NOTES ON THE Vi ANTIGEN OF *BACILLUS TYPHOSUS*

By E. S. HORGAN

Stack Medical Research Laboratories, Khartoum

THE author's attention was first drawn to this subject in 1934 by the earlier papers of Felix & Pitt (1934 *a, b*) and Perry *et al.* (1933, 1934), and a series of sporadic cases of enteric fever in Khartoum during the latter part of 1934 and 1935 afforded an opportunity of examining certain claims advanced in these papers. As the following observations on various aspects of the subject have been carried out over a period of 18 months the results are inevitably somewhat scrappy, but in view of the importance of the subject it was thought that they might not be altogether without interest for other workers.

As a strictly chronological record would be somewhat confusing the results obtained are grouped under the main points investigated.

When the work was commenced no inagglutinable cultures of local strains of *B. typhosus* were available, and such cultures were kindly supplied by Dr A. Felix and Major H. J. Bensted, to both of whom the author is much indebted for their assistance.

PRESERVATION OF CULTURES

To avoid as far as possible too many subcultures, the method of infected mouse spleen dried *in vacuo* over calcium chloride as recommended by Perry *et al.* (1934) was followed, the only difference being that the white rat was used in place of the mouse, the latter not being available. It may be stated here that young white rats seem to be equally susceptible to virulent strains of *B. typhosus* as mice. The method has given excellent results, the claim of these authors that cultures could be obtained after 4 months being confirmed, and when tested on young rats the organisms seemed to have lost none of their virulence and inagglutinability by O serum. As no ascitic fluid was available, Kauffmann's method (1935*b*) of passage of cultures through complement and growth on fresh ascitic agar was not tried, but it seems to offer no particular advantages at least to workers in dusty tropical countries. Where no mention otherwise is made all agglutination tests were carried out with living suspensions in saline from agar cultures of 18–20 hours old. The macroscopic technique was used in all agglutination tests, incubation being carried out in a water bath for 4 hours at 37° C. and the results were read after standing at room temperature for 22–24 hours.

Effect of growth at various temperatures

It is unnecessary to detail the results which were completely confirmatory of those of Felix *et al.* (1934).

Relation of composition of the medium to Vi agglutination

It was first noted as the result of an accident that emulsions prepared from MacConkey's medium gave much higher titres against a standard Vi serum than those prepared from nutrient agar. In these laboratories the composition of the latter is peptone (Parke Davis) 10 g., NaCl 5 g., agar 30 g., heart extract 1000 c.c. (The heart extract is prepared by extracting 500 g. bullock's heart in 1500 c.c. water.) The pH of the nutrient agar is 7·6. MacConkey's medium is prepared according to the standard formula. It was at first thought that the result might be related to the presence of bile salt in the latter, but a series of experiments of testing the constituents separately and in all possible combinations showed that the result was one of inhibition related to the presence of heart extract in the nutrient agar. Cultures were then put up on a peptone salt agar pH 7·4—the concentrations of the peptone varying from 1 to 5 per cent. The actual amount used did not appear to affect the result, and the following experiments were carried out with a concentration of 1 per cent. peptone. The following strains were used: Ty 2, Giglioli, Harwood, Watson, Rawlings (rejuvenated strain supplied by Major H. J. Bensted), with the results recorded in Table I.

The above series is typical of a large number carried out and indicates the distinct inhibiting effect of the heart extract on Vi agglutination of all strains examined. It is rather curious that the peptone cultures showed also a slightly higher O agglutination which perhaps merely indicates a generally increased sensitivity of the culture to agglutination. It was also noted as a point of interest that the Vi agglutination with the peptone suspensions was more clear cut, and gave a sharper end-point.

On the author's return from leave some months later, the experiments were recommenced using different peptones and Lab-Lemco as well as heart extract. This series gave more irregular results than the clear-cut results of Table I, and while it would be valueless to detail all the tests Table II represents an average finding.

The use of Lab-Lemco in place of heart extract gave approximately similar results.

