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EXPERIMENTAL CHOLERA IN INFANT RABBITS: A METHOD FOR CHEMOTHERAPEUTIC INVESTIGATION

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Laboratory investigations on the chemotherapy of cholera have been handicapped by the absence of a suitable animal in which a disease resembling the human disorder could be reproduced. Sulphathiazole, sulphadiazine, sulphaguanidine (Griffitts, 1942a), chloramphenicol (Gauld, Schlingman, Jackson, Manning, Batson, and Campbell, 1949) and oxytetracycline (Terramycin) (Olejnik and Davidovitch, 1951) were shown to be effective in the treatment of mice experimentally inoculated with *Vibrio cholerae*. In all this work mice were inoculated intraperitoneally, and died with massive bacteraemia. Collier, Hall, and Waterhouse (1949) have developed the "mouse faecal suspension test" for evaluation of remedies for cholera, but the authors admit that "there can be little direct evidence that any laboratory tests provide reliable indication of clinical usefulness."

Several workers gave the vibrios to laboratory animals orally, but the results were not sufficiently consistent to have any practical application. Sabolotny (1894) demonstrated the susceptibility of *Spermophilus guttatus* to cholera vibrios given by mouth. Only half of the animals became infected; diarrhoea did not always occur and septicaemia was common. Metchnikoff (1894) could not induce the infection in kittens, puppies, mice, or gerbils. Diarrhoea and death were produced in 25 to 50% of suckling rabbits, but the results were irregular and unpredictable. Similar results were obtained by Sanarelli (1921), and Crendiropoulo (1921) showed that *V. cholerae* was destroyed by gastric juice. Klemperer (1894) tried unsuccessfully to produce cholera in rabbits by intra-intestinal inoculation. An unsuccessful attempt was also made to produce cholera in monkeys by damaging the intestinal wall with warm water or deep *x*-ray, followed by intra-intestinal inoculation of vibrios (Report, Scientific Advisory Board, 1946). Griffitts (1942b) demonstrated that the virulence of *V. cholerae* for mice was enhanced if the organisms were injected together with mucin.

The aim of the present work was to develop a method by which cholera could be produced readily in a laboratory animal, and to use it for the evaluation of anti-cholera drugs. If the results of study on experimental animals were to run parallel with the clinical findings with known drugs, the method would provide a means for further research on cholera. Our attempts to infect young mice, rats, and guinea-pigs by intra-intestinal inoculation of *V. cholerae* did not meet with success, but it was found that infant rabbits under certain conditions were highly susceptible.

METHODS

Experimental Animals.—Infant rabbits, ten days old, of either sex, weighing 100–200 g. were used. The young ones live on their mothers' milk and do not generally open their eyes before the 10th or the 11th day. The litters were kept with their respective mothers in separate cages.

Preparation of Infective Dose.—A strain of *V. cholerae* (Inaba 569 B), maintained at this Institute for preparation of vaccine, was used. The freeze-dried culture was regenerated in broth, plated on agar slants, and incubated overnight. A 1 mm. loopful of the culture was suspended in 10 ml. of nutrient broth and again incubated for 3 hr. At the end of this period the broth contained approximately 1,000 million vibrios per ml. This was suitably diluted before use. The viable cholera vibrios were counted by the technique of Sokhey (1939) described for plague organisms.

Evolution of a Virulent Strain (Table I).—When infant rabbits were fed with 100 million vibrios per 100 g. body weight, only 1 of 8 suffered from severe diarrhoea, and collapsed after 48 hr. In the remaining animals *V. cholerae* failed to cause the infection. When 100 million organisms per 100 g. body weight were injected directly into the small intestines of infant rabbits under ether anaesthesia, the mortality

was increased. Of 4 animals in the first expt. 2 died within 72 hr. with severe diarrhoea; watery stools were not observed in the remaining 2. Of the 4 rabbits of the second group, 3 had liquid motions. Two of them recovered, while the third became emaciated and died after 4 days. One animal from this group was completely resistant.

TABLE I

INFECTION OF INFANT RABBITS WITH *V. CHOLERAE* (INABA)

Strain	Infective Dose/100 g. Body Wt.	No. with Diarrhoea/ No. Infected	No. Dead/No. Infected
Unpassaged, without mucin ..	100×10^6 (oral)	1/8	1/8
,, ,, ..	100×10^6 (i.i.*)	5/8	3/8
Unpassaged, with 5% mucin	50×10^6 ,,	7/25	11/25
Passaged, with 5% mucin	50×10^6 ,,	12/12	12/12
Passaged, without mucin ..	50×10^6 ,,	2/2	2/2
,, ,,	10^4 ,,	86/86	86/86
,, ,,	10^3 ,,	11/11	11/11

* i.i. = intra-intestinal.

