Researches on the bacteriological diagnosis of cholera carried out by medical officers of the Sanitary, Maritime and Quarantine Council of Egypt / under the direction of Marc Armand Ruffer, President of the Council.

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In 1897, during the return of the pilgrimage from Mecca, Dr. Gal-
i showed me microscopical preparations from the intestinal
tents of a pilgrim, who had been found dead on a ship returning
n Jeddah. No history of the man's illness could be obtained.
I was greatly astonished to find in these preparations a large
nber of microorganisms, not to be distinguished microscopically
n cholera vibrios. At the autopsy of the pilgrim, dysenteric
ons were found, together with fibroid kidneys and hea[illegible]. Cultures
n the intestinal contents, made first in peptone water and then in
tine, showed the presence of comma-shaped, monociliated, motile,
-phosphorescent vibrios, which were extremely virulent for
nea pigs, did not coagulate milk, and gave a marked indol reaction.
act, the cultures could not be distinguished from those of cholera.
The agglutination test with the serum of a horse repeatedly
cted with cultures of cholera obtained from Professor Pfeiffer
wed that the vibrios agglutinated and gave Pfeiffer's reaction
ctly like those isolated from cholera cases. No quantitative or
litative differences were noticed.
My first idea was that the patient had either suffered from, or had
n in contact with cholera. After careful enquiries, this theory

had to be abandoned, for it was found that no cases of chol had occurred among the pilgrims in the Hedjaz, nor on the retu journey. A sharp look-out was kept at El Tor for cases of sudc diarrhoea, vomiting and cramps, but in vain. No disease resembli cholera was observed.

On the days following this discovery, the intestinal contents every pilgrim who died in the camp were examined bacteriologica I superintended the autopsies and examined all the patients hospital, together with Dr. Zachariadis Bey; who, having seen seve epidemics, was well acquainted with the clinical signs and lesions cholera. Nevertheless no case of cholera was discovered, thou in no less than 5 typical cases of colitis and dysentery we isolat cultures not to be distinguished from those of cholera, even by t agglutination test and Pfeiffer's reaction. The pilgrims finally l El Tor five days after the finding of the vibrio, and no cases of chol occurred either on the ships, or after the pilgrims reached home.

During the four following years, the work of reorganisation a the lack of skilled assistance prevented the systematic prosecution these researches; though I frequently observed vibrios in the sto of pilgrims free from all symptoms of cholera.

In 1902, the pilgrims brought back typical cholera from Jedd and Yambo to El Tor. The observations made at the time show three facts quite clearly:

1.—The stools of almost every pilgrim suffering from diseas other than cholera (e.g. pneumonia, rheumatism, dysentery, cons pation, diarrhoea etc.) contained vibrios numerous enough to recognised microscopically.

2.—Vibrios agglutinating with cholera serum were isolated fr patients showing no symptoms of cholera. On the other hand, so vibrios isolated from typical cases of cholera did not agglutinate w cholera serum.

3.—Although it was unavoidable that pilgrims carrying vibri should mix with other pilgrims, yet only one fresh case of chole occurred in the camp; all the others were imported. In the gene hospital, where the majority of patients were excreting vibri in every stool, not a single case of infection occurred among t patients, the European or native attendants. Further, not a sing case of cholera occurred on the ships leaving El Tor after the peri of quarantine was over.

In 1905, after the Hedjaz and El Tor had been free from cholera or nearly two years, Dr. F. Gotschlich examined systematically for vibrios the stools of every patient who died at El Tor. The autopsies were made by myself, and it may be stated once for all that no lesions resembling cholera were found in any case.

The results of these bacteriological examinations may be summed up as follows: Vibrios were found in 38 patients out of 107 cases examined. Thirty-two of these vibrios did not agglutinate, but six agglutinated readily with cholera serum. The characteristics of the latter were as follows: they were monociliated, non-phosphorescent, very virulent (with one exception) for guinea pigs, and not virulent for pigeons, did not coagulate milk, liquefied 10 % gelatine and gave a well marked indol-reaction. The morphological characteristics were not the same in all, but the differences were not greater than those existing between cholera vibrios isolated from different cases.

The results of the agglutination test are shown in the following tables.

TABLE 1.

of Culture.	Name of pilgrim from whom vibrios were isolated.	Nationality	Po mortem Diagnosis.	Horse cholera Serum (Standard 1 : 3000).	Goat cholera Serum Dilution 1:500	Rabbit cholera Serum	
						Dilution 1: 200	Dilution 1: 1000
1	Osman Hussein	Anatolian	Colitis	Limit 1:1000	+ + +	+ +	+ (limit)
2	Sanka Uschalinga	Russian	Dysentery	Limit 1:2000	+ + +	+ + +	+
3	?	Turk	Colitis gangrenosa	Limit 1:1000	+ + +	+ + +	+
4	Ahmed Salih	Rumelian	Colitis	Limit 1:1000	+ + +	+ + +	+
5	Bekir Mahomet	Anatolian	Colitis	Limit 1 : 500	+	+ slight	0
6	Ahmet Ali	Rumelian	Dysentery	Limit 1:2000	+ + +	+ + +	+

N.B.—The control tubes containing vibrios, normal sera and physiological salt solution showed no agglutination.