Numerous series of experiments were carried out using different virulent strains of *B. typhosus* and several Vi sera, but while on the whole the titres of the peptone-agar cultures were higher the difference, as indicated by the results in Table II, was not marked, and occasionally (as above with Ty 2 strain grown on Parke Davis peptone and heart extract) the titres of the heart extract suspensions were if anything higher. The brand of peptone used

Table I. *Effect of presence of heart extract on sensitivity of "inagglutinable" strains to Vi agglutination*

Serum	Giglioli		Ty 2		Harwood		Watson		Rawlings	
	A	B	A	B	A	B	A	B	A	B
Vi II	3000±	500± 1250–	3000±	500± 1250–	3000 tr.	1250 tr. 2000–	3000 tr.	1250± 2000–	3000±	500± 1250 tr.
O 901 (titre 1 in 10,000)	250 tr.	50 tr.	250± 500–	50 tr.	250 tr.	50 tr.	250 tr.	50 tr.	250± 500–	50 tr.

A = peptone only.
± = agglutination just visible to naked eye.
B = peptone + heart extract.
tr. = agglutination visible with hand lens.
– = absence of agglutination with hand lens.

Table II. *Effect of different peptones with and without the addition of heart extract on Vi agglutination (Vi II serum)*

Strains	Difco		Fairchild's		B.D.H.		Witte's		Parke Davis	
	A	B	A	B	A	B	A	B	A	B
Ty 2	1250± 2500 tr.	1250± 2500–	1250+ 2500±	1250+ 2500–	1250+ 2500 tr.	1250+ 2500–	1250+ 2500±	500± 1250 tr.	1250 tr. 2500–	1250± 2500 tr.
Giglioli	1250+ 2500–	500+ 1250 tr.	1250+ 2500 tr.	500+ 1250 tr. 2500–	1250+ 2500 tr.	500+ 1250 tr. 2500–	1250+ 2500 tr.	1250+ 2500–	1250+ 2500–	1250 tr. 2500–

appeared to make no difference to the agglutination, but on Witte's peptone agar it was difficult to obtain growths of several of the strains. During the progress of the above studies another interesting point was noticed, viz. that the Vi agglutinability of repeated (daily) subcultures of the same strain on the same culture medium varied considerably, a period of lessened agglutinability of several days' duration being sometimes succeeded by a period of increased agglutinability. It must be admitted, however, that this variation was very irregular, but it was noted that on the whole the more virulent strains (Ty 2 and Giglioli) grown on peptone agar showed little variation through repeated subcultures.

It has long been known that different suspensions of the same bacterial strain may vary widely in agglutinability by the same serum, but as much of the experimental work was carried out before the distinction between H and O agglutination was realized, a repetition of such work would be desirable. Such is outside the scope of this paper, but a few experiments were carried out with a highly agglutinable strain (H 901) grown under the same conditions as the virulent strains mentioned above, H and O agglutination being separately estimated. Neither the presence of heart extract nor repeated subcultures appeared to make the slightest difference in the end-titres.

It seems reasonable to conclude that just as the Vi antigen of virulent strains of *B. typhosus* shows a greater susceptibility to differences in the temperature of growth than the ordinary H and O antigens, it may also be more readily affected by changes in the composition of the medium.

The above work was carried out before the value of formolized suspensions for Vi agglutination was pointed out by Kauffmann (1935 a), and there is little doubt that such will be the routine method of choice for future work, but the above findings would at least indicate the desirability of all workers preparing such suspensions from a culture medium of standard composition.

PREPARATION OF Vi SERA

Living virulent strains. Before the appearance of the paper by Felix & Pitt (1935) advocating the use of rough Vi strains, considerable difficulty was experienced by the author using living suspensions of smooth virulent strains (Felix & Pitt, 1934b), as most of the rabbits died shortly after the second dose. It was found that the difficulty could be overcome by giving three or four earlier doses (250 millions at a few days' interval) subcutaneously followed by a series of four intravenous injections of 200, 200, 400, 400 millions living organisms. The blood was taken 8 days after the last dose. The method is admittedly somewhat tedious but it appeared safe, for in a series of four rabbits and one goat no ill effects were noted—and two of the sera obtained showed high Vi titres (rabbit 1 in 3000, goat 1 in 2500).