When 50 million vibrios were injected into the intestine together with mucin, 11 out of 25 died. Of the dead ones only 7 animals had diarrhoea. Vibrios were isolated from heart blood shortly after death in three animals. The strain was preserved by freeze-drying.

The strain was regenerated as described above and infant rabbits were infected intra-intestinally with varying numbers of vibrios. Mucin was not used, but 86 animals which received 10,000 vibrios per 100 g. body weight all showed signs of a disease resembling human cholera in many respects. The virulent strain isolated from heart blood was used in all subsequent experiments. Virulence was maintained by animal passage every 6 months.

Another strain of *V. cholerae* (Ogawa 41), taken from our stock cultures, when tested on infant rabbits behaved in the same way as Inaba 569 B.

Biochemical Studies.—Measurements were made of the cell volume, urea, and non-protein nitrogen contents of samples of oxalated heart blood obtained from infected animals. Specimens were not collected until life, in our estimation, could not have been possible for more than 2 hr. Owing to the difficulty of obtaining blood from dehydrated animals, most of the samples were pooled from 2 animals. In normal rabbits each sample was obtained from a single animal.

Susceptibility of Rabbits of Various Ages.—Infant rabbits were infected intra-intestinally with a heavy dose of 100 million vibrios per 100 g. body weight. Animals less than 10 days old were not sufficiently mature to withstand the injurious effect of anaesthetic ether, and therefore were not used.

Habitat of the Vibrios.—For this experiment groups of infected rabbits were killed at varying intervals 8–49 hr. following inoculation. From each animal 0.25 ml. of heart blood and a portion of the liver were cultured in peptone water for isolation of the vibrios. The liver was approached through the chest cavity and the diaphragm to avoid handling intestines.

Drugs.—For *in vitro* tests formo-sulphathiazole ("Formo-Cibazol," Ciba; formaldehyde sulphathiazole), sulphaguanidine, chloramphenicol, and chlortetracycline (aureomycin) hydrochloride were suspended in 6% aqueous gum acacia. The first three were sterilized at 10 lb./sq. in. for 10 min.; the chlortetracycline suspension was prepared aseptically. Oxytetracycline hydrochloride was dissolved in sterile aqueous solution of HCl (*p*H 2). For *in vivo* tests all the substances were suspended in 6% aqueous gum acacia and administered orally with a small glass pipette.

In vitro *Experiments.*—A serial dilution method was employed in which the total volume of the medium in each tube was kept constant (10 ml.) but the concentrations of the compounds were varied. The substances were tested in peptone water (*p*H 7.4) and in a protein-free casein hydrolysate medium (Sokhey, Habbu, and Bharucha, 1950). The potency of the drug was assessed against 10^3, 10^6, and 10^8 vibrios per ml., obtained by suitably diluting a 3 hr. growth (rabbit-passaged strain of Inaba 569 B) of *V. cholerae*. A large inoculum was used in the belief that it might more nearly represent the condition in which the drugs were required to act in the body. The culture tubes were incubated at 37° C. for 24 hr.

Testing of Drugs in Experimental Cholera

Standard Dose Schedule.—Preliminary study suggested that a nine-dose treatment, one dose every 8 hr. for 3 days, was necessary to obtain the optimum effect of a drug. Chloramphenicol, chlortetracycline, and oxytetracycline hydrochlorides were administered in doses of 100 mg./kg. Sulphaguanidine and formo-sulphathiazole were given in amounts of 750 and 650 mg./kg. respectively. Those animals which recovered from the attack received the full regimen of 9 doses; otherwise the amounts of drug administered depended on the survival periods.

Treatment was begun 1 hr. before the infection, or 8, 16, or 24 hr. following it. The onset of diarrhoea in the infected rabbit was taken as the first definite sign of the beginning of the attack. The prophylactic and curative values of a drug were judged by the results obtained from beginning treatment before inoculation and 24 hr. after inoculation. For each experiment, a group of infected untreated animals was used as a control.

