indirect role for duodenum—one of activating trypsinogen. Nascent trypsin appears to be the direct activating agent for pro-elastase. The absence of elastase activity in trypsin has been shown by Balo and Banga(5), and we have found it to be absent in both trypsin and α-chymotrypsin.

Several investigators have reported active elastase in extracts of desiccated pancreas without a duodenum component(5,7,8). These observations might be reconciled in the light of varying amounts of active protease in fresh mammalian pancreas tissue(9). Unless the starting tissue has an exceedingly low concentration of active trypsin or the investigator has taken special steps to avoid autoactivation, a given pancreas extract may be expected to contain sufficient trypsin to activate pro-elastase.

The data shown in Table I suggest that the duodenal factor is enterokinase. The activation mechanism strongly resembles that of chymotrypsinogen(10) and pro-carboxypeptidase(11) and may be represented as

(A) Trypsinogen $\xrightarrow{\text{enterokinase}}$ trypsin

(B) Pro-elastase $\xrightarrow{\text{trypsin}}$ elastase

Summary. Dog pancreatic juice and extracts of hog pancreas contain elastase in an inactive form. Trypsin or a duodenal factor is capable of activating pro-elastase. The activation is blocked by pancreatic and soybean trypsin inhibitors, an indication that the duodenal factor is enterokinase.

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Clostridium perfringens Iota Antitoxin Levels In Convalescent Sera from Hemorrhagic Fever Patients. (22003)

JOHN D. MARSHALL, JR. AND RALPH O. ANSLOW. (Introduced by Oscar Felsenfeld.)

From the Department of Bacteriology, 406th Medical General Laboratory, APO 500, San Francisco.

The resemblance of the anatomic-morphologic changes in men who died of hemorrhagic fever (HF) to those in animals which succumbed to enterotoxemias caused by *Clostridium perfringens* led to an inquiry into the possible role of *Cl. perfringens* in the Korean variety of that human disease(1-4). The occurrence of *Cl. perfringens* in the human intestine is well known. Past studies concerned with the distribution of toxigenic types of *Cl. perfringens* have demonstrated that types A and F constitute all of the human intestinal isolates with a few exceptions(5-8). The classification of the strains isolated from the colon of HF patients has not yet been undertaken due to the present limited supply of typing antitoxin on hand. It seemed, however, that the demonstration of serum antitoxins against known species of *Cl. perfringens* in convalescent HF patients would be another approach to this problem.

Materials and methods. Sera. Sera were obtained from 17 patients on the Hemorrhagic Fever Wards of an Army Hospital in Korea, during their convalescence from a disease answering the clinical criteria of HF. These

sera were drawn 35 to 51 days after onset of the disease and were compared with sera of 50 individuals from Japan and areas other than Korea who had never suffered such an illness. *Toxins.* Crude toxins were prepared from the following known type strains of *Cl. perfringens*: type A, No. 146 from Dr. L. S. McClung, Univ. of Indiana; type B, No. CN 667; type C. No. CN 882; type D, No. CN 1635; type E, No. CN 1241, and type F, No. CN 2048 from Dr. H. Warrack of the Wellcome Laboratory, Beckenham, England. The *Cl. perfringens* strains were grown in a horse muscle infusion containing 1% papain digest of horse muscle and 30% horse muscle particles, as recommended by Ross(9). The cultures were incubated for 18 hours at 35°C. centrifuged at high speed for 15 minutes to remove the large particles and sterilized by Seitz filtration. A later batch of type E toxin was sterilized by passage through a millipore filter with less loss of toxigenic material. All filtrates, except type A, were neutralized with alpha antitoxin to eliminate that toxin which is common to all recognized types of *Cl. perfringens.* The epsilon toxin of type D was activated with trypsin prior to use, as recommended by Batty and Glenny(10). This activation was not applied for the iota toxin of type E, as no enhancing value was noted after 7 hours incubation(9). The filtrates were tested against known antitoxins to confirm their type.* The L+ of each toxin was determined by the Ross method(9). *Procedure with HF sera.* Screening tests were performed by adding 0.6 ml sera which had been inactivated at 56°C for 30 minutes to 1.8 ml toxin containing 6 L+ mouse units, incubating the mixture for 30 minutes at room temperature and inoculating 0.5 ml of this mixture intravenously into each of 4 mice weighing 17-20 g. Neutralization was considered to occur if the mice survived for 72 hours. Quantitative determinations were performed as follows: The sera were diluted 1:2, 1:4, 1:6, 1:8 and 1:10 with physiological saline. One ml of each dilution was mixed with 1.2 ml toxin containing 6 L+ units and tested as in the screening test. Control sera were tested in the same manner using undiluted and 1:2 diluted sera. All titrations were repeated on 3 different days to confirm the reproducibility of results.

