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Cholera: possible infection from aircraft effluent

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SUMMARY

This paper presents the hypothesis that some cases of cholera might be due to effluent discharge from aircraft. The theoretical case is borne out by inspection of data on the physical conditions pertaining between high altitudes and ground level. A study of the distribution of isolated outbreaks and single cases of disease and their relation to major airline routes showed a reasonable correspondence. Sporadic outbreaks of cholera in Europe between 1970 and 1975 were found to lie within the flight paths of regular airline services from Calcutta, where cholera is endemic, to the Northern Hemisphere. Laboratory studies on the stability of *Vibrio cholerae* to conditions likely to be encountered in droplets falling from high altitude to the ground suggested that significant numbers of organisms might survive. It should be noted that in this study no account was taken of the effect of ultra-violet light on viability and it is known that at high altitudes the ultra-violet light component of solar radiation is much higher than at ground level. Results of experiments where small numbers of organisms were inoculated into relatively poor media showed that rapid growth ensued and that sufficient organisms were produced to give an infective dose of *Vibrio cholerae* in 1–10 ml/fluid. It could be concluded that human infection could easily occur by ingestion of fluids such as milk or soup which had some time earlier received a fortuitous but slight contamination from the air. Investigation of one disinfectant (chloramine T) showed that it reacted rapidly and in a complex manner with peptone. One effect of this reaction was the elimination of bactericidal activity and it seems likely that, as at present employed, chloramine T is of doubtful value in aeroplane hygiene. One important conclusion that arises from this work is that if cholera can be spread, even only occasionally, by effluent from aircraft then close investigation should be made of the possibility of other bacteria and viruses being spread in a similar way.

INTRODUCTION

In 1974 Bruce-Chwatt described the possible role of air transport in the spread of disease. He pointed out that modern aircraft could speedily transfer diseases from one place to another; also that some flying times, even on relatively long flights, were shorter than the incubation periods of some diseases. Hence incubating cases could be transferred from place to place and might initiate foci of infectious diseases away from airport terminals. The result negates the usual rules of quarantine and increases the problems of the epidemiologist.

Table 1. *Pressure and temperature variations with altitude**

Altitude (ft 10^{-3})	Pressure		Temperature (°C)	Vapour pressure† (Torr)
	Millibars	Torr		
0	1012	760	1	4·58
10	770	525	−10	1·95
24	400	300	−50	0·03
30	300	225	−60	0·008

* Pressure and temperature figures are approximate except for zero altitude where 'standard' conditions are quoted. The data was supplied by the Meteorological Office.

† Saturation pressure of water vapour over ice at stated temperature. The data was taken from *Documenta Geigy, Scientific Tables*, 6th ed., published by Geigy Pharmaceutical Company Limited, Manchester, England.

Mansueto & Migneco (1974) made similar comments with special reference to cholera, discussing the importance of aircraft in the spread of the seventh pandemic.

Not considered was spread of infectious disease by atmospheric pollution. Most modern aircraft, but not all aircraft in service, have holding tanks for semi-solid sewage. Nearly all aircraft discharge water from handbasins and possibly also overflow material from holding tanks directly into the atmosphere from inbuilt 'drain masts'. At least one major airline is known to add a sterilizing agent, chloramine T, to all water used on its aircraft. Whilst this precaution certainly reduces the risk of transmission of infection from drinking water, its effect on waste water discharge from aircraft will be considered later. Modern jet aircraft fly up to 30000 ft and above. Approximate figures for some of the physical conditions met by an aircraft in the course of a flight are shown in Table 1. Conditions are such that at high altitudes discharged effluent would be frozen immediately. Since the discharge is from a pressure of approx. 600 torr ('pressured' cabins are kept at a simulated altitude of approx. 8000 ft) inside the aircraft to a pressure of less than half this externally then, due to expansion, it is probable that the effluent would freeze as droplets. These droplets would not evaporate (i.e. freeze-dry) because of the relatively large pressure difference between the surrounding atmosphere and the saturation pressure of water over ice at the stated temperature. Droplets would remain frozen until in falling they reached an area where the temperature was above 0 °C. Melting would then occur but the rate of evaporation, if any, would depend upon the difference between the partial pressure of water vapour in the atmosphere and the vapour pressure of water in the droplet. In areas of relatively high humidity there is no reason why droplets of effluent from aircraft should not safely reach the ground. If these droplets contained pathogenic micro-organisms which could survive one or more cycles of freezing and thawing then they could present an infectious hazard.