Saline Treatment.—Within two hours of the onset of diarrhoea in infant rabbits suffering from cholera, each animal received a dose of 100 mg./kg. of chlortetracycline hydrochloride followed by 2 ml. hypertonic saline (8 g. sodium chloride and 0.25 g. calcium chloride in 570 ml. water) and 1 ml. of alkaline saline (6 g. sodium chloride and 11 g. sodium bicarbonate

in 570 ml. water) intraperitoneally. It was not possible to give salines intravenously. The dose of chlortetracycline was repeated after 6 hr. and, later on, at intervals of 8 hr. Both hypertonic and alkaline salines were given every 3 hr. until each of the animals had received 12 ml. of fluid. Control animals did not receive either the drug or the salines.

RESULTS

Experimental Cholera in Infant Rabbits

Signs and Symptoms.—About 16–24 hr. following infection with 10,000 vibrios (passaged strain), the animals showed signs of illness. They became less active and huddled together. On an average the diarrhoea made its appearance 22 hr. after inoculation, but the onset was delayed up to 44 hr. in a few rabbits. The diarrhoea was mild at the beginning but became severe. The animals survived for a mean period of 32 hr. from the time the infective dose was given, or 10 hr. after the first appearance of the liquid motions. Death occurred as early as 22 hr. or as late as 52 hr. after infection. In some cases death took place 4 hr. after the diarrhoea began, whereas in a few instances a day elapsed before the animal died. During the terminal stage, the animal lay exhausted and did not like to move unless seriously disturbed. The skin became cold and the hair rough. Cramps in the muscles were occasionally noticed before death. Vomiting was never observed. *V. cholerae* was frequently isolated from the watery stools, which also contained lumps of mucus.

When rabbits were inoculated with 50 million vibrios/100 g. body weight, diarrhoea was observed 14 hr. after infection, and most died within 20 hr.

Post-mortem Changes.—The animal looked emaciated. The hair was rough and lustreless and the whole undersurface of the body was smeared with liquid discharges from the bowel. On opening the abdomen the distended large intestine stood out prominently. It was rather pale in appearance and full of watery fluid containing flakes of mucous membrane. The vibrios were recovered easily from the liquid content of the bowel. The stomach was always distended with coagulated milk. The small intestine was generally hyperaemic and in places was scarlet in colour. In some animals no congestion was noticed, except that a few vessels stood out prominently. The lower part of the ileum was distended but not so much as the upper part of the large intestine. The small intestine contained a thick viscid liquid, and parts of the mucous membrane had been destroyed. The liver, spleen and kidney were either normal in appearance or congested. The auricles contained thick dark blood and the ventricles were empty and contracted.

Biochemical Studies

A summary of the biochemical findings is given in Table II. A significant degree of haemoconcentration was observed in infected rabbits. The average non-protein nitrogen and urea contents of the blood of rabbits suffering from cholera were significantly higher than normal.

TABLE II

BIOCHEMICAL CHANGES IN BLOOD OF INFANT RABBITS INFECTED INTRA-INTESTINALLY WITH 10,000 *V. CHOLERAE* (INABA) PER 100 G. BODY WEIGHT

	Rabbits	No. of Animals	Range	Mean	t	Level of Significance
Blood cell volume %	Normal	10	32–41	36·70	6·733	1%
	Infected	10	38–52	47·60		
Plasma non-protein nitrogen mg./100 ml.	Normal	11	29–60	42·73	4·433	1%
	Infected	10	41–85	65·50		
Blood urea mg./100 ml.	Normal	11	13–28	18·64	5·181	1%
	Infected	13	22–53	37·07		

Susceptibility of Rabbits of Different Ages

The susceptibility of young rabbits to *V. cholerae* at different ages is shown in Table III. Up to the sixteenth day of life, the animals remained highly susceptible. Beyond this period the rabbits progressively resisted the infection until they were a month old, when it became impossible to infect them. As the susceptibility lessened, the incidence of diarrhoea decreased. Post-mortem changes in all of the rabbits were suggestive of the infection. Susceptibility was not related to body weight, nor to seasonal variations.