Five of these vibrios therefore agglutinated with specific sera just like cholera vibrios, and did not agglutinate at all with ordinary sera. One of them agglutinated far less than the others, but similar differences occur among vibrios isolated from typical cholera cases.

The six vibrios all belonged to the same class, for on immuni rabbits with each vibrio, the sera of all the rabbits diluted to one in thousand, agglutinated not only all the six vibrios, but the cho microorganism also.

Pfeiffer's reaction.—One culture, being avirulent, could not tested on animals. The rabbits inoculated with cholera serum cultures either recovered or lived much longer than the controls.

TABLE II.

(Showing results of Pfeiffer's reaction).

Number of Culture.	a. Specific bacteriolytic Rabbit-serum.			Control. Normal Rabbit ser
	Serum 0,005. Culture. 1 loop.	Serum 0,01. Culture. 1 loop.	Serum 0,02. Culture. 1 loop.	Serum 0,05. Culture. 1 loo
1	Complete bacteriolysis. Death after 24 h.	Complete bacteriolysis. Death after 60 h.	Complete bacteriolysis. Animal survived.	No bacteriolys Death after 24 ho
2	Complete bacteriolysis. Animal survived.	Complete bacteriolysis. Animal survived.	do.	do.
3	Complete bacteriolysis. Animal died after 48 h.	do.	do.	do.
4	do.	do.	do.	do.
5	Avirulent	See Table II	—	—
6	Complete bacteriolysis. Animal survived.	Complete bacteriolysis. Animal survived.	Complete bacteriolysis. Animal survived.	No bacteriolys Death after 24 hou

Further, the six sera of animals inoculated with any of these vibr protected animals against every one of these microorganisms.

These researches were continued during the pilgrimage season 1906, when the intestinal contents of 127 dead pilgrims were examin

ibrios were found in 18 cases of dysentery and colitis, which resented no lesions or symptoms of cholera. Two of these vibrios ave with cholera serum all the reactions of true cholera vibrios, and ere agglutinated also by the sera of animals injected with one of ie six vibrios isolated in 1905.

In order to establish the relations existing between the microor-anisms isolated at El Tor from dysenteric and colitis cases (in future alled « El Tor vibrios ») and the vibrio of cholera, all the monociliated omma-shaped microorganisms isolated by us during the epidemic f 1902, and the El Tor vibrios, were studied by bacteriological ethods. Some vibrios kindly sent by Professor Gaffky of Berlin, r. Rogers of Calcutta, Mr. E.H. Hankin of Agra, and Dr. Gotschlich f Alexandria were examined also.

Agglutination test.—Two sera were used; firstly, a highly agglutin-ing serum kindly provided by Dr. Gaffky; and secondly, an agglutin-ing and bacteriolytic serum, prepared in these laboratories with a icrobe having all the characteristics of the true cholera vibrio, and olated from a cholera stool (vibrio CK). The Berlin serum had very ighly agglutinating properties, and was tested in solutions not weaker an 1 in 3000 (Berlin Institute standard) which agglutinated in half an our, at the temperature of the laboratory, all the vibrios reacting this test. The serum prepared with vibrio CK was far weaker and id not act equally on all the vibrios. Nevertheless the action of both era was identical, in so far that all the agglutinating microorganisms ere acted on by both sera. The method employed was the usual one. he agar cultures were always 18 hours old, and the sera were diluted 1 9 per thousand NaCl solution.

The following table gives the main results of this examination as ar as the agglutination test is concerned.

TABLE III.

Total number of vibrios olated from cases of cholera: 15		Total number of vibrios isolated from patients who had no symptoms of cholera: 23		Vibrios of doubtful origin: including sea-water vibrio. 4	
Agglutinating with cholera serum:	Not agglutinating with cholera serum:	Agglutinating with cholera serum:	Not agglutinating with cholera serum:	Agglutinating with cholera serum:	Not agglutinating with cholera serum:
11 (73 %)	4 (27 %)	7 (30 %)	16 (70 %)	0 (0 %)	4 (100 %)

Table IV gives the details of all the other tests as well.

Table IV.

