on as long as the bar was depressed. Total time of bar depression was shown to be a function of hours of food deprivation.

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Received October 20, 1958. P.S.E.B.M., 1959, v100.

Immunization of Mice with Polysaccharides of *Histoplasma capsulatum.** (24625)

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There are significant reports on enhancement of resistance to bacterial infection following immunization with specific bacterial polysaccharides(1-4). However, little or no data are available concerning the immunizing effect of fungal polysaccharides. Salvin(5,6, 7) presented evidence of increased resistance following intraperitoneal injection of acetone-dried yeast phase *Histoplasma capsulatum*. Mice that had been immunized had fewer organisms in their tissues (spleen, liver, kidney) than nonimmunized mice. The difference between the 2 groups was most noticeable during the first 3 weeks following challenge. In nonimmunized mice, rapid growth of the fungus occurred during the first few hours after challenge; a gradual decrease in numbers of organisms was observed during the following months [Salvin(8), Grayston and Salvin(9)]. Rowley and Huber(10) showed the same sequence of events in mice that had been superinfected; however, they were unable to demonstrate any increase in resistance following immunization with a vaccine prepared from yeast phase *H. capsulatum*. Salvin and Ribi (11) found that the cell wall contained the antigenic fraction(s) responsible for the protection observed against *H. capsulatum*.

The present investigation was conducted to determine the immunogenic capacity of polysaccharide fractions of *H. capsulatum* when compared to immunizing activity of killed whole yeast phase vaccine of the same organisms.

* This investigation was supported, in part, by grants from Nat. Inst. of Allergy and Infect. Dis., N.I.H.; and Office of Naval Research.

Methods. Our previous studies on *H. capsulatum* strain G17(M) have shown this culture to have a LD_{50} for mice of 1.2×10^6 (95% confidence limits of $0.8\text{-}1.8 \times 10^6$) yeast phase organisms when injected intravenously. This culture is significantly more virulent than other stock strains of *H. capsulatum* maintained in this laboratory, and was used throughout. The method for extraction of *H. capsulatum* polysaccharides has been reported (12). The polysaccharide was prepared for use as skin test antigen. *H. capsulatum* G17 (M) broth polysaccharide was injected intraperitoneally into 60 albino mice at 4 day intervals according to following schedule: On days 1, 5, 9 and 13, the following amounts of *H. capsulatum* polysaccharide were given, mg .005, .01, .1, and 1. A second group of 60 mice was injected intraperitoneally with 1 mg of *H. capsulatum* G17(M) broth polysaccharide when fourth injection was given to the first group of mice. Mice of a third group of 60 were immunized by 2 weekly intraperitoneal injections of 1 ml of *H. capsulatum* G17 (M) formalin-killed whole yeast cell antigen (about 10^7 organisms/ml). A fourth group of 60 mice served as nonimmunized control. Ten days after last injection the 4 groups of mice were challenged by intravenous injection of varying numbers of viable *H. capsulatum* G17(M) yeast phase cells. These organisms were grown on antibiotic containing blood agar slants. The yeast phase cells were removed from the blood agar by suspension in brain heart infusion broth. Number of cells in the suspension was estimated from hemacytometer cell counts. The immunized and

TABLE I. Mortality Ratios of Immunized and Nonimmunized Mice 21 Days after Challenge with *H. capsulatum* Strain G17(M).

Immunizing antigen	Challenge dose 1×10^6	2.5×10^6	5×10^7
Whole cell	1/16	2/16	9/16
Polysaccharide—4 inj.	5/18	2/17	13/18
1 "	3/12	2/12	8/15
Control (nonimmunized)	11/19	12/20	20/20

control groups of mice were each divided into 3 subgroups of 20 and challenged with 1 x 10^6, 2.5 x 10^6 and 5.0 x 10^6 yeast phase organisms. Deaths were recorded on day they occurred; however, the results were analyzed on the basis of cumulative mortality ratios after 21 days.

Results. Animal losses during immunization and within 24 hours after intravenous challenge reduced some subgroups of 20 mice to smaller numbers. The data presented in Table I suggest that some degree of protection was induced in the immunized groups. The significance of the magnitudes of differences among these data can be more clearly estimated by statistical analysis; therefore, the data were analyzed by the method of Wilcoxon and Litchfield(13).

Table II presents the calculated LD_{50} values and 95% confidence limits for each group. The results indicate that there is significant protection of mice immunized with either whole cell or polysaccharide when compared to nonimmunized mice following challenge. The LD_{50} values for mice receiving 4 immunizing injections of polysaccharide or a single dose of the same antigen were close and no significant difference could be demonstrated by the analytical method used. The relative efficacy of immunizing procedures, determined by comparison of the control group with immunized groups, was further investigated by calculation of potency ratios: these values are shown at the bottom of Table II.

TABLE II. Analysis of Mortality Ratios of Mice Immunized with Whole Cell or Polysaccharide Antigen.

