoculated individuals in Dreyer and Inman's series and to our series of cases, he finds that the febrile cases show a marked diminution in inoculation agglutinins. This limit of 25 agglutinin units was chosen by us as an arbitrary point of comparison as being very far above the titre of typhoid agglutinins found in normal non-inoculated persons who have not had typhoid fever, since such persons only very exceptionally exhibit the presence of as much as 4 standard units. It in no way represents a diagnostic limit. But this method of comparison was equally inapplicable as employed by Tidy for the reasons already given—namely, that the normal individuals and the febrile cases constituted dissimilar materials, owing to the fact that the two series were examined at very different times from the dates of their protective inoculation. On the other hand, the material to which we applied this form of comparison was not subject to any such objection.

In this connexion we must advert to an astonishing statement made by Tidy in the course of his arguments—namely, that a serum titre of 100 Standard Agglutinin Units “apparently corresponds to a ‘positive’ reaction in non-inoculated persons.” It is difficult to understand how so entirely fallacious a statement could commend itself to anyone acquainted with the ordinary limits which have been recognised for years as forming a reliable standard in the serum diagnosis of typhoid infection, and still less to anyone who had read attentively the communications which he undertook to criticise. For 100 standard units correspond to standard agglutination in a serum dilution of anything from 1 in 250 to 1 in 700 or 800 according to the sensitiveness of the particular standardised agglutinable culture employed. Whereas it is definitely laid down in the directions for the use of standardised agglutinable cultures, which since the middle of July, 1915, have been issued from this department on behalf of the Medical Research Committee, as follows: “In *non-inoculated persons* who have not had typhoid (or paratyphoid) fever agglutination in a dilution of 1 in 25 justifies a strong suspicion of typhoid (or paratyphoid) infection. But the test must be applied again in the course of a few days to ascertain whether there is any change in the titre of agglutination. Marked agglutination in a dilution of 1 in 50 or more is (nearly always) diagnostic of active typhoid (or paratyphoid) infection.” That is to say that the presence of from 4 units to 10 units of agglutinin justifies strong suspicion; and the presence of 10 units to 20 units is (nearly always) definitely diagnostic. These limits of serum dilution were clearly stated by one of us (G. D.) as long ago as 1906,<sup>9</sup> and have been maintained in all subsequent communications, since all experience showed them to be fully adequate. It was on this account that we were able to deny Tidy's statement that “a positive agglutination reaction to B. typhosus after the fifth day of pyrexia has the same value in an inoculated as in a non-inoculated person,” and that “other febrile conditions” cause disappearance of typhoid agglutinins. Since, as has been shown, normal non-inoculated persons who have not had typhoid fever only very exceptionally exhibit an agglutinin titre of as much as 4 standard units, while more than 98 per cent. of the “febrile cases” which we quoted had a titre of from 6 to 875 units per cubic centimetre of serum

But Tidy further claims that the tables published by Dreyer and Torrens<sup>10</sup> in their first contradiction of his statements also show “that it is evident that the febrile cases have distinctly less agglutinin than the normal inoculated persons.” How he arrived at this conclusion from the data given we cannot conceive. But we can easily show that it is not the case by now giving, as an example, the data for the 149 paratyphoid B. cases (from the 171 cases of paratyphoid fevers then referred to) in greater detail, expressing the observations in Standard Agglutinin Units. This form of expression was not employed on the previous occasion because the tables as they stood contained ample evidence to refute the contentions then put forward by Tidy.

Table II A contains the individual observations summarised in the table published by Dreyer and Torrens from the — Stationary Hospital, British Expeditionary Force. In all the cases the B. paratyphosus B was isolated in the course of the disease. Tables II B and II C give the material

TABLE II.—Data for Typhoid-inoculated Individuals.  
A. Typhoid Agglutinin Units in Paratyphoid B Fever chiefly during the Acute Stage.