TABLE III

SUSCEPTIBILITY OF YOUNG RABBITS TO *V. CHOLERAE* (INABA) WHEN INJECTED INTRA-INTESTINALLY WITH 100 MILLION ORGANISMS PER 100 G. BODY WEIGHT

Age of Animals (Days)	Symptoms of Cholera	Mortality
16	6/6	6/6
21	3/5	4/5
31	0/5	0/5
45	0/2	0/2

Habitat of the Vibrio

Specimens of blood and liver of 50 rabbits were examined. Vibrios were recovered from the blood of 3 animals 24–29 hr. after in-

TABLE IV

ACTION *IN VITRO* OF CHLORAMPHENICOL, CHLORTETRACYCLINE, OXYTETRACYCLINE, FORMO-SULPHATHIAZOLE AND SULPHAGUANIDINE AGAINST *V. CHOLERAE* (INABA)

Drug	Casein Hydrolysate Medium				Peptone Water Medium
	Vibriostatic Concn. (μg./ml.) Against 10^6 Vibrios/ml.	Vibriocidal Concn. (μg./ml.) Against 10^3 Vibrios/ml.	Vibriocidal Concn. (μg./ml.) Against 10^6 Vibrios/ml.	Vibriocidal Concn. (μg./ml.) Against 10^8 Vibrios/ml.	Vibriocidal Concn. (μg./ml.) Against 10^6 Vibrios/ml.
Chloramphenicol	2·5	2·0	5·0	100·0	2·5
Chlortetracycline hydrochloride	50·0	10·0	100·0	100·0	100·0
Oxytetracycline hydrochloride	10·0	10·0	50·0	250·0	25·0
Formo-sulphathiazole	250·0	20·0	—	—	—
Sulphaguanidine	>2,000·0 (No inhibition)	>2,000·0 (No inhibition)	—	—	—

fection, and from the liver of 1 animal which did not show a positive blood culture. All the rabbits which showed positive blood or liver cultures suffered from diarrhoea, but there were many other animals with diarrhoea in which bacteraemia or the presence of vibrios in the liver could not be demonstrated.

In vitro *Experiments*

The results are given in Table IV. In casein hydrolysate medium, formo-sulphathiazole and sulphaguanidine were ineffective even when the inoculum was small. The antibiotics were active in both types of medium against large and small inocula. A synthetic compound, α-bromo-*p*-nitrocinnamic aldehyde, reported to be highly bactericidal against a varied set of organisms (Affonso and Khorana, 1952), was found to be vibriocidal in a concentration of 20 μg./ml. against 100 million organisms in casein hydrolysate medium. The compound is insoluble in water and sparingly soluble in common solvents and also possesses low oral toxicity in white mice. Unfortunately, the substance is too toxic for the infant rabbit.

TABLE V

THE ACTION OF ORALLY ADMINISTERED VIBRIOCIDAL DRUGS ON INFANT RABBITS INFECTED INTRAINTESTINALLY WITH 10,000 *V. CHOLERAE* (INABA) PER 100 G. BODY WEIGHT

Treatment Started (Hr. Before or After Infection)	Treated Infected Rabbits			Untreated Infected Rabbits (Controls)		
	Mean Time of Onset of Diarrhoea (hr.)	Mean Survival Time (hr.)	Mortality*	Mean Time of Onset of Diarrhoea (hr.)	Mean Survival Time (hr.)	Mortality*
Chloramphenicol (Dose 100 mg./kg.)						
1 hr. before	Nil	—	0/7	21·0	31·5	4/4
8 ,, after	,,	—	0/6	23·3	31·2	4/4
16 ,, ,,	30·0	39·0	3/12	21·8	31·8	8/8
24 ,, ,,	22·6	31·3	9/9	20·5	28·0	8/8
Oxytetracycline Hydrochloride (Dose 100 mg./kg.)						
8 hr. after	Nil	—	0/7	21·3	29·8	4/4
16 ,, ,,	23·3	37·3	3/12	23·0	33·5	4/4
24 ,, ,,	22·0	38·0	6/6	21·0	30·0	4/4
Chlortetracycline Hydrochloride (Dose 100 mg./kg.)						
8 hr. after	Nil	—	0/6	21·0	32·0	4/4
16 ,, ,,	26·5	42·3	4/12	22·3	33·5	8/8
24 ,, ,,	22·0	32·0	6/6	23·5	36·0	4/4
Sulphaguanidine (Dose 750 mg./kg.)						
1 hr. before	Nil	—	0/5	28·0	34·0	1/1
8 ,, after	24·3	32·0	5/9	24·6	33·3	5/5
16 ,, ,,	26·0	33·3	4/4	26·0	34·0	2/2
Formo-sulphathiazole (Dose 650 mg./kg.)						
1 hr. before	36·0	—	0/7	19·5	24·5	4/4
8 ,, after	25·0	30·3	8/8	20·0	26·3	6/6
Chlortetracycline Hydrochloride (Dose 100 mg./kg.) *and Salines*						
After onset of diarrhoea	23·3	36·9	9/9	28·0	33·2	4/4

* Ratio of animals dead to animals infected.