Method of immunization	LD_{50}	95% confidence limits
Whole cell	8.0×10^6	$4.1–15.6 \times 10^6$
Polysaccharide—4 inj.	4.5 "	3.0– 6.75 "
1 "	4.5	2.5– 9.0 "
Control	1.05 "	.6– 1.83 "

$$LD_{50} \text{ potency ratios} = \frac{LD_{50_1}}{LD_{50_2}}.$$

Whole cell and control = 7.62 (3.18–18.2).
Polysaccharide and control = 4.28 (1.76–10.4).

The 4 injection and 1 injection polysaccharide immunized mice reacted equally to the challenge doses. Animals receiving polysaccharide were able to withstand 1.76 to 10.4 times the number of organisms which killed 50% of the controls; between 3.18 and 18.2 times more organisms were required to produce this mortality in whole cell immunized mice as in control mice. It is apparent therefore that the skin test polysaccharide preparation was able to induce a significant degree of protection in mice. One immunizing injection of 1 mg of polysaccharide material gave protection equal to that obtained by 4 injections (total of 1.115 mg) with this polysaccharide. The protection was significant but not as extensive as that induced by the whole cell vaccine.

Summary. *H. capsulatum* strain G17(M) polysaccharide was capable of producing active resistance against intravenously induced histoplasmosis in mice. However, mice immunized with a *H. capsulatum* whole yeast cell antigen showed greater resistance when the *H. capsulatum* polysaccharide immunized, whole cell immunized and nonimmunized control mice were challenged by the intravenous injection of known numbers of viable *H. capsulatum* yeast phase organism.

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Received October 30, 1958. P.S.E.B.M., 1959, v100.

Determination of Carboxypeptidase in Human Pancreatic Juice.* (24626)

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While properties and specificities of proteolytic enzymes of human pancreatic juice are well characterized, few quantitative measurements are available. Lack of data stems from 2 difficulties: obtaining samples of juice and presence of enzymes as zymogens. Human pancreatic juice is now available in adequate amount by collecting it through plastic catheters left in pancreatic duct for drainage after sphincterotomy(1). Total proteolytic activity of pancreatic juice in healthy and diseased pancreas has been determined(2). However, few data are available concerning relative amounts of trypsinogen, chymotrypsinogen, and procarboxypeptidase enzymes activated by trypsin. Procarboxypeptidase was recently isolated and studied by Neurath, *et al.*(3), although its existence as proenzyme requiring activation by trypsin had been established by Anson(4) as early as 1937. Measuremnt of carboxypeptidase in pancreatic juice is facilitated by availability of the synthetic substrate, carbobenzoxyglycyl-l-phenylalanine, first synthesized by Bergmann (5). This substrate is quite specific for carboxypeptidase and is not affected by trypsin or chymotrypsin(6). The enzyme splits off the phenylalanine moiety, liberating alpha-amino group which can be conveniently followed by ninhydrin color. The method used here is a modification of assay described by Neurath *et al.*(6). The ninhydrin reagent of Stein and Moore(7) is used for color development. The method previously described (2) for activation of total pancreatic juice proteolytic activity (utilizing calcium and minute amounts of trypsin) required 18 hours at 4°C and was somewhat variable. We devised a method utilizing commercial duodenal extract for production of enterokinase preparation which is simple to prepare, stable, and which completely activates trypsinogen of pancreatic juice in 60 to 75 minutes at room temperature. The presence of calcium ion in this extract retards autolysis of the trypsin.

Methods. These procedures were applied to measure active carboxypeptidase and total carboxypeptidase after activation of procarboxypeptidase with duodenal extract in human pancreatic juice. Pancreatic juice samples were collected from 12 patients with surgical fistulas at various intervals during their postoperative course. 1. *Reagents*—A. .1M veronal buffer (PH 7.8). B. .06M carbobenzoxyglycyl - l - phenylalanine (Mann Fine Chem.). C. 5% LiCl. D. 8% ninhydrin (Dougherty Chem. Co.) in methyl cellosolve mixed with equal volume of freshly prepared stannous chloride (16 mg dissolved in 10 ml of pH 5. citrate buffer). E. *Duodenal extract containing enterokinase*—400 mg Viodenum (whole duodenum defatted and dehydrated at low temperature, VioBin Corp., Monticello, Ill.) is suspended in 10 cc distilled water containing 200 mg $CaCl_2$ and shaken gently 18 hours at 4°C. After centrifuging, the supernatant is used as activator. This preparation is stable for at least one month if kept frozen. F. 50% N-Propanol. 2. A *standard curve* was prepared by subjecting known amounts of carboxypeptidase, varying 1.5-39 mg/per ml to procedure described below. Values were plotted against optical density. Carboxypeptidase suspension (Worthington Co.) was dissolved in 10% LiCl at 0°C. The exact amount of carboxypeptidase in this solution

* Supported by grant from U.S.P.H.S., N.I.H.