Use of formolized extracts. The method as advocated by Felix et al. (1934) was followed in detail, three rabbits being used. The results were disappointing, as none of the sera gave a titre higher than 1 in 125 dilution.

Use of formolized cultures (0·2 *per cent. formalin*). In the first series of experiments one goat and two rabbits were used, the suspensions being from the strain Giglioli. The goat received a series of doses of 2000, 2000, 4000, 6000, 8000 millions, the rabbits 1000, 2000, 3000, 3000 millions at intervals of 5 days. Blood was taken 8 days after the last dose. The sera were put up against the homologous suspension for Vi agglutination and against an alcoholic suspension O 901 for O agglutination. The results were:

	Agglutination	
Animal	Vi	O
Goat	<10	10,000
Rabbit 1	<10	10,000
Rabbit 2	<10	5,000

A possible although most unlikely explanation of these negative results was the insusceptibility of all the animals to Vi antigen, and to test this each rabbit was given the usual course of doses of the living culture (Giglioli) and the blood taken 5 days after the last dose. The sera were put up against the formolized suspension and also against living suspensions of several Vi strains. The results were:

	Vi agglutination				
				Giglioli	
Rabbits	Ty 2 (living)	Watson (living)	Harwood (living)	(living)	(formolized)
1	1250	1250	1250	500	500
2	300	1250	1250	500	500

Another attempt was then made using a formolized vaccine from the strain Ty 2. To ensure the virulence of the strain it was first tested on three mice and three young white rats, all of whom died in about 24 hours after an intraperitoneal injection of 70 million organisms and subcultures were made from the heart blood of one of the mice and used for the preparation of the suspension. The doses were 1000, 2000, 2000, 3000 millions at intervals of 5 days, and the blood was taken 5 days after the last dose. Using the same emulsion for agglutination the titres were:

	Agglutination	
Rabbit	Vi	O
3	1 in 10 tr.	5,000
4	1 in 10 tr.	5,000
5	1 in 5 tr.	10,000
6	1 in 10 tr.	5,000

A second course of 2000, 4000, 4000 millions was then given, but blood taken after the end of this series showed no change in the Vi titres of any of the animals.

In the meantime Felix & Bhatnagar (1935) had published their results with formolized Vi vaccine, and while they appeared to have no difficulty in obtaining Vi serum of comparatively high titre, they emphasized the fact that the sera so obtained were deficient in both opsonic and protective values. In view of this conclusion it appeared useless to investigate the matter further,

although no explanation can be given of the complete failure of this method in the authors' hands.

USE OF HEATED SUSPENSIONS

The "thermal death-point" of *B. typhosus* as stated in the text-books appears to be in the range 53–55° C., but as the variables, time density of suspension, etc., are rarely stated, it was considered desirable to confirm this point. Suspensions (1000 millions per c.c. in saline pH 7·4) of an 18 hours' agar culture were held over this range of temperature for the minimum period, to avoid overheating of the Vi antigen as far as possible. They were tested for sterility by inoculation on to broth and agar which were incubated for 12 days to allow for any lag in reproduction of a few organisms.

It was found that suspensions kept at 53° C. were not completely sterilized after 90 min.; suspensions kept at 55° C. were invariably killed in 1 hour, and so the latter was taken as the "thermal death-point", optimal for the present work. The heated suspensions were examined together with living controls for both Vi and O agglutination, and while the O titres of the killed organisms were slightly higher, there appeared to be no appreciable difference in the Vi titres.

Experiments. It was first considered desirable to test the four rabbits (above) who had not responded to large doses of a formolized suspension, and each was given 2 doses of 1500 millions at intervals of 5 days. Blood was taken 5 days after the second dose. The Vi titres (using the formolized suspension) were as follows:

Rabbit	Agglutination (Vi)
3	50 + 50 tr.
4	125 + 250 tr.
5	125 + 250 ±
6	50 + 125 ±

The results indicated that cultures heated at 55° C. were capable of producing Vi agglutination of moderate titres, but to confirm this another trial was carried out with four more rabbits, each receiving 500, 1000, 2000, 2000 million organisms; the larger doses being used in an attempt to produce a high-titre Vi serum.

