routes were effective. As already indicated we now believe that it was the *Salmonella* infection in our stock mice rather than the autolyzed brain diluent which was responsible for the apparent enhancing effect of the latter which we previously reported(6).

When the present studies were nearly completed, the paper by Findlay and Howard(8) appeared in which they reported similar findings in mice injected with diphtheria toxoid or pertussis vaccine plus diphtheria toxoid following intracerebral inoculation with the Lansing strain of poliomyelitis virus. Their experiments differed from ours, however, in that all of the vaccines were given intravenously rather than intraperitoneally. They also reported that T.A.B. vaccine given intravenously had a similar effect.

Summary. Swiss mice infected intracerebrally with the Lansing strain of poliomyelitis virus show a significantly decreased incubation period before onset of paralysis following intraperitoneal inoculation with pertussis vaccine, pertussis-diphtheria toxoid or diphtheria toxoid alone. The results obtained were based on a series of 20 experiments carried out by 2 technicians working independently and involving over 650 mice. Similar results were also obtained in 6 out of 7 other experiments in which infected mice were injected intravenously with *Salmonella typhimurium* vaccine. Evidence is presented that *Salmonella typhimurium* is apparently responsible for the previously reported enhancing effect of autolyzed brain tissue diluent.

Addendum. We have found that 30,000 units of procaine penicillin G in peanut oil given intraperitoneally at the same intervals as the pertussis vaccine had no significant effect on the incubation period before onset of paralysis.

8. Findlay, G. M., and Howard, E. M., *J. Path. and Bact.*, 1950, v62, 371.

Received June 4, 1951. P.S.E.B.M., 1951, v77.

Beneficial Effect of Liver Feeding on Swimming Capacity of Rats in Cold Water.* (18824)

BENJAMIN H. ERSHOFF.
From the Emory W. Thurston Laboratories, Los Angeles, Calif.

Considerable data are available indicating that in addition to the known nutrients substances are present in our diet which may be required in increased amounts during conditions of stress. Such factors are apparently dispensable under normal conditions, or their requirements are so small they may readily be met by amounts present in the diet or through the synthetic activity of the intestinal flora or the animals' own tissues. Certain drugs or other "stress factors" may, however, increase requirements for these substances to such an extent that deficiencies occur, manifested by retarded growth or tissue pathology, and preventable by the administration in appropriate amounts of the missing nutrient (1,2). Whole liver is a potent source of such unknown nutrients. In the present communication data are presented which indicate that whole liver contains a factor, apparently distinct from any of the known B vitamins, which significantly increased the capacity of rats to withstand the stress of swimming in cold water.

Procedure. The basal ration employed in the present experiment consisted of sucrose,

* This paper reports research undertaken in cooperation with the Quartermaster Food and Container Institute for the Armed Forces, has been assigned number 341 in the series of papers approved for publication. The views or conclusions contained in this report are those of the author. They are not to be construed as necessarily reflecting the views or indorsement of the Department of the Army.

1. Ershoff, B. H., *Physiol. Rev.*, 1948, v28, 107.

2. Ershoff, B. H., Nutrition Fronts in Public Health, 1951. The National Vitamin Foundation, Inc., New York.

61%; casein,† 24%; salt mixture,‡ 5%; cottonseed oil (Wesson), 10%, and the following synthetic vitamins per kg of diet: thiamine hydrochloride, 20 mg; riboflavin, 20 mg; pyridoxine hydrochloride, 20 mg; calcium pantothenate, 60 mg; nicotinic acid, 60 mg; ascorbic acid, 200 mg; 2-methyl-naphthoquinone, 10 mg; and choline chloride, 2 g. To each kg of diet were also added 8000 U.S.P. units of vit. A§ and 800 U.S.P. units of vit. D.‖ The vitamins were added in place of an equal amount of sucrose. Each rat also received once weekly a supplement of 4.5 mg alphatocopherol acetate. In addition to the basal ration the following diets were also employed: (1) basal ration plus the following additional vitamin supplements per kg of diet: thiamine hydrochloride, 20 mg; riboflavin, 20 mg; pyridoxine hydrochloride, 20 mg; calcium pantothenate, 60 mg; nicotinic acid, 60 mg; biotin, 5 mg; folic acid, 10 mg; p-aminobenzoic acid, 400 mg; inositol, 800 mg; and vit. B_{12}, 150 μg; and (2) basal ration plus 10% whole liver powder.¶ The vitamin supplements and the whole liver powder were added in place of an equal amount of sucrose. Sixty female rats of the Long-Evans strain were selected for the present experiment at 22 to 25 days of age and an average weight of 44.2 g. Animals were kept in metal cages with raised screen bottoms to prevent access to feces and were fed the above diets *ad lib.* (20 animals per group). Diets were made up weekly and stored under refrigeration when not in use. Animals were fed on alternate days. All food not consumed 48 hours after feeding was discarded. These measures were employed to minimize oxidative changes in the diet. After 12 weeks of feeding the following body weights were obtained for rats in the various dietary groups: Basal ration, 215.4 $\pm$ 6.8 g;** basal ration plus B vitamins, 228.8 $\pm$ 9.0 g;** and basal ration plus 10% whole liver, 247.7 $\pm$ 6.9 g.** Subsequent to this period swimming tests were conducted on all animals. The procedure employed was as follows: Rats were placed in a barrel approximately 33 inches in height and 28 inches in maximum diameter, with smooth vertical sides, and filled to a depth of 18 inches with water. Tests were conducted at a water temperature of both 36°C and 20°C. Eight rats from each dietary group were tested at the higher temperature; the remainder (12 animals per group) at the lower. Measurements were made of the length of time that rats would swim before remaining submerged for a period of 15 seconds.

