lite CG-45 resin in acetate form. Yields were 500 mg of lysine vasopressin (≧300 U/mg) and 200 mg of α-MSH (1.3-1.5 MSH U/mg, pressor activity 0.2 U/mg). Data from experiments with synthetic materials led us to believe that this minimal pressor activity may be inherent to the molecule of α-MSH.

α-MSH could be freed of small amount of fast moving contaminant by re-chromatography on CMC. Potency of final material was 1.5-2.0 x 10^7 U/mg. This α-MSH preparation was homogeneous on electrophoresis and chromatography in BuOH: HOAc:H_2O = 4:1:5, R_f = 0.6.

Tryptophan was detected spectrophotometrically and only the other 11 amino acids of α-MSH were found after acid hydrolysis. Overall recovery of α-MSH after precipitation with organic solvents(6), CMC chromatography and countercurrent distribution was 40% of total MSH activity in Pitressin Intermediate.

Summary. α- and β-MSH were isolated on preparative scale with high recovery of starting activity. The initial step in purification of both hormones is chromatography on carboxymethylcellulose. β-MSH is further purified (4-5 x 10^6 U/mg) by use of DEAE cellulose. α-MSH was not completely freed of vasopressin and other contaminants by zone electrophoresis on large vertical column. Countercurrent distribution of concentrate of vasopressin and α-MSH, yields α-MSH with a potency of 1.5 x 10^7 U/mg, free of vasopressin.

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Received April 4, 1960. P.S.E.B.M., 1960, v104.

Studies of Gastrointestinal Viral Flora of Cholera Patients.*† (25813)

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Pathogenesis of cholera is poorly understood. Presence of the organism in the environment or even in the individual person does not necessarily result in the clinical disease(1). In the disease state, cholera vibrio appears to exert a profound effect on the gastrointestinal tract without entering into the tissue deeply enough to be found in the blood stream(2). Many theories have been postulated to explain the pathogenesis of this disease. Among them has been presence of symbiotic or synergistic microorganisms including viruses. With the newly developed tissue culture technics for isolation of virus, and the discovery that large numbers of virus inhabit the gastrointestinal tract, a study was under-

* This study supported by contract between Office of Naval Research and Univ. of Chicago and by funding under Public Law 480 Section 104(c).

† Opinions and assertions contained herein are those of the authors and are not to be construed as official or as reflecting views of Navy Dept. or naval service at large.

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taken of viral flora of the gastrointestinal tract of cholera patients as revealed by culture in monkey kidney and HeLa cell tissue cultures. This study was carried out during cholera epidemic in Bangkok, Thailand in 1958 and 1959.

Materials and methods. Stool specimens were collected from patients with cholera, some of which were being treated by the NAMRU-2 team(3), and others from the general ward at Chulalongkorn Hospital, Bangkok in 1958. All patients had severe classical cholera with rice water stools and severe dehydration. In 1959 patients with cholera at Municipal Infectious Disease Hospital in Bangkok, were cultured. Many of these patients had only mild clinical symptoms, but cholera organisms were isolated from each(1). During study of epidemiology of the cholera epidemic in 1958, rectal swab specimens were collected from family members of cholera patients(4). None of these family members were ill, and cholera organisms were isolated from only 3 out of 243. Virus isolation attempts were made on 57 of these specimens collected from persons whose stool culture did not contain cholera organisms. All specimens to be cultured for virus were packed in dry ice and sent to NAMRU-2. Technics used for virus isolation and identification have been reported(5). Primary monkey kidney cultures were prepared with trypsin from Taiwan monkeys (*Macaca cyclopis*)(6). Eagle's medium(7) with 5% calf serum was used for both tissues. Cultures in HeLa cells were observed 40 days prior to being declared negative. Cultures of cholera patients' specimens in monkey kidney cells were observed 34 days prior to being declared negative. Specimens from well persons were observed in monkey kidney tissue culture only through one passage of variable length. Isolates were identified by neutralization tests with the following type specific antiserums; adenovirus 1-10, poliovirus 1-3, Coxsackie virus A9 and Bl-5, ECHO virus 1-14.