Blood taken 6 days after the last dose showed:

Rabbit	Agglutination	
	Vi	O
7	250 + 500 tr. +	5000
8	50 ± 125 tr.	5000
9	125 ± 250 tr.	2500
10	250 ± 500 –	5000

It would appear from this small series that the larger doses on the whole had comparatively little effect on the end-point.

It is reasonable to conclude from the above results that a suspension of a virulent strain killed at 55° C. can be used as a vaccine for the production of Vi antibodies. Owing to the lack of sufficient animals it has so far been impracticable to test the protective value of such a vaccine, but as Felix & Pitt (1934 a) have shown that a vaccine heated for 1½ hours at 58° C. confers good protection on mice, notwithstanding the absence of Vi agglutination, there seems no reason to doubt the efficiency of a similar vaccine prepared at a lower temperature.

Further, if it be established that the production of Vi agglutinins is associated with a correspondingly greater protective value, the above method might be safely used in preparing a vaccine for human inoculation.

Thermostability of the Vi antigen (agglutinogen)

It had been noted by the author in preparing a pure anti-Vi serum (absorbed) using a stock strain of Rawlings for the absorption, that living suspensions of the latter were capable of absorbing Vi agglutinins. Robertson & Yu (1936) report a similar result. It was found that to destroy the Vi agglutinogen, emulsions had to be heated for at least 15 min. at 100° C.

Felix *et al.* (1934) state that suspensions of "inagglutinable" strains heated at 100° C. for 1 hour were still capable of specifically absorbing to some extent Vi agglutinins. Vi agglutination was, however, no longer demonstrable "after heating to 60° C. for 1 hour or to 100° C. for 10 min.", and Table IV of their paper also indicates that the same periods of heating completely abolished the resistance of the suspensions to O agglutination.

Robertson & Yu (1936), however, state that it was necessary to heat their suspensions at 100° C. for 1 hour to obtain strong O agglutination.

Effect of heating at 100° C. on the agglutinability (Vi and O) of virulent strains

Emulsions (1000 million per c.c.) in saline from an 18 hours' agar culture heated at 100° C. for varying intervals were examined for Vi and O agglutination. Six virulent strains were examined, and Table III records a typical result:

The results show:

(1) Vi agglutination is completely abolished in suspensions heated to 100° C. for 15 min.

(2) Heating for at least 1 hour is necessary to break down the resistance to O agglutination.

(3) Strains whose resistance to O agglutination is not so complete appear to lose their resistance correspondingly rapidly.

(4) Zonal agglutination of the heated suspensions—this was present in the six strains tested.

Table III. *Heat resistance of the Vi agglutinogen as shown
by Vi and O agglutination*

		Ty 2		
		Heated (min.)		
Sera	Unheated	15	30	60
Vi	1250 ±	—	—	—
O	50 tr.			
(titre 1 in 10,000)	125 –	50 tr.	50 tr.	50 tr.
		125 tr.	125 tr.	125 tr.
		250 –	250 –	250 ±
		500 –	500 –	500 +
				1250 +
				2500 +
				5000 tr.

		W 477 (see below)		
		Heated (min.)		
	Unheated	15	30	60
Vi	2500 +	—	—	—
	125 tr.			
O	250 tr.			
(titre 1 in 10,000)	500 –	125 tr.	125 tr.	25 tr.
		250 tr.	250 tr.	250 ±
		500 ±	500 ±	500 +
		1250 tr.	1250 ±	1,250 ±
			2500 ±	2,500 ±
			5000 tr.	10,000 tr.

The present results are therefore in general agreement with those of Robertson & Yu (1936) and further indicate a satisfactory correspondence between the methods of agglutinin absorption and resistance to O agglutination for the determination of the thermostability of the Vi antigen. On the contrary, Vi agglutination gives completely erroneous results.