This paper describes a study of the possible spread of cholera by droplet infection from aircraft discharge. In the first part of the paper the incidence of cholera is considered with respect to some major airline routes. In the second part laboratory experiments are described which were made to test the survival of *Vibrio cholerae* under some of the conditions thought to occur in the field. Cholera was

chosen as a model because case incidence of the disease is published regularly. Moreover the major causative organisms are relatively delicate and can act as a sensitive indicator of the possible behaviour of more robust micro-organisms.

MATERIALS AND METHODS

Incidence of cholera

Data for 1970–5 inclusive was obtained from the World Health Organization Weekly Epidemiological Record. Total incidence over 5 years for a number of areas (usually an area was a country) was plotted on a suitable map.

Airline routes

Major routes were taken from maps supplied by British Airways and Air India. Some of these routes are plotted on a map identical with that used for the cholera incidence.

Organisms used

A laboratory strain of *Vibrio cholerae* was used throughout. In some supporting experiments a laboratory strain of *Vibrio el Tor* was used also. In any one series of experiments serial cultures were made. Peptone-saline, 5 ml of 1% peptone in isotonic saline at pH 8, was inoculated each day with 0·02 ml of the previous day's growth. Incubation was for 16 h at 37 °C.

Total counts

These were done using a Helber chamber and a phase-contrast microscope. Before test, organisms were immobilized with 1% (v/v) formalin.

Colony counts

Performed essentially as described by Miles & Misra (1938) except that sectored as opposed to multiple plates were used. The medium was 1·5% Ionagar No. 3 (Oxoid Ltd) in peptone-saline controlled strictly at pH8. Even slight variations of pH led to meaningless counts.

Reagents

Where possible ANALAR reagents were used. Samples of chloramine T were of reagent grade.

Estimation of chloramine T

The method used was based on an *o*-tolidine reaction with 'free' chlorine described in *A Handbook of Colorimetric Chemical Analytical Methods* (1953). The Tintometer Ltd, Salisbury, England.

The *o*-tolidine reagent was prepared by dissolving 1 g of this substance in 100 ml concentrated HCl and making the solution up to 1l. This operation was done only once for all the experiments performed. The greatest care was taken using a fume cupboard and rubber gloves since the reagent is carcinogenic and its use is

now not recommended. In these experiments it was chosen for expedience and sensitivity.

For test, 0·2 ml reagent was added to 10 ml chloramine T solutions containing 0–20 mg/l of this substance.

After standing 50 min at room temperature the Optical Density (O.D.) of the yellow colour which developed was measured at 436 nm using a Bausch and Lomb 'Spectronic 20' spectrophotometer.

Preliminary tests showed that the peak of the absortion spectrum was 436 nm. Colour development reached a maximum at 50 min, was stable for 3 h and began to fade at 5·5 h; by 16 h all colour had disappeared. Other tests showed that the Beer–Lambert Law was obeyed at least over the range 1–30 mg/l chloramine T.

The 'free' chlorine content of chloramine T was determined for three commercial samples. An excess of 10% (w/v) potassium iodide was added to solutions of known concentrations of chloramine T (5 ml of 0·2–1·0 g/l). The iodine released was titrated against standard sodium thiosulphate solution (N/100) using starch as indicator. In our hands a mean value of 16·5% 'free' chlorine was obtained from seven determinations using three batches of reagent. This differs from the value calculated from the formula given on the bottles (15·6% anhydrous, 12·6% hydrated) the reason being unknown. Nevertheless this value indicates that the *o*-tolidine test as used here is at least as sensitive for 'free chlorine' as the other methods described in the literature.