Results. A total of 6 viruses was isolated from 44 patients with proven cholera. These included 3 adenoviruses and 3 enteroviruses. Serologic type identifications were made on 3 of these viruses. One was an adenovirus type 4, one Coxsackie type B3 and the other ECHO type 9. The one isolation in 1959 from among 17 mild cases was ECHO type 9 virus which came from an 18-month-old child. From among 57 well persons cultured, 12 viruses were isolated. Five were adenoviruses and 7 enteroviruses. One of the adenoviruses was type 6, and 6 of the enteroviruses were ECHO viruses, 2 type 4 and one each type 5, 6, 12 and 14. The remaining isolates produced cytopathogenesis typical of their group on appropriate cell type, but could not be neutralized by antiserums available in this laboratory. They are presumed to be adenoviruses other than types 1-10 and ECHO viruses other than types 1-14.

TABLE I. Isolation of Viruses from 1958 Cholera Patients and Non-Ill Family Contacts by Age Group.

		0-10 yr		11-50 yr	
		No.	%	No.	%
Cholera patients	Adenovirus	1/9	11	2/18	11
	Enterovirus	2/9	22	0/18	0
"Control" persons	Adenovirus	2/25	8	3/32	9
	Enterovirus	7/25	28	0/32	0

Nine cholera patients cultured in 1958 were children to age of 10, the rest were adults. Table I shows a comparison of isolation percentage of adenoviruses and enteroviruses from cholera patients and "control" persons by age group for the 1958 cases. From adults, only adenoviruses were isolated. From children, adenoviruses and enteroviruses were isolated from both groups. The cholera patient with Coxsackie B3 was 17 months old. The ECHO viruses were isolated from children under 5 years of age with the exception of 1 ECHO 4 from a 10-year-old.

Discussion. This study revealed no evidence of any association between viruses of the enterovirus or adenovirus groups with the clinical syndrome of cholera. There was a low percentage of isolation of viruses from cholera patients, and there was no tendency for any one type of virus to be isolated. Viruses were isolated as frequently from well persons as from cholera patients. Although family contacts without evidence of illness

could not be considered a suitable control group, they at least give some idea of distribution of these types of viruses in the population at the same time, for comparison by age group with the cholera patients.

There was no evidence that disturbance of the gastrointestinal tract by cholera increased virulence of the adenoviruses and enteroviruses isolated. All cholera patients from whom virus was isolated recovered promptly after adequate fluid and electrolyte replacement therapy, and no other illnesses were diagnosed during the convalescent period.

Summary. Virus isolation studies on rectal swab specimens of cholera patients were carried out in tissue cultures during the Bangkok, Thailand cholera epidemic of 1958 and 1959. An adenovirus or enterovirus was isolated from 6 of 44 patients with proven cholera. It was concluded that viruses which can be isolated in monkey kidney and HeLa cell tissue cultures were not involved in the cholera syndrome.

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Received April 19, 1960. P.S.E.B.M., 1960, v104.

Response of Peritoneal Exudate Cells to *Brucella melitensis.* Influence of Nature of Inflammatory Irritant.* (25814)

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For many years there has been increasing interest in the use of mononuclear cells from rabbit and guinea pig peritoneal exudates as a tool for study of specific and non-specific resistance to bacterial and viral infections. One difficulty in comparing findings of different inevstigators rests principally with use of different animal species as sources of the monocytes and with inoculation of different inflammatory agents into the peritoneal cavity. This subject has recently been reviewed (1). To learn whether the nature of the irritant influences activity of cells which accumulate in response to intraperitoneal inoculation into the rabbit, a comparison has been made in which such irritants as light mineral oil, sodium caseinate, dextran, glycogen and killed Brucella cells were used. Responses of monocytes from normal and Brucella-immune rabbits to parasitization by *Brucella melitensis* were studied by methods described(1).

Methods and materials. Irritants. "Klearol" (obtained from L. H. Butcher Co., San Francisco). Casein (supplied as sodium caseinate by Mann Assayed Biochemicals, approximately 90% protein). A 2.5% solution of the protein in saline was employed. Glycogen (Nutritional Biochemicals). Dextran (Cutter Labs) in the form of 6% solution for intravenous injection. *Br. melitensis,* scraped off Albimi agar slants after 3 days incubation at 37°C washed twice and resuspended in saline. Monocytes from adult rabbits, both normal and immunized, were injected with one of the inflammatory agents. For example, either sterile Klearol, 1.2% (W/V) solution of sodium caseinate, 6% Dextran (W/V), 0.1 mg % (W/V) glycogen or a formalin-killed, saline suspension (10^9/ml) of a virulent strain

* Supported by funds available from Contract between office of Naval Research and Regents of Univ. of California.