Testing of Drugs in Experimental Cholera

Table V summarizes the effect of the 5 compounds tried in experimental cholera. Formosulphathiazole prevented death only when administration of the drug was begun 1 hr. before the animals were infected.

When the drugs were administered 8 hr. after the infection, the three antibiotics prevented all symptoms of the disease from appearing. Sulphaguanidine had some activity, but formo-sulphathiazole was quite ineffective.

When treatment was begun 16 hr. after infection, sulphaguanidine failed to effect any improvement, but the three antibiotics were of about equal potency in combating the infection. At the beginning of treatment diarrhoea had not yet appeared. It is interesting to record that 2 or 3 rabbits from each group treated with antibiotics later developed diarrhoea, but they ultimately recovered completely under the treatment. The treated animals which died continued to pass watery stools and at autopsy the large intestine was full of fluid. Congestion of the vessels of the small intestine was not seen as frequently as in the controls.

Diarrhoea had already begun in animals treated 24 hr. after infection. Under these conditions none of the antibiotics had any effect upon the course of the illness and all the animals died before the fourth dose could be administered.

The treatment of rabbits with salines and chlortetracycline did not bring about any improvement in survival time or mortality rate.

Discussion

The results of the study show that experimental cholera in the rabbit closely resembles the human disorder. The rapid onset of the disease and its short duration, profuse diarrhoea, dehydration, congestion of the small intestine and desquamation of the intestinal epithelium are common to both. Moreover, there is a significant increase of the non-protein nitrogen, urea and cell volume of the blood of rabbits suffering from cholera. In clinical cases too, appreciable increase in the cell volume (Pasricha and Malik, 1940 ; Taylor, 1941), plasma non-protein nitrogen (Shorten, 1918 ; Dhar, Dhar, and Adhyee, 1930 ; Banerjee, 1936 ; Pasricha and Malik, 1940 ; Chatterjee, 1941) and blood urea (Shorten, 1918 ; Banerjee, 1936 ; Pasricha and Malik, 1940 ; Chatterjee, 1941) is observed. These findings are not so definite as in rabbit cholera—perhaps because the mortality rate of the patients was much lower than in the experimental animals.

There are several features in which the disease in the rabbit differs from cholera in man. For example, vomiting is never seen in the rabbit. Unlike rabbit cholera, the pathological picture is variable in clinical cases (Rogers, 1911). Marked congestion of the small intestine, and, to a less extent, of the large intestine was seen in only 30% of human cases (Chatterjee, 1939). Distension of the large intestine with fluid is characteristic of the animal disease. Such differences are not altogether unexpected in view of the differences of anatomy and physiology in the rabbit and man.

Controversy concerning the role of vibrios in producing septicaemia in cholera has arisen because of failure to isolate them from the circulation during the active stage of the disease. However, there is indirect evidence to show that the vibrios occasionally enter the general circulation. Greig (1913a) recovered vibrios from the urine of cholera patients and also showed their presence in the lung and kidney (Greig, 1913b). Pasricha, de Monte and Chatterjee (1938) isolated *V. cholerae* from a few drops of blood obtained by liver biopsy in a boy suffering from cholera. All attempts to isolate the vibrios from the circulation have so far failed (Greig, 1919 ; de Monte and Gupta, 1938). In the present study, *V. cholerae* remained localized within the intestine of the rabbit and only occasionally entered the blood stream or the tissues.

Adult rabbits were immune to cholera ; young ones were highly suceptible up to the 16th day of life. Sanarelli (1921) believes that the development of immunity is associated with the change of diet from mother's milk to green vegetables. Arnold and Shapiro (1930) believe that an alteration in the reaction of the contents of the upper part of the intestine from slightly acid to alkaline brings about the change in susceptibility.