* *Cl. welchii* antitoxins supplied by Burroughs Wellcome and Company.

TABLE I. Individual Iota Antitoxin Levels of 67 Sera.

Units/cc serum	No. sera showing antitoxin	
	HF sera	Control
0 - .3	1	27
.4- .9	0	14
1 - 2.9	1	9
3 - 4.9	0	0
5 - 6.9	8	0
7 - 8.9	4	0
9 -11.9	2	0
12 -21	1	0
Total	17	50

Results. In the preliminary tests no neutralization of toxins produced by *Cl. perfringens* types A, B, C, D, or F was observed. Sera from 15 of the 17 HF convalescents contained sufficient antibody to neutralize the iota toxin produced by *Cl. perfringens* type E (Bosworth). Table I indicates the antitoxin titers of these 17 test sera as well as of the 50 control sera.

Sera tested against type E filtrates not neutralized with alpha antitoxin failed to protect the mice. Death was accompanied by hemoglobinuria which is common in lecithinase intoxication. This was not observed in mice killed with type E filtrate in which the alpha toxin was neutralized.

Discussion. The only major toxin produced by *Cl. perfringens* type E not formed by the other toxigenic strains is the iota toxin. Iota toxin is lethal, necrotizing, non-hemolytic and is activated by trypsin.

It is assumed that the neutralization observed was due to the presence of iota antitoxin as antitoxins of the other toxigenic types failed to protect mice when inoculated with type E filtrates used in these tests. The occurrence of iota neutralizing antibodies in the sera of convalescent HF patients in higher concentrations than in the 50 control sera is of interest. Further studies are being carried out to determine if an increase in iota antitoxin occurs during the course of HF. Cultural studies are also being conducted to determine the toxigenic types of *Cl. perfringens*

isolated from the stools of HF patients and from the soil of the endemic area in Korea.

Summary. Sera from 17 patients convalescing from hemorrhagic fever and 50 normal controls were tested for the presence of neutralizing antibodies of *Cl. perfringens* iota toxin. Hemorrhagic fever sera contained a significantly higher quantity of neutralizing antibodies than did the control sera.

We should like to express our thanks to Dr. McClung for the culture of *Cl. perfringens* type A, and to Dr. Warrack for the other toxigenic types, as well as for the type antitoxins made available by her from the Wellcome Research Laboratory.

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Improved Procedure for Hypophysectomy of the Mouse.* (22004)

ARDIS J. LOSTROH AND CHARLES W. JORDAN, JR. (Introduced by Choh Hao Li.)
From the Hormone Research Laboratory, University of California, Berkeley.

As early as 1933, Selye, Collip and Thomson(1) reported the successful removal of the pituitary from female albino mice; no data on survival were included, although it was stated that mice hypophysectomized by the parapharyngeal approach tolerate the operation better than do rats. Four years later, Leblond and Nelson(2) published an extensive report on the histological changes observed in the adrenal, thyroid, and gonads of mice of the A and CBAN strains following hypophysectomy; a high mortality during the first postoperative week was reported, although the animals that survived this critical period subsisted thereafter apparently with no special therapy. Subsequently, Thomas(3) described a technic for which he used 2 minute hooks in place of the more customary dental drill to break through the cranium. The procedure for mouse hypophysectomy described in the present communication utilizes the basic technic of Smith(4), but introduces modifications which insure completeness of operation and make possible an operative survival approaching 100%.

Materials and methods. Materials. A cork-topped board of convenient size is outfitted with 2 rubber bands held taut by thumbtacks in order to secure the limbs. For immobilizing the head, a malleable wire is stuck firmly into the board and bent so that the incisors of the animal can be hooked securely beneath it. A 20-gauge one-inch injection needle bent at an angle of 120° serves admirably as a tracheal cannula. Cotton pledgets or miniature swabs are made by winding a small piece of cotton around the end of a pointed toothpick. These materials, together with a view of the operative setup, may be seen in Fig. 1. Other necessary equipment, forceps, dental drill, No. 5 dental burr, binocular magnifier mounted on a stand, and adjustable lighting, are similar to those required for the operation in the rat (5). Nembutal is used for anesthesia. 850 mg of sodium pentobarbital (USP) dissolved in 4 ml of propylene glycol, 2 ml of ethanol, and 14 ml of water serves as a stable stock

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