RESULTS AND DISCUSSION

Cholera incidence and airline routes

Fig. 1 is a map of part of the Eastern and Western Hemispheres showing total cholera incidence reported per area for the years 1970–5. Fig. 2 shows some of the major air routes from Calcutta, India to the West. Clearly some areas are more afflicted by cholera than others. In some of them cholera is endemic, in others a high score is due to a large imported outbreak in one particular year (cf. Portugal, WHO, 1974 *a*, *b*, *c*). Some countries show a few scattered outbreaks or cases and others none at all. A high or persistent incidence has most often been attributed to water or food-borne infection, case to case infection, or carrier transmitted infection. In country to country transfer, spread of organisms along trade routes provides a reasonable explanation of the epidemiology. In some instances, however, where only small outbreaks have occurred (Trucial Oman, WHO, 1970*a*, *b*; Czechoslovakia, WHO, 1970*c*) the source of infection is, to say the least, vague. Single sporadic cases require still more subtle explanation especially where no known contacts have been reported. For example in 1970 at Val Saint-Germain, Essone Department, France, *Vibrio el Tor, Ogawa*, was isolated from a hospitalized 65-year-old woman. The patient had never left her village and had no known contact with anybody coming from a country where cholera had been found. She ate only produce from her own garden and there was no suggestion that human waste was used as a fertilizer. Her water supply met drinking water standards. Examination of all family contacts, medical and hospital personnel did not reveal