Number.	Name of Vibrio.	Origin of Vibrio.	Agglutination Test.		Saturation Test.		Pfeiffer's reaction	Fixation Test.		Haemolysi Test.
			Serum Berlin.	Serum C. K.	Serum non saturated.	Serum saturated.		Bordet's method.	Dopter's method.	
1	C.K.	Cholera stool	3000	1000	3000	250	+	+	+	—
2	Aida	» »	3000	1000	3000	250	+	+	+	—
3	Fatma	» »	3000	1000	3000	250	+	+	+	—
4	Alioglu	» »	—	—	—	—	—	—	—	+
5	LXII	» »	—	—	—	—	—	—	—	+
6	141	» »	3000	800	3000	500	+	+	+	—
7	Berlin 70	» »	3000	1000	3000	300	+	+	+	—
8	C.V.	« »	3000	1000	3000	300	+	+	+	—
9	98	Dysentery (El Tor)	—	—	—	—	—	—	—	+
10	147	» »	—	—	—	—	—	+	+	+ Slight after
11	Berlin 76	Cholera stool	3000	800	3000	500	+	+	+	—
12	Machaout	Pneumonia during cholera epidemic	3000	1000	3000	500	+	+	+	—
13	Tor No 1	Dysentery (El Tor)	3000	500	3000	300	+	—	—	+
14	V	Cholera stool	—	—	—	—	—	—	—	+
15	173	Dysentery (El Tor)	—	—	—	—	—	+	+	+ Very sligh
16	Berlin 115	Cholera stool	2500	1000	2500	250	+	+	+	—
17	Tor No 2	Dysentery (El Tor)	3000	500	3000	250	+	—	—	+
18	C.V.A.	Cholera stool	2000	1000	2000	100	+	+	+	—
19	IV	» »	—	—	—	—	—	+	+	+
20	167	Dysentery (El Tor)	—	—	—	—	—	—	—	+
21	Tor No 3	» »	3000	800	3000	300	+	—	—	+
22	145	Cholera stool	3000	500	3000	500	+	+	+	—
23	218	Dysentery (El Tor)	—	—	—	—	—	—	—	+
24	184	» »	—	—	—	—	—	+	+	+ After 48 h.
25	10	» »	—	—	—	—	—	+	+	+ Slight after
26	Tor No 4	» »	3000	800	3000	250	+	—	—	+
27	» » 5	» »	3000	500	3000	250	+	—	—	+
28	16	» »	—	—	—	—	—	+	+	+ Slight after
29	21	» »	—	—	—	—	—	+	+	+
30	23	» »	—	—	—	—	—	+	+	+
31	Tor No 6	» «	3000	800	3000	300	+	—	—	+
32	Calcutta 2	(?)	—	—	—	—	—	+	+	+ After 48 h.
33	1	Dysentery (El Tor)	—	—	—	—	—	..	..	+
34	2	» »	—	—	—	—	—	..	..	+
35	3	» »	—	—	—	—	—	..	..	Very slight after 48
36	Calcutta 6	(?)	—	—	—	—	—	+	+	+ After 48 h.
37	» 8	(?)	—	—	—	—	—	+	+	+
38	4	Dysentery (El Tor)	—	—	—	—	—	+	+	+ Very slight
39	5	» »	—	—	—	—	—	+	+	+ » »
40	6	» »	—	—	—	—	—	+	+	+ » »
41	Mansone	Sea water	—	—	—	—	—	—	—	+ » »
42	Agra II	Cholera stool	3000	800	3000	250	+	+	+	—

N. B. — The numbers give the dilution at which the serum produced complete agglu tion. + means that a given test was positive. — means that the given test was negative

Saturation test. — Bordet was the first to notice that an emulsion a microbe added to the agglutinating serum of an animal previously ected with the same microbe, absorbed most, if not all, of the specific glutinins of the serum. The specific agglutinins of an immune olera serum, for instance, are completely absorbed by adding olera vibrios to the serum, whereas the non specific agglutinins nain free in the serum.

In our experiments, ten loops of a cholera vibrio (CK), grown for hours on agar, were suspended in 10 ccs. of a 5 % solution of olera serum (Berlin). After standing for two hours at the tempe- ure of the laboratory, the mixture was centrifugalised and the ar supernatant fluid decanted. The agglutinating power of the canted liquid was now tested on all the vibrios which, in previous periments, had been agglutinated by the serum. As a control, the glutinating power of the same serum, not previously saturated th cholera microorganisms, was examined comparatively. The ult was that all the vibrios which agglutinated with cholera serum, ether isolated from cholera cases or not, absorbed the specific glutinins (See Table IV).

Pfeiffer's reaction. — Owing to the number of microbes to be amined, it was impossible to try this reaction on guinea-pigs. The ormous number of animals which would have been necessary, and fact that some vibrios were not virulent, made this experiment impossible one. Metchnikoff had noticed that if one drop of ritoneal lymph from a guinea pig (which had received either no evious injection, or merely one of bouillon) was added to vibrios spended in a little immune serum, a typical Pfeiffer's reaction took ace. Bordet used guinea pig's serum instead of peritoneal lymph. Three to four ccs. of bouillon were injected into the peritoneum an animal, and four hours afterwards the opaque liquid, full of cocytes, was withdrawn and left in the ice-box until the next day. e cholera serum was prepared by injecting rabbits with vibrio CK. hen a trace of this peritoneal lymph was added to vibrios suspended serum (CK), the vibrios which reacted at all were almost completely nsformed into granules. The microscopical appearance of these eparations therefore differed entirely from that of control pre- rations. The main results are given in Table IV, which shows at all the vibrios agglutinated with cholera serum also reacted Pfeiffer's test.

Fixation test. — If a serum (previously heated to 56° C), spe
for the corpuscles of an animal or for a given microorganism,
cholera-vibrio, be mixed with the red corpuscles of the same ani
or with the cholera-vibrio, the corpuscles or vibrios absorb
immune body.

Bordet also showed that if red corpuscles or microbes contai
an appropriate immune body, be added to fresh non-heated alex
(e.g. fresh rabbit's serum), the corpuscles or microbes absorb
alexine, so that none remains free in the liquid.

Accordingly, if fresh guinea pig's serum be added to cholera vib
which have not absorbed any cholera-immune body, the alex
not being absorbed, remains free in the liquid. The proof of thi
that if « sensitised*» corpuscles be added to such a mixture,
globules are quickly haemolysed. If on the other hand, vibrios w
have already absorbed the cholera-immune body be added to the s
quantity of fresh serum, the microbes absorb the alexine; a
provided the amount of fresh serum is not too great, the alexin
absorbed so completely that « sensitised » corpuscles, when adde
the mixture, are not dissolved. If vibrios other than cholera
added to cholera serum, the immune body is not fixed, the ale
added remains free, and the sensitised corpuscles are dissolved.