No.	Standard agglutinin units.	No.	Standard agglutinin units.	No.	Standard agglutinin units.	No.	Standard agglutinin units.	No.	Standard agglutinin units.	No.	Standard agglutinin units.	No.	Standard agglutinin units.
1	20,000	23	1850	45	926	67	500	89	235	111	180	133	65
2	17,900	24	1800	46	925	68	500	90	235	112	163	134	64
3	13,000	25	1700	47	900	69	500	91	235	113	148	135	52
4	8,200	26	1700	48	900	70	500	92	235	114	148	136	50
5	6,100	27	1630	49	870	71	481	93	230	115	135	137	50
6	4,000	28	1540	50	820	72	475	94	225	116	135	138	50
7	3,520	29	1540	51	812	73	465	95	210	117	100	139	50
8	3,370	30	1480	52	810	74	465	96	200	118	100	140	42
9	3,330	31	1350	53	740	75	462	97	200	119	100	141	37
10	3,250	32	1300	54	740	76	370	98	200	120	100	142	34
11	3,200	33	1110	55	690	77	359	99	200	121	100	143	26
12	2,770	34	1040	56	675	78	333	100	200	122	100	144	24
13	2,600	35	1040	57	650	79	330	101	200	123	100	145	24
14	2,580	36	1000	58	640	80	277	102	200	124	100	146	15
15	2,560	37	1000	59	640	81	277	103	200	125	100	147	11
16	2,500	38	1000	60	580	82	275	104	200	126	100	148	10
17	2,500	39	1000	61	562	83	275	105	200	127	100	149	5
18	2,300	40	1000	62	555	84	270	106	200	128	93		
19	2,220	41	1000	63	540	85	270	107	200	129	83		
20	2,050	42	930	64	540	86	256	108	192	130	83		
21	2,000	43	926	65	512	87	236	109	186	131	69		
22	1,850	44	926	66	500	88	235	110	185	132	67		

B. Typhoid Agglutinin Units in Paratyphoid B Fever, observed chiefly during Convalescence.

1	875	6	219	11	88	16	75	21	44	23	37	31	19
2	875	7	219	12	88	17	75	22	44	27	28	32	9
3	650	8	162	13	88	18	65	23	44	28	22	33	6
4	438	9	88	14	83	19	44	24	44	29	22		
5	219	10	88	15	88	20	44	25	44	30	21		

C. Typhoid Agglutinin Units in Paratyphoid A Fever, chiefly during Convalescence.

1	88	7	75	13	44	19	37	25	22	31	21	37	14
2	88	8	75	14	41	20	37	26	22	32	21	38	9
3	75	9	56	15	37	21	37	27	22	33	21	39	7
4	75	10	56	16	37	22	28	23	22	34	19	40	7
5	75	11	56	17	37	23	23	29	22	35	19	41	7
6	75	12	44	18	37	24	28	30	22	36	14	42	6

D. Typhoid Agglutinin Units in Normal Inoculated Persons.

1	1,500	12	348	23	278	34	139	45	76	56	61	67	36
2	1,390	13	348	24	278	35	139	46	76	57	61	68	36
3	1,390	14	300	25	162	36	139	47	76	58	61	69	36
4	1,250	15	300	26	162	37	139	48	76	59	60	70	36
5	750	16	300	27	162	38	139	49	70	60	60	71	35
6	600	17	300	28	150	39	139	50	70	61	60	72	35
7	600	18	300	29	150	40	139	51	70	62	59	73	30
8	580	19	278	30	150	41	135	52	67	63	59	74	30
9	580	20	278	31	139	42	132	53	67	64	56		
10	580	21	278	32	139	43	130	54	61	65	36		
11	350	22	278	33	139	44	130	55	61	66	36		

published by Dreyer, Gibson, and Ainley Walker from the 3rd Southern General Hospital, and Table II D the data for normal inoculated individuals obtained by Dreyer and Inman. All the data are expressed in terms of standard typhoid agglutinin units per cubic centimetre of the serum.

It is obvious on the most cursory inspection of these tables that it would be entirely irrational to attach any kind of value whatever to their arithmetical means or to their middle terms for the purpose of direct comparison between the different series, since they show a range of individual variation so extreme—as, for example, from 5 to 20,000 Standard Agglutinin Units in a series of 149 observations.

To show how utterly irrelevant this method of comparison is for the figures in question, we give in Table III. the figures representing the arithmetical means and middle terms of the series of observations recorded above, either individually or in various combinations. The entire lack of agreement between the value of the different arithmetical means and

TABLE III.

Series.	Average arrived at by	
	Arithmetical means.	Middle terms.
1. Normal typhoid inoculated person (72 cases)...	238.9	139.0
2. Paratyphoid B observed chiefly during the acute stage (149 cases) ... ..	1146.8	462.0
3. Paratyphoid B observed chiefly during convalescence (33 cases) ... ..	150.3	75.0
4. All the cases of paratyphoid B taken together (224 cases) ... ..	966.1	235.5
5. All the cases of paratyphoid B and paratyphoid A taken together (255 cases) ... ..	791.9	200.0

the corresponding middle terms for each series of observations renders obvious the illegitimate character of such a method of comparison in the case of the observations concerned.