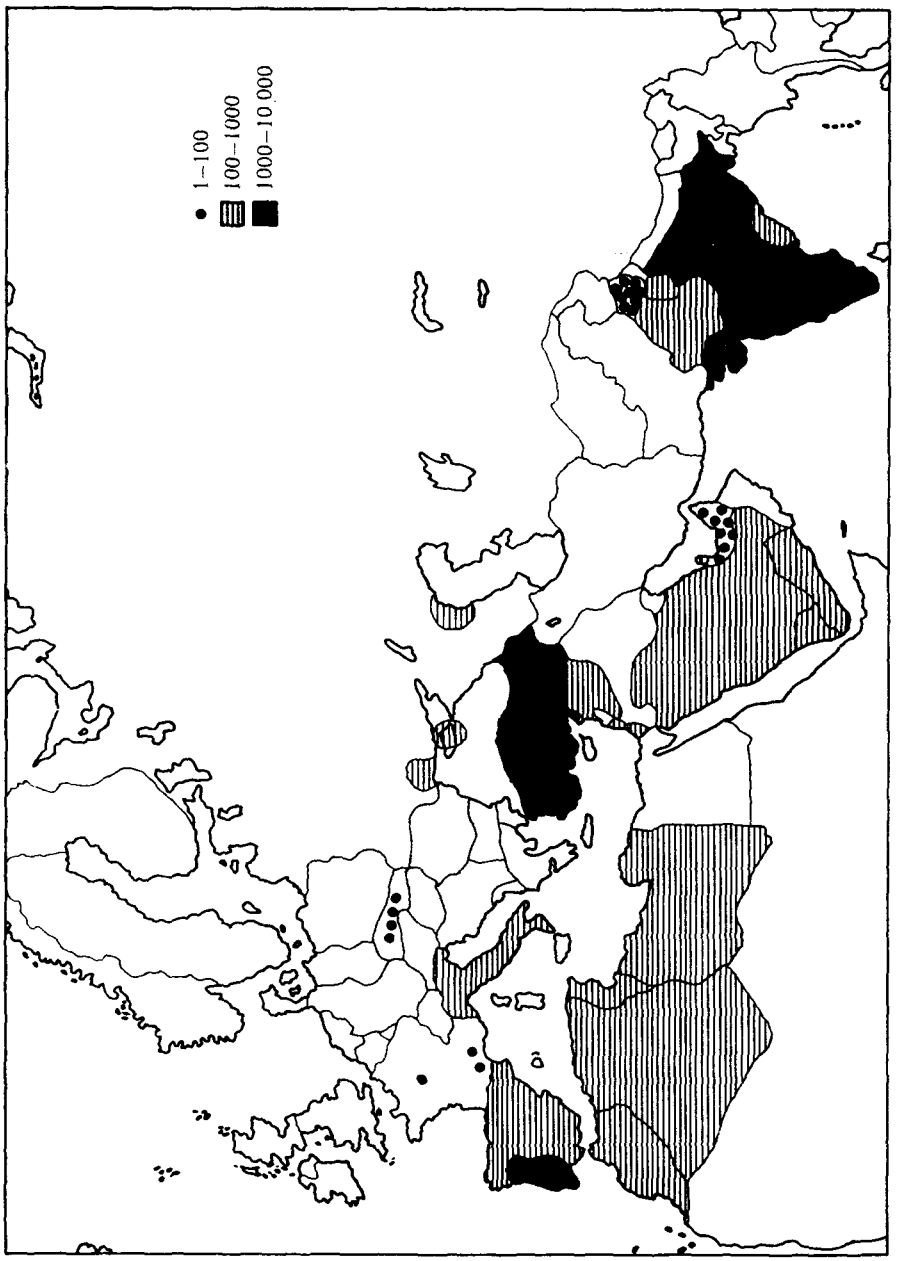

Fig. 1. India to North Africa and Europe: incidence of cholera 1970–5.

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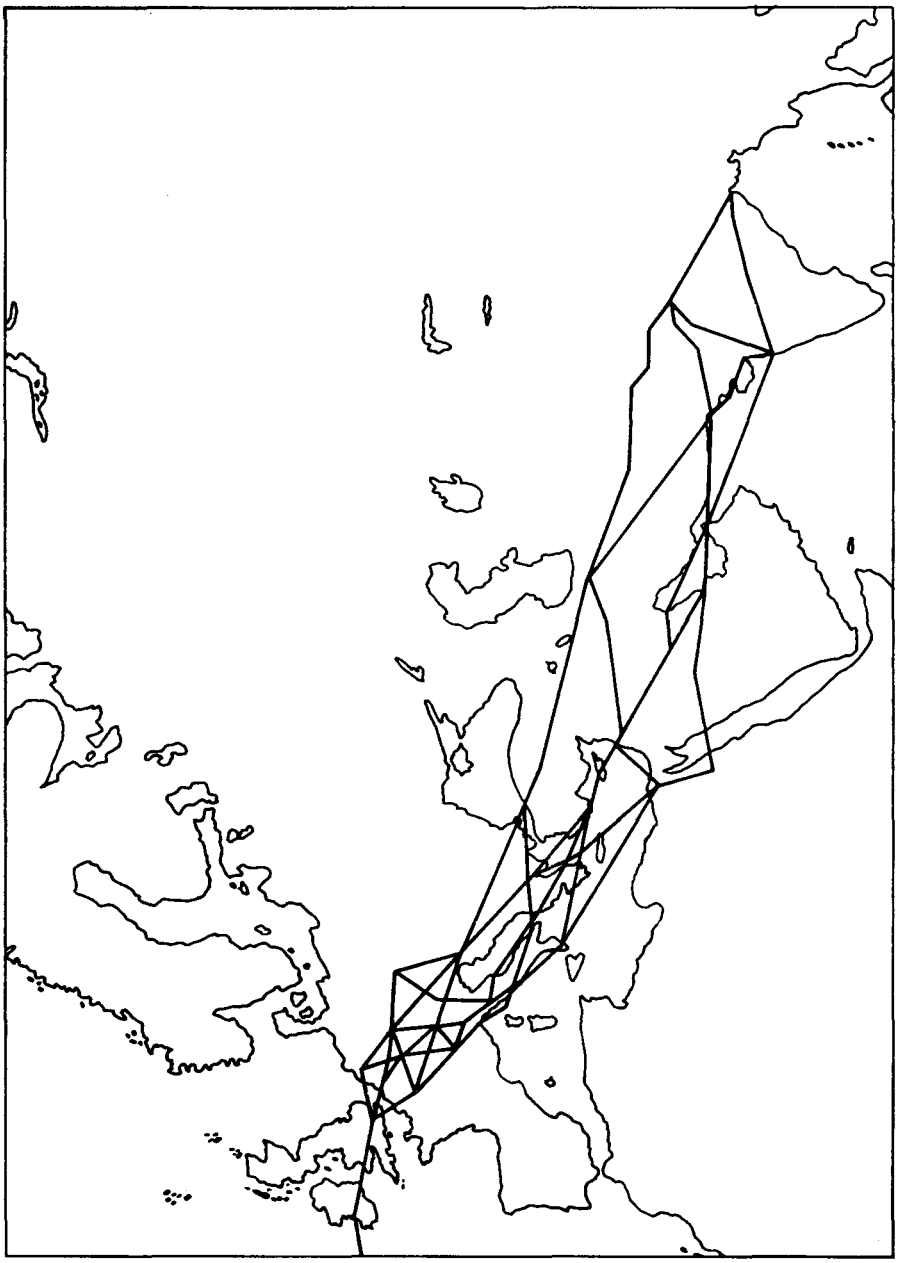