In view of the latter, it is perhaps not surprising that doubts have arisen as to the relation of the Vi agglutinogen to the immunity produced. Thus Kauffmann (1935b) states "that the highly thermolabile Vi agglutinogen must not be placed on a par with the Vi immunogen. It has in fact, been shown that vaccines prepared from V forms still give effective protection after the cultures have been heated to 60° C. or 100° C. although this heating destroys the Vi agglutinogen." This is introducing an unnecessary complication, as an agglutinogen which withstands heating for a considerable time at 100° C. as indicated by the above results and those of Felix et al. (1934) can hardly be regarded as highly thermolabile. In the author's opinion the explanation of the latter workers is much more convincing. "It is well known that the various *in vitro* and *in vivo* activities of an antigen differ widely in their capacity to demonstrate the degree of deterioration of antigen due to heating or treatment with various chemicals. This is also the case with the Vi antigen. The discrepancies...are apparent only and due to different degrees of fineness exhibited by the various reactions."

OCCURRENCE OF STRAINS OF *B. TYPHOSUS* CONTAINING Vi ANTIGEN

A series of strains of *B. typhosus* of which the majority were freshly isolated from the blood (first or second subculture on agar) were examined for Vi agglutination, using a pure (absorbed) Vi high-titre serum.

All these strains were readily agglutinated by a pure O serum. Two "inagglutinable" virulent strains were included in the series as controls, Rawlings (rejuvenated strain supplied by Major H. J. Bensted) and a local strain recently isolated (W 477).

In each case the result was confirmed by agglutinin absorption of Vi serum with living suspensions of the strains.

The results, which it is unnecessary to give in full detail, are presented according to the convenient nomenclature of Kauffmann (1935 *b*).

Table IV. *Type of strains of* B. typhosus *in a series of recently isolated and old stock cultures*

Strains	Type		
	V	V–W	W
12 (freshly isolated from blood culture)	–	+	–
W 477 (freshly isolated)	+	–	–
Rawlings (Bensted)	+	–	–
T 31 (isolated 1933)	–	+	–
T 32 (isolated 1933)	–	+	–
T 34 (isolated 1933)	–	+	–
Staines (old stock strain)	–	+	–
Rawlings (old stock strain)	–	+	–
O 90 (old stock strain)	–	–	+

The results are clear cut and show that with the exception of the stock strain O 90 all strains examined contained Vi antigen. It is of interest that the old stock Rawlings strain was readily agglutinated by the Vi sera up to a dilution of 1 in 400, a result which is in contrast with that of Felix (1934 *b*), who would class this classical strain as a pure W in type. It would appear that differences may exist between different stock subcultures of the same strain in various laboratories. The results of the recently isolated strains are in close agreement with those of Felix *et al.* (1935), who in a series of ninety strains found only two devoid of Vi antigen (W type), and these had been examined after the seventh and eighth subculture, respectively.

Taking all results into consideration it would appear that the great majority of strains of *B. typhosus* in Palestine and the Sudan are of the V–W type, and that the pure W type is more likely to be an artificial variant produced by subculture. It has yet to be shown whether a freshly isolated strain is ever of this type. For the purposes of proof a negative Vi agglutination should be checked by an absorption of a Vi serum with the strain in question.

OCCURRENCE OF Vi AGGLUTININS IN NORMAL HUMAN SERA

One hundred were examined, of which ninety were negative (less than 1 in 5 dilution), four were positive in a dilution of 1 in 5 (trace) and six positive in a dilution of 1 in 10 (trace). Although Felix *et al.* (1934) state that the normal Vi titres of some rabbits may be 1 in 10, and of horses even 1 in 20 dilution, there appear to be no previous observations on human sera.

AGGLUTININS IN TYPHOID SERA

The majority of the cases were proved by blood culture, the twelve freshly isolated strains mentioned in Table IV being from these cases. A few clinically typical cases are included, in which culture had not been carried out. The series is admittedly small, but it was considered preferable to examine a comparatively small number of proved cases which could be followed up, rather than examine at random a larger number of sera sent in for routine diagnosis and which it would have been impossible to check. In several of the cases repeated examinations were made to determine fluctuations of the Vi titres.