Such are the fundamental facts on which Bordet and Geng
method is based. This has been tried on various microbic spe
but, as far as I know, not systematically on vibrios.

This test was tried by two different methods.

Bordet and Gengou's method.—The immune-serum was heate
56° C. during half an hour, and fresh rabbit's serum was treate
the same manner. An agar culture 18 hours old of the microbe t
tested was suspended in 2 ccs. of physiological solution.
alexine was the serum of a rabbit bled on the preceding day,
centrifugalised until freed of corpuscles.

The following mixtures were then prepared in three tubes:

Tube I contains 0,2 cc. of microbic emulsion, 0,6 cc. of imm
serum, and 0,1 cc. of alexine.

Tube II contains 0,2 cc. of microbic emulsion, 0,6 cc. of
(heated) serum, and 0,1 cc. of alexine.

*Note.—The French word « sensibilisé » translated here « sensitised» means
the corpuscles or microbes have fixed a certain amount of immune-body.

Tube III contains the same mixture as the first.

All these remained for 4 to 5 hours at room temperature, and re well shaken from time to time.

One volume of a mixture, consisting of two parts of a heated um hemolysing bovine red corpuscles, and one part of the washed rpuscles of the same animal, was then added to tubes I and II. e volume of a mixture of two volumes of physiological salt ution and one volume of bovine, non-sensitised, red blood rpuscles was added to tube III. After three hours, the tubes were mined and note was taken of any haemolysis which had occurred. hen the vibrios fixed the immune-body, the contents of tube II owed very marked haemolysis after two hours, whereas haemo- is was entirely absent in the other tubes. In tube III, haemolysis peared after 12-18 hours, if the microbes used were themselves emolytic, but when this was not the case, no haemolysis took place. sults are shown in Table IV.

Dopter's method.—The following mixtures were introduced into e different tubes:

Tube I.—Twenty drops of immune serum (CK) heated to 56° C, lrops of alexine, 6 drops of microbic emulsion.

Tube II.—Twenty drops of immune-serum (CK), 4 drops of alexine, lrops of microbic emulsion.

Tube III.—Twenty drops of immune-serum (CK), 5 drops of xine, 6 drops of microbic emulsion.

Tube IV.—Twenty drops of fresh rabbit's serum previously ated to 56° C, 3 drops of alexine, 6 drops of microbic emulsion.

Tube V.—Twenty drops of fresh rabbit's serum heated to 56° C, lrops of alexine, 6 drops of microbic emulsion.

After the tubes had been left 4 to 5 hours at the temperature of the oratory, 2 drops of sensitised corpuscles were added to each tube.

The results obtained are shown in Table IV, and it is noticeable that hough a distinct relationship exists between the agglutination test d Pfeiffer's reaction, in so far that the microbes reacting to one test o react to the other, there is no agreement between these two actions and the fixation test. Fourteen of the 24 vibrios not glutinated by cholera serum fixed the immune body of the same

serum. Conversely, 6 microbes which were transformed i granules and agglutinated by cholera serum did not absorb immune body.

Haemolysis test.—Kraus having noticed that certain vib dissolved red corpuscles, extended his researches and conclu that no haemolytic comma micro-organism was a true cholera vib He used the following method: tubes of agar were liquefied, coole 40° C, and 0,3-0,5 ccs. of defibrinated rabbit's blood were allowe mix slowly and uniformly with the agar in each tube. The agar poured into Petri's dishes, and when cool, was inoculated with vibrio to be examined, in such a way as to obtain separate w defined colonies. After 24 hours in the incubator, the colonies w haemolytic, were surrounded by a clear more or less exten areola, contrasting sharply with the dark opaque colour of agar.

The objection to this method is that the results are not constant not easily observed. A simpler method, by which the slightest t of haemolysis may be readily demonstrated, was therefore used.

An agar culture, 18 hours old, was rubbed up in 4-5 ccs. physiological NaCl solution; 0,1 cc. of this emulsion was mixed tube with 0,9 cc. of physiological salt solution, to which one la drop of washed red corpuscles, or of the defibrinated blood of a ra or any other animal had been added. After 12-24 hours as a r haemolysis was noticeable if it was ever to take place at all, tho in some cases it dit not appear for 24 hours. If no haemolysis taken place after 48 hours, further observation was unnecessary such vibrios would not dissolve blood later on, and were theref non-haemolytic.

The haemolytic properties of vibrios were tested on the corpuscles of rabbits, goats, sheep and guinea pigs, and the corpus of all these animals reacted in the same way. The vibrionic hae lysins therefore were not specific for one special kind of animal.

At first sight, the haemolytic properties of vibrios appear to stan no relationship to the agglutinating and Pfeiffer's reactions, for sev agglutinating vibrios haemolyse and others do not. Neverthel Table IV shows that no vibrio haemolyses when the agglutina test, Pfeiffer's reaction and the fixation test are positive.

Recapitulation of bacteriological results.—The vibrios examined
y be divided into four groups:

1.—Those which give the four principal tests with cholera serum,
nely the agglutination test, saturation, Pfeiffer's reaction and the
ition test. They do not haemolyse, even when remaining in contact
h red corpuscles for three days, at the temperature of the laboratory.