Fig. 2. Some major airline routes from India to Europe.

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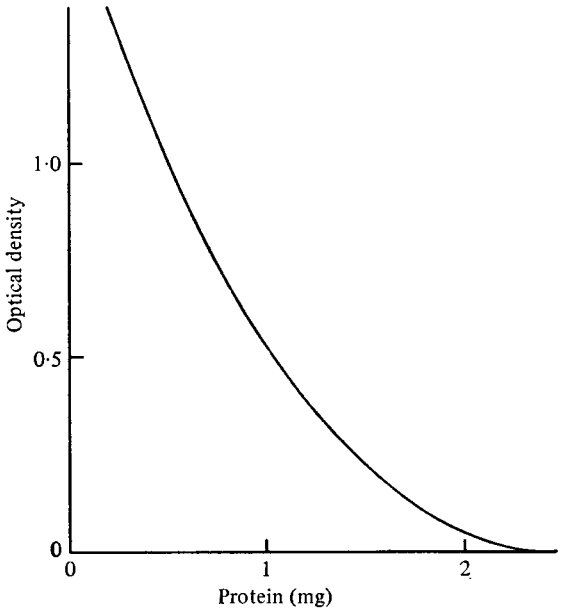

Fig. 3. Inactivation of chloramine T by peptone.

any carriers (WHO, 1970*d*). Similar puzzling cases were reported in France from Marseilles (WHO, 1974*d*) and from St Laurant sur Manoire in the Dordogne Department (WHO, 1975). It is for the scattered outbreaks and sporadic cases that infection from the air should be considered. As shown on the map all the cases cited lie within the flight paths of regular routes from India to the West. At modern flying altitudes crosswinds of 50–100 m.p.h. are known. Hence close correlation of outbreaks and flight paths should not be expected. However, if it could be shown that an organisms such as *Vibrio cholerae* could survive conditions known to occur at flying altitudes, could possibly survive descent and also survive for some time the type and concentration of disinfectant used by airlines then a case could be argued for further investigation.

Growth of Vibrio *in peptone water*

In one series of ten experiments involving some 500 observations the total counts observed for *Vibrio cholera* grown for 16 h at 37 °C ranged from $7{\cdot}2 \times 10^7$ organisms/ml to $4{\cdot}5 \times 10^8$ orgs./ml. The colony counts ranged from $1{\cdot}9 \times 10^7$ c.f.u./ml to $3{\cdot}1 \times 10^8$ c.f.u./ml. In another series of experiments total counts of up to $8{\cdot}0 \times 10^8$ orgs./ml, were found. In these experiments colony counts were not done. Similar results were obtained with *Vibrio el Tor* except that counts were usually, although not always, approximately tenfold higher than with *Vibrio cholerae*.