The selection of the end point was based on preliminary studies in which it was observed that rats almost never remained submerged below the surface of the water for more than 5 seconds and almost never for more than 10 seconds of their own accord. As the end point of a swim was approached, the rat would submerge for periods of 5 to 10 seconds with increasing frequency and could reach the surface only with obvious difficulty. Of 30 rats tested only 2 rose to the surface after remaining submerged for a period of 15 seconds; and these swam for less than 50 seconds before submerging again, at which time they drowned. Rats remaining below the surface for more than 15 seconds almost invariably drowned.

Results. No significant difference was observed in the swimming performance of rats on the various dietary regimes at a water temperature of 36°C. All of the rats in each dietary group survived a test period of 120 minutes at the end of which they were still swimming. When tests were conducted at a water temperature of 20°C, however, a significant difference was observed between the various dietary groups. Rats which had been fed the basal ration swam an average of 13.3

† Vitamin Test Casein, General Biochemicals, Chagrin Falls, O.

‡ Hubbel, Mendel and Wakeman Salt Mixture, General Biochemicals, Chagrin Falls, O.

§ MYVA-DRY Powder, Distillation Products, Rochester, N. Y.

‖ HY-DEE Powder, Standard Brands, New York.

¶ Desiccated Liver, Armour & Co., Chicago, Ill.

** Including standard error of the mean calculated as follows: $\sqrt{\frac{\Sigma d^2}{n}} / \sqrt{n}$ where "d" is the deviation from the mean and "n" is the number of observations.

minutes (range 5 to 29 minutes); those which received the basal ration plus B vitamin diet swam 13.4 minutes (range 10 to 23 minutes); only 3 of 12 rats tested in the liver group, however, swam for less than 2 hours (63, 83 and 87 minutes); all other rats in this group were still swimming at the end of 120 minutes at which time the test was discontinued.

No data are available to indicate what factors were responsible for the improved swimming performance of rats on the liver ration at a water temperature of 20°C. Differences in the rate or degree of heat loss between the various groups while swimming in water at a temperature of 20°C did not appear to be responsible for the diverse effects. In this series the body temperature of rats (as determined rectally in a subsequent experiment) rapidly fell from an initial value of 37°-37.5°C to 23°-25°C. The rate of fall was approximately 1°C per minute for rats fed the basal ration or the basal ration plus vitamin diet and somewhat less (about 0.6°-0.7°C per minute) for rats in the liver group. After 30 minutes, however, the body temperature of rats fed the liver-containing diet was as low or lower than that of rats in other groups: nevertheless, these animals continued to swim for a prolonged period of time. At a water temperature of 36°C no significant changes in body temperature occurred on any of the diets employed.

Swimming tests were repeated on the 4th and 7th day after the initial test on all rats under conditions similar to those first employed. No improvement resulted in successive tests in the swimming performance of rats in the 20°C series on either the basal ration or basal ration plus B vitamin diet. The average swimming time of rats in these groups for the 2nd and 3rd test did not differ by more than 3 minutes from that obtained in the initial test. Similarly no significant differences occurred in other groups between the first and subsequent tests.

Swimming tests were also conducted with rats similar in age and weight to those employed above but which had been raised from weaning on a natural food ration.†† At a water temperature of 36°C all rats tested (6 animals) swam for 120 minutes at which time the test was discontinued. At a water temperature of 20°C, the average swimming time of 8 rats was 24.7 minutes (range 15 to 39 minutes). The average swimming time of rats fed the natural food ration was approximately twice as long at a water temperature of 20°C as that of comparable animals fed the basal ration or basal ration plus B vitamin diet; it was still significantly less, however, than that of rats fed the liver-containing ration. It would appear, therefore, that such protective factors as may have been present in the natural food ration were not present in sufficient amounts for an optimal effect.