2.—The second group contains the vibrios agglutinated and
ing the saturation and Pfeiffer's reactions with cholera serum, but
fixing the cholera immune body. These vibrios are strongly
molytic. This group consists of the El Tor vibrios only.

3.—The third group is formed by vibrios which are not
glutinated by immune serum, do not give the saturation or Pfeiffer's
ction, but fix the cholera immune-body. These vibrios also
molyse, but feebly and late, often after 36-48 hours only.

4.—The last group is formed by strongly haemolytic vibrios not
cting at all to cholera immune serum.

DISCUSSION OF RESULTS.

The discovery at El Tor of vibrios agglutinating with cholera-serum
of great importance, for two chief reasons: (1) It is the first time
t vibrios agglutinating with cholera serum have been found in the
sence of a local or general epidemic. (2) The discovery raises
whole question of the value of the present system of bacterio-
gical diagnosis; for if the agglutinating El Tor vibrios are not cholera
brios, the reliance placed so far on the agglutination, Pfeiffer's,
d saturation reactions as specific tests is not justified.

An opinion regarding the specific nature of the vibrios found at
Tor can be formed only by considering the subject from the
demiological and from the bacteriological points of view.

Discussion of epidemiological data. — Three principal epidemio-
gical questions present themselves.

Firstly. Are there any facts to show that in 1897, 1905 and 1906
olera existed in the Hedjaz or in any country traversed by the
grims on their way to and from Mecca? The answer is: "None."

Secondly. Is it likely that the vibrios agglutinating with cho serum come from a different place than the non agglutinating vibr In my opinion, the probability is that wherever the pilgrims infec themselves with vibrios agglutinating with cholera-serum, there t absorbed the other vibrios also.

Thirdly. Did the pilgrims whose faeces contained vibrios ag tinating by cholera serum, bring these vibrios from their homes the Hedjaz and El Tor, or were they infected en route?

A few details concerning the route followed by pilgrims travel to and from Mecca are here necessary. The pilgrims who unde quarantine at El Tor go to Mecca by two different routes. A num of them, chiefly Turks and people from Asia Minor, land at Yan stay there a few days and then proceed to Medina on foot or camel. After resting a few days at Medina, they travel to Me remaining in the holy city until the feast is over; that is, from a w to a month, or perhaps longer in some cases.

The other pilgrims, consisting for the most part of Egyptia Moghrabis, Algerians, Syrians and Russians, proceed the reve way: that is, they land at Jeddah and go on to Mecca, Medina, Yan and El Tor.

It is an undoubted fact that the vibrios agglutinating with chol serum have so far been found only among pilgrims from Turk Russia and Asia Minor returning via Jeddah, and it has been argu that the latter carried the cholera vibrios, from their own country i the Hedjaz and back to El Tor.

I am not ready to admit this theory and for the follow reasons. In the first place, I do not believe that the presence vibrios among the pilgrims was limited to the year 1897, 1905 a 1906, but consider it highly probable that the phenomenon occ every year. As a matter of fact, vibrios agglutinating with chol serum have been found at El Tor *whenever they have been looked f and exclusively among pilgrims returning via Jeddah.* In 1897, wh the vibrios were first discovered, Asia Minor and the route follow by pilgrims were free from cholera. In 1905 and 1906 cert districts, but by no means the whole of Asia Minor, were infec with cholera; but there is absolutely no evidence to show that t pilgrims, harbouring vibrios in their intestines, came from a chole infected district, or had been in contact with cases of this disea

er at home or on the journey. As will be shown presently, the ditions for the development of cholera at Mecca are simply ideal. these people who excreted vibrios in every stool at El Tor did contaminate the holy city. To argue, as has been done, that use cholera is present in some parts of Asia Minor, therefore y person coming from there must be regarded with suspicion, is ivalent to saying that because cholera exists at Marseille, therefore y one coming from St Petersburg or Moscow may carry vibrios his intestine. The comparison is by no means far-fetched, for urney of one hundred miles in Asia Minor, may and often does longer than that from Marseille to St. Petersburg. As a matter act, moreover, the pilgrims carrying vibrios did not come from same place or even from the same district. Two were from Minor (no place could be ascertained exactly), two were melians, two Anatolians, one Russian and one Turk.

Rejecting as I do the theory that the vibrios were brought by the rims from their homes, I would fain advance another theory based reasons which appear to me overwhelming. I believe that the rims infected themselves with vibrios at Mecca or Jeddah, that the reason why vibrios agglutinating with cholera serum e found only among pilgrims returning via Jeddah, and not ng pilgrims returning via Yambo, was simply that a much rter time elapsed between the date of infection and the arrival El Tor in the case of the former than in the case of the latter. ther, I consider it highly probable also that the infection took e by drinking the water in Jeddah, or, more probably, in Mecca. It is necessary to consider very briefly the possible infection water in both places. The Jeddah water comes from wells in town, or is brought from some distance, and when I tasted it 905, the water sold to the pilgrims was indescribably filthy and y. It teemed with all kinds of microorganisms, though I can not for certain that it contained vibrios. Indeed it was so bad that Europeans drank it only when obliged to, when, in fact, water n ships was not obtainable.