Table 2. *Colony counts of treated and untreated* V. cholerae *cultures*

Storage conditions			
Temperature (°C)	Time (h)	Other	Colony counts (c.f.u./ml)
Control			
37	0	On bench	$1{\cdot}9 \times 10^7$
4	6	On bench	$1{\cdot}1 \times 10^7$
37	6	On bench	$1{\cdot}8 \times 10^7$
Cultures frozen at −80 °C held 1 h			
20	0·3	On bench	$6{\cdot}1 \times 10^5$
20	6	Over $CaCl_2$	$5{\cdot}0 \times 10^6$
20	6	On bench	$4{\cdot}0 \times 10^6$
20	6	Over water	$3{\cdot}8 \times 10^6$
Cultures frozen at −80 °C, held 1 h, then at −20 °C for 6 h			
20	0·3	On bench	$7{\cdot}1 \times 10^4$
37	22	Over $CaCl_2$	$1{\cdot}1 \times 10^8$
37	22	On bench	10*
37	22	Over water	$1{\cdot}2 \times 10^8$

* These samples had dried in the tube.

Survival of Vibrio cholerae *after freeze-thaw treatment*

Preliminary experiments showed that colony counts of *Vibrio cholerae* cultures after two or three cycles of freezing and thawing differed 10- to a 100-fold from the colony counts of untreated control cultures.

More sophisticated experiments were then done. Samples of culture (0·1 ml) were 'snap' frozen at −80 °C and held at this temperature for 1 h. The temperature was raised to −20 °C over approximately 30 min to 2 h and samples held at this temperature for 2–6 h. Finally culture samples were raised to 20 or 37 °C and held for 6–24 h either in a dessicator containing water (100 % humidity) or on the bench ('intermediate' humidity) or in a dessicator containing dried calcium chloride (very low humidity). Experiments were done in duplicate or triplicate. Results were similar in all experiments and the result of one comprehensive experiment is shown in Table 2. It can be seen that after freezing at −80 °C the colony count fell somewhat. A further loss occurred if cultures frozen at −80 °C were stored at −20 °C for some time. However, further storage at 20 or 37 °C enabled the organisms to recommence growth. The exception was with those cultures which had dried out before reconstruction to the original volume when only a few colonies were observed in the viable counts. It is perhaps interesting to note that attempts to freeze-dry *Vibrio cholerae* cultures resulted in our hands in a 1000- to 10000-fold drop in viable counts.

One implication of these results is that if cholera organisms were discharged from an aircraft at high altitude there is no reason why some of them should not remain viable when they reached the ground. The infective dose of *Vibrio cholerae* has been measured as 10^8 organisms, falling under special circumstances to 10^4 organisms (Cash *et al.* 1974). Frank cases of cholera secrete large numbers of

Table 3. V. cholerae: *effect on growth of inoculum size and concentration of peptone*

Inoculum* (Orgs./0·02 ml)	Concentration of peptone (mg/ml) In water 10	In water 1	In water 0·1	In saline 10	In saline 1	In saline 0·1
$2·6 \times 10^4$	8·1†	0·5	1·0	n.t.	4	1·4
$2·6 \times 10^3$	7·1	1·3	1·4	n.t.	2·8	1·0
260	7·8	1·4	0·88	3·0	1·6	1·2
26	0·4	n.d.	n.d.	0·2	n.d.	n.d.

n.t., Not tested. n.d., Not detectable. All growth was at 37 °C for 16 h.
* Control counts of inoculum $1·3 \times 10^6$ orgs./ml.
† Total counts, orgs./ml $\times 10^{-8}$.

Table 4. Vibrio el Tor: *effect on growth of inoculum size and peptone concentration*

(Orgs./0·02 ml)	Concentration of peptone (mg/ml in saline) 10	1	0·1	0·01
10^4	12*	34	0·9	0·4
10^3	30	44	12	n.d.
10^2	25	40	15	n.d.
10	34	20	0·7	n.d.

n.d., Not detectable.
* Total counts, orgs./ml $\times 10^{-7}$.

organisms and in 'rice water' stools bacterial counts can reach 10^9 c.f.u./ml. Cholera vibrios may, however, be present in the stools in the absence of clinical disease or even diarrhoea (Cash *et al.* 1974). In all these cases contamination of the hands is highly probable and washing in an aircraft handbasin would provide a suitable culture for aerial discharge. Even if the number of survivors at ground level were small a short incubation period in a fortuitous medium such as milk or even dirty water might give rise to sufficient organisms to cause infection.