The results call for little comment; eight of the cases had at some stage of the disease a Vi titre in a dilution of 1 in 25 or over, four had titres in dilutions of over 1 in 100, while forty were negative. It is possible that No. 19, although clinically typical of an enteric fever, was not due to *B. typhosus*. It is true that some of the negative cases were only examined on one occasion, and it is of course possible that examination at a later stage of the disease might have shown Vi agglutination.

Type of cases. The majority of the cases could be described as clinically mild to moderately severe, and of the three severe cases one died. The severe case (No. 28) was a native girl aged 7 years, and on the 17th day of her illness a strain of *B. typhosus* was isolated from blood culture which proved to be of the V type. This strain (W 477) has already been noted above. On the 40th day, when the patient was progressing towards recovery, *B. typhosus* was isolated from the faeces, and in view of the previous inagglutinable strain was carefully examined; it proved to be of the ordinary agglutinable (V–W) type.

It is interesting to note that this is the first case of typhoid in the Sudan from which an "inagglutinable" strain (V type) has been isolated, although it has been the routine practice since July 1934 to examine all freshly isolated strains of *B. typhosus* for O agglutination, and up to date 150 strains have been examined.

Table V. *Agglutination titres in the sera of typhoid patients*

Case no.	Clinical course	Day of illness serum taken	Agglutination titres H	O	Vi
1	Moderately severe	12 (approx.)*	5000	2500	125 ±
2	Mild	18	250	125	5 ±
3	Mild	20	2500	50	5 + 10 tr.
4	Mild	30	—	500	5 tr.
5	Mild	21	1250	50	0
6	Moderately severe with a mild relapse	16	5000	250	5 ± 10 tr.
7	Mild	12	500	250	0
8		15	1250	500	0
9	Moderately severe with a relapse	14	1250	50	0
		38	250	250	5 ± 10 tr.
		49	—	50	50 tr.
		61 (convalescent)	—	50	125 tr.
10	Moderately severe	8 (approx.)	25	25	0
		18 ,,	500	250	0
		31 ,,	—	250	5 ± 10 tr.
11	Mild	17 (approx.)	250	50	5 ± 10 tr.
		27 ,,	—	—	10 ±
		39 ,,	—	—	50 ± 125 tr.
12	Moderately severe	28 ,,	250	50	25 ± 50 tr.
		38 ,,	—	—	25 tr.
		50 (convalescent)	—	—	0
13	Moderately severe	12 (approx.)	125	250	5 + 10 tr.
		22 ,,	—	250	10 ± 25 tr.
		34 ,,	—	—	25 tr.
14	Mild	21 ,,	250	50	0
		37 ,,	—	—	25 tr.
†15	Moderately severe with a mild relapse	13 ,,	500	25	0
		26 ,,	—	—	10 tr.
16	Very mild	10 ,,	50	25	0
		21 ,,	—	—	0
†17	Mild	14	125	50	0
18	Mild	(3rd week)	250	250	0
†19	Moderately severe	30	0	0	0
†20	Moderately severe	38	—	125	5 ± 10 tr.
21	Mild	37	250	125	0
22	Severe with a severe relapse	14	—	125	0
23	Mild (sister of 22)	12	—	—	25 ± 50 tr.
24	Mild	10	500	125	0
		25	—	—	0
25	Moderately severe	22	125	125	5 tr.
26	Mild	30	—	125	5 tr.
27	Very severe (death 18th day)	12	5000	500	—
		16	—	500	5 ± 10 tr.
28	Severe (W 477)	10	250	125	—
		19	—	500	125 250 tr.

* It is sometimes difficult to obtain from natives an exact history of the number of days of illness before admission to hospital. The general tendency is, however, to underestimate this period.   † Not proved by culture.

DIAGNOSTIC AND PROGNOSTIC SIGNIFICANCE OF Vi AGGLUTINATION

Vi agglutination as a guide to specific treatment.[1] Taking into consideration the Palestine results and the smaller series of this paper, Vi agglutination in the patient's serum appears to be devoid of any diagnostic value. Felix *et al.* (1935) concluded that "Vi antibody is not readily elaborated as a result of an attack of typhoid fever. They (their recorded observations of Vi agglutination) further strengthen the conclusion that an early application of a therapeutic serum containing Vi antibody as one of its constituents is an essential requirement of typhoid patients." With the first sentence the author is in complete agreement. It is difficult, however, to see how the data given in their paper strengthen the case for the early administration of serum, as from an analysis of the cases it appears that only eight out of ninety-two were positive for Vi agglutination, and no information is given as to the clinical type or results of the negative cases. The statement would seem to rest chiefly on the successful result obtained in a severe case treated with anti-Vi serum and which subsequently elaborated Vi agglutinins to a high titre.