At Mecca, part of the ceremonies consists in drinking water from Zemzem well. A bacteriological examination made at El Tor shown this water to be swarming with vibrios, though those lutinating with cholera serum have not been found so far.

Addendum.

Since this paper was written, a vibrio agglutinating with cholera-um (1 in 2000) was isolated this year at El Tor from the intestinal tents of an *Algerian* pilgrim who had died from carcinoma of the r and colitis.

Moreover, it is part of a religious ceremony for the pilgrims to w in certain tanks. I have obtained photographs showing the pilgri some drinking in these tanks, others filling their vessels, oth performing their ablutions (including the cleaning of the anus) the very place where, a few seconds afterwards, a pilgrim may que his thirst. One pilgrim carrying vibrios may contaminate the wh water supply, and that this actually does occur is shown by explosive character of the epidemic, and the appalling morta following on this promiscuous bathing and drinking, when a case cholera has occurred at Mecca. If these Asia Minor people re carried the cholera vibrios from their homes to Mecca, why did an explosive outbreak of cholera follow on their using these tan More favourable conditions for the spread of cholera can hardly conceived than water contaminated with vibrios, drunk by thousa of people, of whom many are suffering from gastro-intest disturbances, and whose constitutions are undermined by hardsh of every kind. Yet no case of cholera occurred at Mecca.

Should the pilgrim return home via Jeddah, he arrives at El after a period averaging three weeks; should he return via Yam he does not reach El Tor till six weeks at least have elapsed at leaving Mecca. In his report on the pilgrimage and the chol epidemic at El Tor in 1902, Dr. Crendiropoulo drew attention to fact that the faeces of almost every pilgrim entering the hosp contained vibrios, but that this was only the case during the five eight days following the arrival of the pilgrims. As time went the vibrios became rarer and rarer, and after 13 to 14 days at El (corresponding on an average to 33-34 days after leaving Mec almost none were found in the faeces. The pilgrims returning Yambo therefore, whose journey to El Tor lasts from 5 to 8 weeks, ha certainly had time to eliminate many of the vibrios absorbed at Mec

Lastly a most important fact is that not a single case of chol occurred on the voyage home, although these ships were overcrowd with people coming from Asia Minor. I conclude therefore th epidemiologically, there is no evidence to show that the vibrios fou at El Tor were true cholera-vibrios.

Discussion of bacteriological results. — In the literature concerni the part played by Koch's vibrio in the causation of Asiatic chole there is one fact, and one only, which stands out as being of supre

portance; namely, that at a time when no epidemic of Asiatic lera existed in Europe, certain persons wilfully or accidentally llowed a small quantity of microbes isolated from cases of cholera, that these persons either died, or presented more or less acute ptoms of the disease. The rest of the evidence, as compared to cardinal fact, is of minimal importance.

The value of even this observation, however, may be overesti- ed; for the symptoms of cholera, whether mild or acute, are those characteristic of other intoxications of intestinal origin. se symptoms have been produced also by vibrios, isolated from cholera patients but from water; and at a time when the ase did not exist anywhere near the locality where the vibrios e found. In this connection, the experiments of Metchnikoff are icularly valuable. This observer produced in man the symptoms holera with vibrios isolated from Seine water at a time when e was no cholera in Paris. Nevertheless, these experiments do invalidate those previously noted, as the symptoms produced by chnikoff's microorganisms were neither so definite nor so acute hose due to vibrios isolated from cholera cases.

I admit therefore that the cause of cholera is the microbe which produced typical cholera, experimentally so to speak, in several an beings.

The sera of animals inoculated with this vibrio acquire new and nite properties. Minimal doses agglutinate cholera vibrios; if lera serum and cholera vibrios be introduced into the peritoneum of nea pigs, the microorganisms are first converted into granules and nately dissolved; when the cholera-vibrios are added in vitro to serum, they absorb the specific agglutinins. The conclusion ally drawn from these facts is, that any microorganism giving e reactions with sera so prepared is the true vibrio of cholera, that any vibrio, not so reacting, belongs to another class. Several ons compel me to reject such a conclusion.

Ever since the discovery of biological reactions, more especially of precipitation and agglutination tests, attempts have been made to ch an absolutely specific value to these reactions. To my mind, n attempts have failed. The precipitation test, for instance, is not rely specific; for although it is easy to obtain a serum precipitating of humans only, yet a serum which precipitates that of other

animals also, e.g. monkeys', may be prepared at will. The t
therefore specific under certain conditions only; conditio
defined, but nevertheless existant.

Similarly, typhoid serum agglutinates, less actively it is
paratyphoid bacilli also. The agglutination test therefore, is
relatively, but not absolutely specific.

Further, it is unusual to rely on one or two characteristics o
the classification of plants and animals. In every classification,
characteristic should be taken into consideration and its po
value weighed.

A comparison of the vibrios found at El Tor in cases of dyse
with those of the specific vibrios of cholera, shows that although
are equally affected by the agglutination, Pfeiffer's and satu
tests, they react differently to the haemolysis and fixation tests.
reactions appear to me as important as the former.