Nevertheless, it may be of interest to note for the benefit of anyone to whom the elementary principles of mathematics are unfamiliar what extraordinarily adverse evidence as to the validity of Tidy's views would be afforded by this erroneous method of comparison. Thus it is seen that the agglutinin titre in those cases of paratyphoid B fever which were for the most part examined during the acute stage of the disease yield an arithmetical mean 4.8 times as great as the arithmetical mean of the titres found in normal inoculated individuals. Expressed by middle terms the former series shows a titre 3.3 times as great as that in the latter. Assuredly there is here no evidence of diminution or disappearance of typhoid agglutinins as the result of the pyrexial attack. Similarly, when all the paratyphoid B cases are taken together the arithmetical mean of their agglutinin titres and the middle term are respectively 4 times and 1.7 times as great as the corresponding figures for the normal inoculated individuals. In the same manner, taking all the paratyphoid fever cases A and B together, the arithmetical mean and the middle term of their agglutinin titres are respectively 3.3 times and 1.4 times as great as in the case of normal inoculated individuals.

On the other hand, if we consider by themselves the cases of paratyphoid B fever observed chiefly during convalescence it is seen that the typhoid agglutinin titre of normal inoculated individuals is 1.6 times, or 1.9 times as great as that of the former, according as we take arithmetical means or middle terms. But this difference is an entirely artificial and meaningless one, since the cases of paratyphoid B fever observed in the acute stage have themselves a titre 7.6 times, or 6.3 times as great as that of convalescent cases according as we take arithmetical means or middle terms. There is therefore no evidence whatever in any of the data given above which lends the least support to Tidy's views. Moreover, our emphatic and repeated contradiction of his statement that inoculation agglutinins are removed or markedly diminished by pyrexial attacks, and that a positive Widal reaction after the fifth day of pyrexia has the same value in an inoculated as in an uninoculated person, is not based exclusively on the extensive material which has accumulated during the present war. It is in absolute agreement with the important and careful observations carried out by Grattan and Harvey, and Grattan and Wood some years ago in their investigation of paratyphoid fever in India. Thus Grattan and Harvey<sup>11</sup> showed that in undoubted cases of paratyphoid A fever in typhoid-inoculated individuals (in which the bacillus was recovered from the blood) a marked increase in the agglutinins for B. typhosus was noted. They further stated that "these cases would have been diagnosed on the Widal reaction as mild cases of true enteric fever." In a later communication Grattan and Wood<sup>12</sup> repeated and amplified the foregoing conclusions, and laid particular stress on the danger of errors in diagnosis arising from this increase in inoculation agglutinins.

The fact of the matter is that the question whether the inoculation agglutinins are diminished at all or not after the

fourth or fifth day of pyrexia can never be settled finally by comparing different individuals. Since the range of individual variation is so enormous as regards the agglutinin content of the serum, whether in normal inoculated persons or in those attacked by fever, it can only be decided by following the serum titre of individuals during the acute infection by means of exact quantitative measurements capable of reduction to a constant standard so as to ascertain what are the changes which do actually occur in their agglutinin titres. This is done in all our cases as a matter of routine, since, as has been fully explained on a previous occasion (as well as in the directions for the use of Standard Agglutinable Cultures) it is necessary to do so in order to arrive at a reliable serum diagnosis.

The effect of the pyrexial condition associated with paratyphoid B infection is exemplified in the cases shown below in Table IV., which are taken from cases where the titre of typhoid agglutination was definitely affected by the febrile

TABLE IV.—Paratyphoid B Fever in Typhoid-inoculated Individuals.

Case.	Day of illness.	Standard agglutinin units per c.c. of serum.		
		Typhoid.	Paratyphoid A.	Paratyphoid B.
1	9	556.0	< 1.4	2060.0
	13	3700.0	< 1.4	10600.0
	17	13090.0	< 1.4	29400.0
	21	3700.0	< 1.4	16480.0
2	8	37.0	< 1.4	29.4
	15	83.0	< 1.4	735.0
	22	55.6	< 1.4	1040.0
	32	25.9	< 1.4	470.0
3	10	46.3	< 1.4	588.0
	14	278.0	< 1.4	2940.0
	18	650.0	< 1.4	11180.0
	22	593.0	< 1.4	8240.0
4	9	481.0	< 1.0	588.0
	13	2960.0	< 1.0	7650.0
	17	3520.0	< 1.0	26400.0

attack. In other cases, as we have shown elsewhere, the typhoid agglutination titre may remain unchanged or only slightly increased throughout the course of paratyphoid fever. But wherever the typhoid agglutination titre is affected at all the change produced is one of active rise, subsequently followed by a fall towards the former level.

