highly purified mercury salt of a glutamic acid precursor was isolated from urine of patient with leukemia during amethopterin therapy, and on the basis of its chemical and microbiological properties, concluded to be FIGLU. 3. It is postulated that folic acid antagonists induced a folic acid deficiency, resulting in insufficient tetrahydrofolic acid needed for catabolism of FIGLU. Consequently FIGLU accumulated. It is suggested that determination of FIGLU in human urine may be a useful aid in diagnosing folic acid deficiency in man.

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Effect of Food Deprivation on Thermal Behavior of the Rat. (24624)

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Ordinarily the laboratory rat has little or no control over his environmental temperature. When cold stressed, this organism, unlike man, does not have access to external heat control devices. Recently McCleary *et al.* (unpublished) investigated behavioral results of providing the rat with some control over environmental temperature. Rats were exposed to low ambient temperature and given a response pedal, which when depressed, activated a 10 second burst of heat from an infra red heat lamp. Under these conditions the rate of bar pressing was shown to be a function of ambient temperature. Weiss(1) has extended this work to show that the bar press rate under above conditions is also a function of restricted food intake and pantothenic acid deprivation. The present study is a further extension of the above work, using a modified dependent variable with 3 levels of food deprivation.

Method. Six adult male Sprague Dawley rats were used. The experimental apparatus was a rectangular box of galvanized steel, 15 by 24 by 10 inches. A 3/8 inch round response bar protruded into the box 4¼ inches and was located 4½ inches in front of 2 GE 250 watt infra red heat lamps with built-in red filters. The lamps were separated from the bar compartment by a partition of ¼ inch hardware cloth. Depression of the bar closed a microswitch and activated the heat lamps. At the same time a cumulative timer and response counter were energized. This apparatus was placed in a conventional refrigerator with ambient temperature at 4° ± 1°C at beginning of experimental session. Auxiliary lighting was supplied by 2 NE 40 GE glow lamps at top of side opposite the heat lamps. Preliminary training of animals consisted of exposure to experimental conditions for 2 hours before first day of recording. The animal's coat was clipped and it was placed in the apparatus and left to discover the response bar and its func-

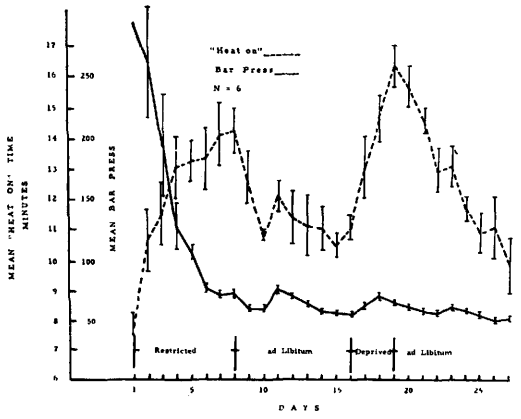

FIG. 1. Mean heat on time and bar press rate (with S.E. of means plotted) as a function of various feeding schedules.

tion. Thereafter each animal was run one hour a day for 27 consecutive days. From day one through day 8 the rats were placed in the apparatus approximately 22 hours after feeding 1½ hours on powdered chow. Days 9 through 16, the rats were placed on an *ad lib.* diet of Purina Chow pellets. After one hour session on 16th day, food was removed from the cages. The following 3 days the animals were run 24, 48, and 72 hours deprived of food. After the 72 hour run the animals were again placed on the *ad lib.* diet. Two response measures were recorded—total time that heat lamps were on, and number of bar presses.

Results. Fig. 1 summarizes the results. A sharp decline of bar press rate and a rise of heat were noted during the first 5 days. These are taken as evidence that the rats quickly learned first to press the bar and then to hold it down for longer periods of time. Further evidence for learning to hold the bar down was shown during period of starvation, when the mean heat on time was increased by 48% while the mean number of bar presses increased by only 17%. Each subject showed increase in heat on time over the 3 day period. The range of these increases from day 16, the last day of *ad lib.* feeding, to the 72 hour deprivation period was 3.2-7.5 minutes. It is on this point that the present response measure appears to present an advantage over that of bar press rate alone. Bar press rates, under conditions of predetermined 10 second heat bursts are likely to be a function, not only of heat requirements, but also of high activity level during food deprivation. In addition, high activity level would result in increased internal heat production which in turn might attenuate external needs. The present data suggest that by using heat on time as the dependent variable, a more valid picture is obtained of heat requirement.

The apparent rise in heat drive during the starvation period, as reflected in increased heat on time, probably results from the animal's deficiency of energy supply. This deficiency logically leads the rat to attempt to conserve body heat or to reduce heat loss, since body temperature is the result of a balance between heat production and heat loss.

It is pertinent to discuss the nature of reinforcement in this work. Since a bar press is not only followed by heat but also by a change in illumination it might be argued that for some reason the rat on the deprivation schedule is being reinforced by this illumination change. Davis(2) has shown that if the onset of dim illumination is made contingent upon bar pressing, the response rate rises as a function of hours of food deprivation. He believed the onset of illumination to be the reinforcing condition. In the present heat reinforcement study, however, it has been shown that the bar press rate changes very little with increasing hours of food deprivation. It is unlikely, therefore, that onset of illumination (and not the heat) is the reinforcement. The remaining possibility, that deprived rats for some reason prefer longer periods of illumination, seems equally unlikely, but future studies will utilize a filter between the lamps and the rat compartment.

A further qualification of the conclusions is needed because of the nearness of the response bar to the heat lamp. It is conceivable that under food deprivation conditions, resulting in prolonged bar depression, the heat becomes noxious. This would tend to attenuate the heat on time. Studies have been initiated to investigate this variable by placing the response bar at various intervals from the heat lamp.

Summary. Rats exposed to low ambient temperatures were trained to depress a response bar to obtain heat. The heat remained

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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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.

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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