All the El Tor vibrios haemolyse under certain conditions
all the vibrios isolated from cases of cholera and agglutinating
cholera serum, do not. This fact, in my opinion, is a sufficient r
for classing the El Tor and the cholera vibrios into two distinct gr

It has been urged, however, that haemolytic vibrios agglutin
with cholera serum have been isolated from cholera stools, an
conclusion drawn that the true cholera vibrio may occasional
haemolytic. The fact is correct, but the conclusion would be
only if typical cholera stools contained but one kind of vibrio
the East at any rate, this is far from being, and many case
cholera are cases of mixed « vibrionic » infection. In many stoo
one finds not one but several comma-shaped bacilli, some ag
nating with cholera serum and others not, some monociliated, o
multiciliated etc. It is possible, nay likely, that in some cases
the cholera and the El Tor vibrios exist in the same stool.

In this connection I may state that some years ago, I wrote
distinguished bacteriologist, who was watching an epidemic of ch
in the East, to send me cultures isolated from cholera stools.
cultures of vibrios were sent, of which onlyone agglutinated
Berlin serum. The three others although monociliated did
agglutinate but were intensely haemolytic. I quote this fact
to show how very complicated the bacteriological examination
cholera stool is in reality.

The fact therefore that vibrio, not only agglutinating but and molytic also, is occasionally found in cholera stools, does not ssarily prove that a cholera vibrio haemolyses at times, but it simply show that several kinds of vibrios, some haemolytic some non haemolytic, may be isolated from cholera stools. ed, in view of the facts recited in this paper, the last conclusion is to me the only possible one.

What has been said regarding haemolysis applies equally to the ion test. All the vibrios isolated from the cholera stools which utinated with cholera serum gave the fixation test; all the El Tor ios, without exception, did not. On the other hand, several ios, though neither agglutinated nor converted into granules by era serum, reacted to both the fixation and the haemolysis test. conclude therefore that all these reactions are relatively and not olutely specific and that any one test, or group of tests is insufficient stablish a true classification. The only possible classification is roup together all the vibrios reacting in the same way to all , separating them from those which, under the same conditions, ve in a different way. If this method be applied to the vibrios d at El Tor, there is no difficulty in distinguishing them from the cholera vibrios, in spite of several of the reactions of both being lar. And it follows also, that *the agglutination, saturation and ffer's tests, are not, in themselves, of absolute diagnostic value for era vibrios.*

ON VIBRIOS FOUND IN SHIP WATER.

During the second quarter of the year 1905, the drinking water hips calling at Port Said was examined for vibrios by Dr. Zirolia. samples of water submitted to bacteriological examination were in number and in 82 cases, vibrios were found.

Six only of these 82 vibrios gave a positive agglutinating lt with high dilutions of cholera serum. (Fluid horse-serum lly sent by Dr. Gaffky of Berlin. Standard 1 in 3000. Date /1905). Special attention was therefore paid to these 6 vibrios, representing in a natural classification, a group most closely ted to that of the cholera vibrio.

The following table gives the port of departure of the ship the origin of the water containing these vibrios.

TABLE V.

Date of arrival at Port-Saïd.	Name of Ship.	Flag.	Port of departure.	Origin of drinking water.	Observations.
July 15.05.	Clan-Ronald.	British.	Liverpool.	Glasgow.	In a preceding jo the ship sailed fro cutta and called a Saïd on May 12th.
Aug. 22.05.	Torbryan.	»	Swansea.	Swansea.	Usually runs b Yokohama & Sing
Sept. 22.05.	Benarty.	»	Colombo.	Colombo.	Usually sails to calling at Yokohan gapore, Bombay, Co
Sept. 26.05.	Knowsley Hall.	»	Karachi. Colombo.	Karachi.	
Octo. 19.05.	Congo.	French	Alexandria.	Alexandria.	Runs between M les, Alexandria, Po and Beyrouth. H crossed Suez Can many years.
Octo. 20.05.	Vesta	Russian.	Odessa.	Constantinople.	Runs between C Constantinople, Sm Beyrouth, Jaffa, Por Alexandria. The sh not been through th Canal in 1905.

TABLE VI.—SHOWING THE CHIEF MORPHOLOGICAL & CULTURAL CHARACTERISTICS OF THE VIBRIOS.

Name of Culture	Form and size.	Motility.	Cilia.	Reaction with Gram.	Optimum temperature.	Culture in bouillon and peptone water.	Cholera-roth.	Gelatine Culture.	Cultures on potatoes.	Cultures in milk.	Inverting action.	Haemolytic action.
CLAN RONALD...	Same form and size as V. cholera. Involution and S. forms.	Motile. Serpentine movements quick. Slow rotatory and forward movements.	Monociliated	Does not retain stain	Optimum 37° C.	Well developed surface veil.	Present.	Growth slow. Colonies transparent, yellowish brown with irregular borders, characteristic bulla in stab cultures.	Whitish layer.	No coagulation. Slight acidity.	Well marked reaction.	Very marked clear, 3mm. areola round colonies.
TORBRYAN.....	Same length but thinner. Curve accentuated. Many S shaped forms.	Very motile. — Movements like those of preceding one.	Monociliated (2)	do.	do.	No surface veil. Uniform clouding.	Present but feeble and slow. More marked by addition of Pot. Nit. solution.	Very slow liquefaction. Brown culture with irregular contours and dark nucleus. No bulla in stab cultures.	Yellowish to dark brown layer.	No coagulation. Reaction unaltered.	Reaction very feeble.	None.
BENARTY.....	Same length but thinner. S shaped and filamentous forms.	Motile. Slow serpentine movements. Slow rotatory and forward movements.	do. (2)	do.	do.	do.	do.	No liquefaction. Brown, yellowish colonies, granular, with irregular contours.	Yellowish layer.	do.	do.	Very slight, not clearly shown.
KNOWSLEY HALL	Short curve, very marked. No filaments or S shaped forms.	Motile. — Movements slow.	do. (2)	do.	do.	do.	do.	No liquefaction. Brown colonies with irregular contours & central nucleus.	Yellowish brown layer.	do.	do.	do.
CONGO.....	Short but slightly longer than preceding one.	Very motile. Serpentine movements very quick. Rotatory and forward movements quick.	do. (4)	do.	do.	do.	do.	Very slow liquefaction. Brown yellowish, transparent colonies. No bulla in stab cultures.	Very light yellowish layer.	do.	do.	do.
VESTA.....	Short, curve accentuated. No filaments or S shaped forms.	Very motile. Serpentine movements very quick. Rotatory and forward movements quick.	do. (4)	do.	do.	do.	do.	Liquefaction very slow. Colonies reddish yellow with irregular contours. No bulla in stab cultures.	Dark yellow layer.	do.	do.	do.