When such rise occurs its beginning is either somewhat antecedent to, or synchronous with, the beginning of the rise in paratyphoid B agglutination titre. Its maximum is reached either before or at the time that the paratyphoid B agglutinins reach their maximum. And, as seen from the examples given in Table IV., in cases where the typhoid maximum precedes the maximum of paratyphoid B agglutination the subsequent fall in typhoid titre progresses rapidly, while the paratyphoid B titre continues to rise sharply to its maximum. This maximum occurs between the sixteenth and twenty-fourth (usually the eighteenth to twentieth) day of the disease (as is also the case in typhoid fever and in paratyphoid A fever), after which the usual fall in paratyphoid B titre sets in.

The cases quoted in Table IV. are a few examples from a long series. They prove clearly that there is no diminution or disappearance of typhoid inoculation agglutinins as the result of the pyrexial condition in paratyphoid fever. It follows that in view of the great mass of evidence produced by other workers, as well as by ourselves, it is obvious that Tidy's startling conclusions, which we have been at pains to discuss in detail, are entirely erroneous. Moreover, he has not himself as yet offered any substantial body of data in their support. The real question at issue is a simple question of facts, and there we now propose to let the matter rest.

Abortive Cases of Typhoid and Paratyphoid Fevers.

The occurrence of mild cases of enteric fever has long been recognised, and Woodruff and others have called special attention to the danger of neglecting these cases, which may only have slight fever lasting a few days. In practice they are often taken for other conditions, and are not uncommonly put down as febricula. Sometimes, as Osler and McCrae point out, they may only be recognised through the routine Widal reaction. If these facts were true before the introduction of protective inoculation, still more must they be



true since the widespread introduction of inoculation against typhoid and the paratyphoid fevers.

The effect of prophylactic inoculation is threefold: firstly, it diminishes the incidence of these infections; secondly, it lessens the mortality among those infected; and, thirdly, it increases the proportion of mild and atypical cases among the latter. Now, in non-inoculated persons the diagnosis of mild cases is not difficult where the Widal reaction is employed as a matter of routine. But in inoculated individuals the mere detection of agglutinin is, of course, without significance. Accordingly, all doubtful febrile cases, of however short duration, occurring in inoculated persons, require a quantitative determination of their agglutinin titre for typhoid and paratyphoid bacilli on three or more successive occasions at a few days' interval before the presence of typhoid or paratyphoid infection can be excluded.

In a certain number even of the mildest cases of these infections the rise and subsequent fall in the agglutinin titre are so definite that the diagnosis could never be in doubt. But in other cases the rise and fall of the curve are much less marked, and differences of opinion may exist as to whether the case is one of active infection or not. In order to assist in the elucidation of such cases it may be stated that the following points are of importance in the interpretation of the agglutinin curves.

1. The maximum agglutinin titre of active typhoid or paratyphoid infection occurs between the sixteenth and twenty-fourth day of the disease, and most frequently about the eighteenth to twentieth day.

2. If the maximum is reached at what appears to be an earlier date, it is important to institute a careful inquiry into the actual date of onset of the illness.

3. If it is clear that the maximum falls markedly outside the limits given above (day 16-24) a diagnosis of typhoid or paratyphoid fever should not be based on a rise in titre of only moderate extent—i.e., a 100 or 200 per cent. increase in agglutinin titre. Because experience is not at present sufficient to exclude the possibility that a rise of this extent may be due to other febrile conditions.

4. In following out the titration of the patient's serum on several successive occasions it will frequently be found that the maximum has fallen between two dates of observations. And two successive observations at about the same level do not mean that the curve is stationary at this point, but merely that the maximum has occurred between there. Similarly, if the two highest observations are at different levels, it does not follow that the highest titre observed represents the maximum of the agglutinin curve. But it does follow that the maximum has occurred between these points.

5. In inoculated persons among whom mild and atypical attacks of typhoid (or paratyphoid) fever are likely to occur with fever of perhaps only a few days' duration, and with few if any of the usual symptoms, the diagnosis of typhoid or paratyphoid fever must not be rejected without the most careful consideration. As far as experience at present goes, if a regular rise and subsequent fall, even of only 100 or 200 per cent., occurs in the typhoid (or paratyphoid) agglutinin titre of the serum, and its maximum clearly falls between the sixteenth and twenty-fourth day from the onset of illness, the case is likely to be one of typhoid (or paratyphoid) infection. And we must not be misled by the fact that the patient may be convalescent or quite well again long before the date when his maximum agglutinin titre is reached.