The difficulties of clinical assessment of drugs in the treatment of cholera are shown by the contradictory statements made by different workers about the efficacy of the same drug. The problem is complex, because no really effective drug therapy is known, and the activity of a new drug can be measured only against the effect of saline administration. Sulphaguanidine is stated to be potent against cholera (Chopra, de Monte, Gupta, and Chatterjee, 1941 ; Pasricha, Paul, Das Gupta, and Das, 1947), and to show low toxicity at an effective dose. The beneficial effect is statistically significant (Gupta, Chatterjee, Paul, and Ghose, 1945 ; Chu, Huang, Chang, and Kao, 1946 ; and Seal, 1947). The effectiveness of the drug has been questioned by Carruthers (1942) and Lahiri (1948,

1951). Lahiri demonstrated that, although the drug seemed to produce a beneficial effect, the results in his patients were not statistically significant. Formo-sulphathiazole was also stated to be an effective drug (Bhatnagar, Fernandes, De Sa, and Divekar, 1948). Subsequent workers (Chaudhuri, Ghosal, and Rai Chaudhuri, 1950; Lahiri, 1951; Roy Chaudhuri, Chaudhury, and Chadha, 1952) could not confirm this. In experimental cholera, both sulphaguanidine and formo-sulphathiazole possess no curative value.

Clinical trials of chloramphenicol (Chaudhuri, Ghosal, Mondal, and Chakravarty, 1952; Roy Chaudhuri *et al.*, 1952), chlortetracycline (Roy Chaudhuri *et al.*, 1952; Seal, Ghosh, and Ghosal, 1954) and oxytetracycline (Das, Ghosal, Gupta, and Chaudhuri, 1951; Konar and Sen Gupta, 1951; Roy Chaudhuri *et al.*, 1952; Das, Ghosal, Gupta, and Mondal, 1953; Das, Ghosal, and Gupta, 1953) do not suggest that they are therapeutically effective. The results of experimental trials of these drugs in rabbit cholera confirm the clinical findings, but suggest that these remedies might prove valuable in preventing the disease in man. The low toxicity and ease of administration of the antibiotics would make them especially valuable for contact cases.

From the therapeutic point of view the results of the *in vitro* tests have little importance because all these drugs have proved to be of little value in experimental and clinical cholera. Nevertheless it seems that the vibriocidal power of the compounds runs parallel with their ability to render the stools of cholera patients free from the vibrio. It has been reported that chloramphenicol (Chaudhuri *et al.*, 1952; Roy Chaudhuri *et al.*, 1952), chlortetracycline (Roy Chaudhuri *et al.*, 1952), and oxytetracycline (Das *et al.*, 1953) quickly remove the vibrios from the stools of patients. Formo-sulphathiazole, a poor vibriocidal compound, exerts a slight beneficial effect (Lahiri, 1951; Roy Chaudhuri *et al.*, 1952). It is doubtful whether sulphaguanidine, which is devoid of vibriocidal power, causes disappearance of the vibrios from stools earlier than in the controls (Chu *et al.*, 1946; Lahiri, 1951).

Experimental evidence suggests that the great mass of vibrios is confined to the intestinal lumen, and toxic substances are formed there as a result of their growth. These are probably absorbed early in the disease and diarrhoea may be a manifestation of the toxaemic state. Such a condition calls for immediate neutralization of the circulating toxin and prevention of further growth of the vibrios in the intestine by oral administration of compounds which are powerful vibriocides as well as relatively non-absorbable. The role of antitoxic serum in the treatment of experimental cholera is under investigation.

Summary

1. A method has been described for inducing cholera in infant rabbits which closely resembles the human disease.

2. The disease is produced in ten-day-old rabbits by infecting them intra-intestinally with animal-passaged strain of Inaba or Ogawa subtypes. It is characterized by severe diarrhoea, dehydration and occasional cramps in the muscles. A significant increase in the plasma non-protein nitrogen, blood urea and blood cell volume is observed.

3. Post-mortem examination shows congestion of the blood vessels of the small intestine, areas of desquamation of the epithelium, and distension of the large intestine with watery fluid containing flakes of mucous membrane.

4. The vibrios are generally localized within the intestine and only occasionally make their entry into the circulation.

5. Infant rabbits are highly susceptible to *V. cholera* up to the sixteenth day of life, but later progressively resist infection and are completely immune by the end of a month.

6. A method for evaluation of drugs against cholera is described. Chloramphenicol (" chloromycetin "), chlortetracycline (aureomycin) and oxytetracycline (" terramycin ") possess excellent protective action, but they lack curative property even when administered at the early stage of the disease. The preventive value of sulphaguanidine is less than that of the antibiotics, but is superior to formaldehyde sulphathiazole (formo-sulphathiazole, " Formo-Cibazol ").

7. The quantitative nature of the test is well demonstrated for the chemotherapeutic substances studied, and the results are in agreement with the reported clinical findings.

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