gglutination test.—Cholera serum was diluted in physiological solution. The macroscopic test only was applied. In one cc. of solution of serum, one loop or 2 mg. of agar-culture grown 8 hours at 37°C., was suspended. The result was considered as ive when agglutination took place in two hours' time, at 37°C.
s the Berlin cholera-serum contained carbolic acid, it was necessary e whether carbolic acid possessed any agglutinating properties ese microbes. For this purpose, chemically pure carbolic acid tested in 0,5-0.25 % dilutions, either in distilled water or physio-al salt solution, but none of these vibrios were agglutinated by olic acid, not even by a 0.5 % solution.
everal rabbits were immunised also by intravenous injections of ually increasing doses of living and dead cultures, against each ese 6 vibrios and a cholera vibrio isolated during the last otian cholera epidemic of 1902.

E VII. — SHOWING THE RESULTS OF THE AGGLUTINATION TEST OF THESE VARIOUS VIBRIOS WITH DIFFERENT SERA.

Name of Sera.	V. Clan Ronald.	V. Tor-bryan.	V. Benarty.	V. Knows-ley Hall.	V. Congo	V. Vesta.	Cholera.
cholera agglutinates bbit)	500	100	—	—	500	100	500
cholera (Horse) »	10000	500	500	500	500	100	3000
Ronald ..... »	10000	50	—	100	500	—	2000
yan ..... »	—	200	1000	—	—	—	—
ty ..... »	500	—	20000	—	100	100	2000
sley Hall ..... »	500	500	—	20000	—	—	—
..... »	100	1000	—	—	500	2000	100
..... »	200	—	100	50	1000	—	50

athogenic effects and Pfeiffer's reaction.—Pfeiffer's reaction obtained with the V. Clan Ronald only, and the result is shown e following table. In the case of the other vibrios the requisite lence could not be obtained.

TABLE VIII.—PATHOGENIC EFFECTS. PFEIFFER'S PHENOMENON

Name of culture	Result of injection into pigeons	Result of injection into guinea-pig.		Pfeiff react
		Result	Minimum mortal dose	
Clan Ronald	Negative	Positive	1/4 loop of culture 24 h. (guinea pig gr. 300)	Posi
Torbryan	»	»	3 loops	—
Benarty	»	»	2 »	—
Knowsley Hall . . .	»	»	2 »	—
Congo	»	»	3 »	—
Vesta	»	»	3 »	—

The conclusion to be drawn from the recital of the more mar characteristics of these 6 vibrios is, that V. Clan Ronald posses more than any of the others, the characteristics of the true cho vibrio. The five other vibrios markedly differ from cholera vib by their general properties. Their most marked characteristic, nam their agglutination by a very active cholera serum in 1/500 solut means that there is a certain degree of relationship between them the true cholera vibrios. In other words, the agglutination-react must not be regarded as a specific test but as a group-reaction.

The results of agglutinafion tests made with the 7 sera, altho apparently surprising, show there is a distinct relationship betw the V. Clan Ronald and cholera vibrios, as the sera of animals injec with either vibrio agglutinate both. I do not believe that V. C Ronald is a true cholera vibrio, as it is haemolytic.

The experiments also show the degree of relationship exist between the 6 vibrios and between each and the cholera vibrio.

The conclusion which I base on the experiments carried out Port Said are as follows:

1.—A certain number of vibrios, although agglutinating to so extent with cholera serum, *are sharply differentiated morphologic from the cholera vibrio, by the fact that they are multiciliated.*

2.—Whereas some vibrios found in ship water are agglutina by very dilute solutions of cholera serum, others are agglutinated

nger solutions only. The latter form a transition stage, so to between the non-agglutinating and the highly agglutinating s.

- Although an active cholera serum agglutinates all the vibrios e extent, yet only two of these vibrios when injected into ani- roduce sera having a powerful agglutinating effect on cholera ; two sera have the same property to a slighter extent, and ve none.

ese experiments therefore support the conclusion previously l at, namely that *it is not advisable to trust to the agglutination ly in the bacteriological diagnosis of cholera. The test is useful specific.*

El Tor,
February, 22. 1907.