If the hypothesis of Felix *et al.* as to the part played by the Vi antigen be correct the clinician would naturally wish to administer anti-Vi serum therapeutically, as early as possible in the course of the disease. But the Palestine results indicate that the majority of the sera taken during the early stages of the disease are negative, and the present series also shows that one rarely obtains even low titres of Vi agglutination before the end of the second week. It is therefore difficult to see how a negative finding in the first fortnight of the disease can help the clinician one way or the other. In the later stages of the disease there appears to be no correlation between the titres of Vi, H, and O agglutinins, and in the present series, at least, there is no relation between the Vi titres and the patient's clinical progress. In brief, the little evidence so far obtained indicates that the clinical course of the disease must be the clinician's sole criterion for specific treatment.

It is admittedly dangerous to draw general conclusions from the cases with a low mortality at present occurring in Palestine and the Sudan, and it is possible that in the later stages of severe cases or in cases with relapses, a high-titre Vi agglutination may be of favourable prognostic significance. It is at least of interest that one of the severe cases above (No. 28) which eventually recovered gave the highest Vi agglutination which I have so far obtained from a case of typhoid fever, and several of the severe cases recorded by Felix *et al.* (1935) also showed good Vi agglutination.

Repeated examination of the serum of severe cases for Vi agglutination, especially from outbreaks, as in Shanghai where V types of *B. typhosus* appear to be commonly isolated (Robertson & Yu, 1936), would be of considerable interest in this connexion.

[1] From a study of the paper by Felix *et al.* I see no suggestion made to employ Vi agglutination with the patient's serum, as a guide to specific serum treatment.—ED.

SUMMARY

1. A peptone salt agar appears to be the medium of choice in the preparation of suspensions of V strains of *B. typhosus* for Vi agglutination. The presence of heart extract may have a definite inhibitory effect on Vi agglutination but the effect is irregular.

2. A safe method for the preparation of anti-Vi serum with virulent cultures is described.

3. Repeated endeavours to produce an anti-Vi serum by the use of formolized vaccines were unsuccessful.

4. A suspension of a V strain killed at 55° C. can be used as a vaccine for producing Vi agglutination.

5. The thermostability of the Vi antigen is discussed.

6. An analysis is made of the presence of Vi antigen in a number of freshly isolated and stock strains of *B. typhosus*.

7. A series of typhoid cases has been examined for Vi agglutination, and it is concluded that the latter is unlikely to be of any clinical value either in diagnosis or prognosis.

REFERENCES

FELIX, A. & PITT, R. M. (1934a). *J. Path. Bact.* **38**, 409.
—— —— (1934b). *Lancet*, **11**, 186.
—— —— (1935). *J. Hygiene*, **35**, 428.
FELIX, A. & BHATNAGAR, S. S. (1935). *Brit. J. Exp. Path.* **16**, 422.
FELIX, A., BHATNAGAR, S. S. & PITT, R. M. (1934). *Ibid.* **15**, 346.
FELIX, A., KRIKORIAN, K. S. & REITLER, R. (1935). *J. Hygiene*, **35**, 421.
KAUFFMANN, F. (1935a). *Z. Hyg. Infektkr.* **116**, 617.
—— (1935b). *League of Nations Quart. Bull. of the Health Organization.* Geneva, **4**, 482.
PERRY, H. M., FINDLAY, H. T. & BENSTED, H. J. (1933). *J. Roy. Army Med. Corps*, **61**, 81.
—— —— (1934). *Ibid.* **62**, 161.
ROBERTSON, R. C. & YU, H. (1936). *J. Path. Bact.* **42**, 53.