Experiments similar to the above were also conducted with young rats. Forty-eight female rats of the Long-Evans strain were selected at 21 to 23 days of age and an average body weight of 42.4 g and were fed *ad lib.* the 3 experimental diets employed above (16 animals per group). After 24 days of feeding the average body weight of rats in the various groups was as follows: Basal ration, 138.1 g; basal ration plus B vitamins, 146.4 g; and basal ration plus whole liver, 149.2 g. Swimming tests were conducted at this time on all rats. At a water temperature of 36°C, all rats tested (6 animals in each dietary group) swam for 120 minutes at which time the test was discontinued. At a water temperature of 20°C, rats fed the basal ration swam an average of 9.8 minutes (range 6 to 13 minutes); those on the basal ration plus B vitamin diet an average of 10.7 minutes (range 7 to 15 minutes); and those on the liver-containing ration an average of 37.4 minutes (range 9 to 155 minutes) (10 rats per group). The beneficial effect of liver on the swimming capacity of rats at a water temperature of 20°C was thus evident in young animals as well as adults. Three of the rats in the liver group, however, swam for less than 15 minutes and did not differ significantly in swimming capacity from animals on the basal ration or basal plus B vitamin diet. It would appear, therefore, that more than 24 days of feeding is required, under conditions of the present

†† Purina Laboratory Chow, Ralston Purina Co., St. Louis, Mo.

experiment, for liver to exert its optimal effect.

Subsequent experiments were conducted in an effort to determine whether the greater swimming capacity of rats on the liver ration at a water temperature of 20°C was due to an increased production of adrenal cortical hormone(s). Eighteen female rats of the Long-Evans strain were selected at 23 to 25 days of age and fed *ad lib.* the basal ration plus B vitamin diet employed above. After 12 weeks of feeding the animals were divided into 3 groups and swimming tests were conducted at a water temperature of 20°C (6 animals per group). Each rat in group I received an intraperitoneal injection of 5 mg ACTH‡‡ 30 minutes before the test; each rat in group II received an intraperitoneal injection of 5 mg cortisone acetate§§ seven minutes before the test; rats in group III served as untreated controls. No significant difference was observed in the swimming performance of rats in the various groups. The average swimming time of animals in group I was 11.8 minutes; in groups II, 11.4 minutes; and in group III, 12.6 minutes. It would appear, therefore, that an increased production of ACTH or cortisone in response to stress was not the cause of the greater swimming capacity of rats fed the liver-containing diet at a water temperature of 20°C.

Discussion. Findings indicate that immature rats raised to maturity on a purified ration containing 10% whole liver powder swam for a significantly longer time at a water temperature of 20°C than rats fed a similar ration in which the B vitamins were provided in synthetic form. The beneficial effect of liver was apparently not due to its content of known B vitamins. Available data do not indicate, however, whether its protective effect was due to an unidentified factor or to its content of known nutrients. In view of the fact that military personnel and others may on occasion be forced to spend prolonged periods of time in water at a temperature of 20°C or below, it would appear that the identification of a factor which might be employed to increase resistance to this form of stress may have considerable practical value.

Summary. Immature rats raised to maturity on a purified ration containing 10% whole liver powder swam for a significantly longer period at a water temperature of 20°C than rats fed a similar ration containing the B vitamins in synthetic form. The protective factor in liver was distinct from any of the known B vitamins.

‡‡ ACTHAR, Armour and Co., Chicago, Ill. The material employed was diluted with saline solution to a concentration equivalent to 5 mg of the standard LA-1-A per cc.

§§ Saline Suspension of Cortone Acetate, Merck and Co., Rahway, N. J. Each cc contained 25 mg of cortisone acetate.

Received June 4, 1951. P.S.E.B.M., 1951, v77

Effect of ACTH on Whole Blood Coagulability.* (18825)

JOHN L. FAHEY. (Introduced by G. W. Thorn.)

From Medical Clinics, Peter Bent Brigham Hospital, and Department of Medicine, Harvard Medical School.

Administration of ACTH in sufficient dosage induces numerous metabolic changes. Reports of the effect of this hormone on the coagulation time of whole blood, however, have been conflicting. Cosgriff *et al.* conclude that ACTH shortens the clotting time, induces hypercoagulability and a state conducive to thromboembolic complications(1). Smith *et al.*, on the other hand, reported a

* The author is indebted to Drs. Albert E. Renold and Peter H. Forsham for continued interest in this study, and to Dr. H. Cluxton, of the Armour Laboratories, Chicago, Ill., for the ACTH used in this work.

1. Cosgriff, S. W., Diefenbach, A. F., and Vogt, W., *Am. J. Med.*, 1950, v9, 752.