Vibrio growth: Inoculum size and peptone concentration

Experiments were done to determine the smallest number of organisms which could form an inoculum in solutions containing only small amounts of protein. Peptone was considered convenient for this purpose. Stock peptone-saline was diluted with water or saline and 5 ml amounts dispensed in sterile tubes. These were then inoculated with 0·02 ml volumes of carefully made dilutions of 'master' culture. Since the total counts of each 'master' culture were also done, the number of organisms per inoculum was easily calculated. Inoculated cultures were incubated at 37 °C for 16 h and total counts done on each culture. The results of two experiments are shown in Tables 3 and 4. Table 3 shows that a small inoculum (260 organisms) of *Vibrio cholerae* readily produced quite high counts (approx. 10^7 orgs./ml) even when grown

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in solutions containing only 0·1 mg/ml peptone. An inoculum of only 26 c.f.u. grew detectably in solutions containing 10 mg/ml peptone. Indeed, since this experiment was done measuring total counts only, the number of viable organisms present would be less. Using the colony count/total count ratios calculated from results given earlier in this paper the number of organisms used could lie between 3 and 22. Growth was not detected in solutions containing less peptone but the failure of detection might have been due to the limitations of the total count method used. Unfortunately the 'non-detectable' tubes were not further subcultured in peptone at 10 mg/ml to see if small numbers of organisms were present.

Table 4, which summarizes an experiment with *Vibrio el Tor* shows similar results to those given in Table 3. Here an inoculum of 10 organisms showed growth in 0·1 mg/ml peptone.

These and other experiments not given here suggest that a small inoculum of *Vibrio cholerae* or *Vibrio el Tor* could grow sufficiently well in a minimal medium to produce 10^7–10^8 c.f.u./ml and this is compatible with an infective dose of 1–10 ml of culture.

It should be noted that these experiments refer only to growth at 37 °C as it was not possible to expand this part of the work while it was in progress. It would be interesting to extend this study to the effect of temperature on inoculum size and also to use media other than dilute peptone.

Effect of chloramine T on Vibrio cholerae

At least one major airline adds chloramine T at 16 mg/l to all water used on its aeroplanes. The effect of this disinfectant on the growth of *Vibrio cholerae* was therefore studied.

It was found that the colony counts of cultures grown for 16 h at 37 °C in 1 % (w/v) peptone-saline, pH 8·0 were unaffected by exposure for 1–7 h to concentrations of chloramine T within the range 16–160 mg/l. Moroever the growth of *Vibrio cholerae* in peptone-saline was unaffected by the addition of chloramine T to the medium at a final concentration of 160 mg/l.

Since it is known that 'chlorine disinfectants' combine readily with organic matter, experiments were done to investigate the inter-reaction of chloramine T and peptone.

In an extensive series of tests it became apparent that for a given chloramine T concentration the uptake of this substance increased with increase of peptone concentration but that the uptake of chloramine T per mg peptone decreased. Moreover for a given peptone concentration the uptake of chloramine T increased with the concentration of this substance and the uptake of chloramine T per mg peptone also increased.

Clearly a complex situation exists but an important practical effect is shown in Fig. 3. In this experiment 10 ml amounts of chloramine T at 15 mg/l were mixed with different amounts of peptone. The peptone was added as a 1 % (w/v) solution using an 'Alga' micrometer syringe in an attempt to keep the volume of each test as similar as possible (i.e. approximately constant). The 'contact time' of chloramine T and peptone before testing with *o*-tolidine was 2 min. It is evident that

chloramine T is rapidly inactivated and the 2 mg peptone eliminated 10 ml chloramine T at 15 mg/l within the time of the test.

Hence the washing of hands contaminated with faecal material or even hands with scaly skin could easily introduce enough organic matter to neutralize the disinfectant present. It might appear that the simple remedy would be to increase the concentration of chloramine T. Unfortunately higher concentrations of disinfectant render the water unpalatable. However a possible solution to the possible 'handbasin hazard' is the enforced use of medicated soaps.

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