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## A METHOD OF DROP-MEASURING LIQUIDS AND SUSPENSIONS.

(SUPPLEMENTAL NOTES AND NOTES ON APPLICATIONS.)

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REFERENCE to the various topics of the two articles—the article in *THE LANCET* of Dec. 4th, 1915, and this article—will be facilitated by the following summary of contents. The roman numeral refers to the article, the ordinary number to the paragraph.

Surface tension promotes accurate delivery by drops and hinders accurate delivery otherwise of small quantities of liquids: I., 1-3; II., 61. Outline of this drop-measuring method: I., 4-17; II., 2. The conditions determining drop-size briefly stated: I., 4; cf. II., 24. Tubing required: I., 6; II., 15. Drawing pipettes: I., 7. Gauging: I., 8. Testing drop-count of a liquid: I., 9, especially by small measure-flask: II., 23. Effect of drop-rate on drop-volume: I., 10; II., 26. Mercury-plunger tube: I., 11; II., 64. A method of using the test: I., 11. Separator cylinder with Mariotte tube: I., 15, 16. Resistance in pipettes by capillary, cone-valve, &c.: I., 10; II., 17.

Apologia for the second article: II., 1-3. Fundamental physical phenomena: II., 4-8. Apparently these phenomena are misunderstood in some quarters: II., 9, 10. Errors in "Standard" dropping-pipettes: II., 9, 10; 34-42. Corrective experiments: II., 12. Table of gauge-sizes, Lancashire (i.e., Stubbs) and Morse; drops per c.c.; c.mm. per drop: II., 20. Explanation of table: II., 19, 21, 15. Various liquids have various surface tensions: II., 22 *et passim* in the applications. Making and use of small measure-flask for checking drop-count: II., 23. Acid-fast marking with blue-black ink: II., 23. Recapitulation of essentials for correct drop-measuring: II., 24. Drop-volume increases more and more rapidly as pipette slopes from vertical: II., 25.

List of applications of the method: II., 28. Bibliography of the method: I., 1, note; II., 28. Applications in stalagmometry and in viscosimetry: II., 29. In the Widal test: II., 30-33. Critique on the drop-measuring part of "Standard" "Directions" and pipettes: II., 34-42. Micro-Wassermann technique: II., 43-55. Counting suspended bacteria and other cells: II., 56-66. General features of technique: II., 56. Staining bacteria in milk, in vaccines, in water: II., 58. Removing stain-adsorbing salts: II., 59. Method of using oil-immersion lens on stage micrometer: II., 60. Importance of direct bacteria-count of water and of milk: II., 61. Staining drop-films of c.s.f. cells: II., 62. Blood dilutions: II., 63, 65. Steady filling of hæmocytometer pipette by mercury-plunger tube: II., 64. Super-lability of some red blood corpuscles: II., 66. Summary: II., 67.

In this and in the earlier article is described a simple method designed to make practically uniform drops of any required size "grow" readily in the serological or in the chemical laboratory, whereas hitherto practically accurate drops, and those of a limited choice of sizes, have been produced only in the physical laboratory, or from some more or less mysterious "plant" raised in the physical laboratory. The physical principles underlying the method have been more or less widely known for generations, and full general acknowledgment is here rendered to the authors of scores of papers on the investigation of those principles.

2. This method of simply gauging the pipettes as required merely places in the hands of the ordinary laboratory worker the means of applying those complex component forces, or rather the resultant of them, to suit his purpose.

3. Inquiries made by correspondents and circumstances observed by me since the former article indicate the need for the early publication of these further practical details, introduced by a brief exposition of the fundamental phenomena on which a trustworthy method of drop-measuring must depend. The opportunity is taken of adding a few notes on some applications of the method.

4. *Fundamental physical phenomena.*—A small drop of water on a paraffined surface maintains its almost spherical form by surface-tension—the force exerted on the surface layer of liquid molecules by all the molecules within that layer, which layer consequently behaves as if it were an elastic skin, constantly tending to compel the liquid drop to take the shape that will allow it to have a minimum of surface, that shape being, of course, spherical.

5. The amount of force exerted by the surface of a water-drop varies inversely as its radius. The smaller the drop, the