or 60.5% lactose. This was observed on both diets A and B. Cecal distension was also present on diets $A_2$ and $B_2$ but was less pronounced.

A significant difference in ovarian weight was observed in lactose-fed rats on the various diets (Table II). Ovaries appeared immature both in weight and microscopic appearance in animals fed diets $A_2$, $A_3$, $A_4$ and $B_4$. No mature follicles or corpora lutea were present in any of these rats with the exception of 1 animal on diet $A_2$. Six of the 8 rats on diet $B_2$ and 5 animals on diet $B_3$, however, had ovaries which were indistinguishable grossly and in histological appearance from normal controls (diets $A_1$ and $B_1$). In these animals mature follicles were numerous and corpora lutea were present.

Previous findings(10,11) indicate that the failure of rats to survive on diets containing high levels of lactose was due, at least in part, to an inability to hydrolyze adequately large amounts of this disaccharide. Present findings suggest that the administration of defatted and desiccated whole liver powder increased the rats' ability to digest lactose, although a period of approximately 10 days elapsed before this effect occurred. No data are available concerning the factor or factors in liver responsible for the increased growth, survival and ovarian development on lactose-containing diets. Recent findings by Hartman *et al.* (12) suggest, however, that vitamin $B_{12}$ may be responsible, at least in part, for these effects.

*Summary.* The oral administration of 10% defatted and desiccated whole liver powder increased significantly the gain in body weight of immature rats fed purified diets containing 30.2, 45.3 and 60.5% lactose. It prolonged the survival time of immature rats fed a purified diet containing 60.5 per cent lactose, and it counteracted the inhibition of ovarian development which occurred on purified rations containing 30.2 and 45.3% lactose.

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Received November 9, 1949. P.S.E.B.M., 1949, v72.

## Effect of Protein Level and Inanition on Glutamine Content of Rat Tissues.* (17531)

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A study by Roine(1) suggested the importance of glutamine in protein synthesis in yeast and indicated the need for investigation of the glutamine content of blood and tissues of higher animals. Wiss(2) observed that the blood glutamine content of rats increased when the rats were placed on an inanition routine and decreased when protein was fed. These results implied that blood glutamine concentrations are governed to some extent by the level of protein ingested. This work was undertaken to relate various dietary factors to changes in glutamine concentration in animal tissues.

* Published with the approval of the Director, Oklahoma Agricultural Experiment Station. Supported by a grant from the United States Public Health Service through the National Institutes of Health. Appreciation is expressed to Merck and Company, Rahway, N.J., for supplies of crystalline vitamins and to the Lederle Laboratories Division, American Cyanamid Co., Pearl River, N.Y., for pteroylglutamic acid.

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2. Wiss, O., *Helv. Physiol. et Pharmacol. Acta*, 1948, v6, C35.

TABLE I.
Composition of Rations Used for Rat Dietary Studies.

| Ingredient* | 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| Casein (crude) | 0% | 10% | 20% | 40% |
| Sucrose | 91 | 81 | 71 | 51 |
| Cottonseed oil | 5 | 5 | 5 | 5 |
| Salts† | 4 | 4 | 4 | 4 |

* Each kilogram of ration was supplemented with the following vitamins: Thiamin, 4 mg; riboflavin, 6; pyridoxine, 3; calcium pantothenate, 20; nicotinic acid, 20; pteroylglutamic acid, 1; inositol, 20; p-amino benzoic acid, 20; choline chloride, 1 g. In addition vitamins A and D and alpha tocopherol were administered by dropper twice a week.

† Hegsted *et al., J. Biol. Chem.*, 1941, v138, 460.

*Experimental.* Experimental animals were young adult male rats of the Sprague-Dawley strain. Animals were placed on a 10-day equilibration period on the control ration (Ration 3, Table I) before use. In all studies food and water were available *ad libitum.*

After the equilibration period, animals were placed on rations of varying protein levels (Table I) for a period of 10 days. During the inanition experiment the animals were allowed access only to water. At the end of the experimental periods, the animals were killed by exsanguination under light ether anesthesia, the tissues rapidly removed, frozen and stored at −15°C until analyzed. Plasma glutamine determinations, however, were made immediately.

Glutamine was determined by nesslerization of the ammonia liberated after incubation with beef kidney preparations, essentially according to the procedure of Archibald.(3) The preparation of the tissues for the enzymatic determination involved homogenization of the weighed sample with 15 ml of 0.04 N potassium cyanide solution, dilution of the blended mixture to 20 ml with the potassium cyanide solution, and centrifugation of the resulting mixture for 10 minutes at 1500 R.P.M. Two ml aliquots (one ml in the case of liver) of the supernatant liquid were taken for each determination.

*Results.* The effect of varying the protein level upon the glutamine content of rat tissues is presented in Table II. Each value represents the average of two separate experiments using a total of 12 animals for each treatment.

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TABLE II.
Relation of Protein Level of Ration to the Glutamine Content of Rat Tissues.

| | Glutamine (mg/100 g or ml) | | | |
|---|---|---|---|---|
| Tissue | Ration 1 | 2 | 3 | 4 |
| Plasma | 3.4 | 3.1 | 2.8 | 3.3 |
| Liver | 189 | 148 | 97 | 79 |
| Lung | 174 | 97 | 74 | 50 |
| Kidney | 258 | 135 | 119 | 87 |
| Heart | 484 | 273 | 212 | 143 |
| Spleen | 356 | 172 | 156 | 136 |
| Muscle | 214 | 148 | 178 | 150 |
| Protein level ingested, % | 0 | 10 | 20 | 40 |

TABLE III.
Effect of Inanition on Glutamine Content of Rat Tissues.

| | Glutamine (mg/100 g or ml) | | | | |
|---|---|---|---|---|---|
| Tissue | 0 hr | 12 | 25 | 49 | 95 |
| Plasma | 2.9 | 3.8 | 3.7 | 2.8 | 2.4 |
| Liver | 92 | 85 | 99 | 148 | 183 |
| Lung | 73 | 63 | 89 | 140 | 171 |
| Kidney | 117 | 122 | 146 | 200 | 259 |
| Heart | 212 | 184 | 219 | 335 | 418 |
| Spleen | 156 | 145 | 204 | 267 | 332 |
| Muscle | 189 | 162 | 213 | 246 | 339 |

The relation between the glutamine content of rat tissues and length of inanition is shown in Table III. After a 10-day equilibration period on the control ration (Ration 3, Table I), the animals were subjected to starvation for the indicated periods, sacrificed, and the tissues analyzed as before.

*Discussion.* These studies indicated that there was a definite relationship between the glutamine content of rat tissues and the level of protein in the ration consumed. As the protein level increased, all tissues showed a decrease in glutamine content. Muscle tissue was different than the others studied in that this decrease was not found when protein was increased above the ten percent level. In contrast to tissues generally, the plasma glutamine level declined less sharply and then rose again; at 40 percent protein intake, values similar to those of animals consuming the protein deficient ration were found.

In the tissues of rats which have been

on an inanition regimen there was a definite initial trend towards a decreased glutamine content with deprivation of food. This trend was reversed, however, after 24 hours; and after 95 hours starvation, values similar to those found in rats subsisting on a protein-free ration were attained. In contrast to the report of Wiss(2) plasma glutamine levels did not increase, in fact, there was a suggestion of somewhat decreased values at 95 hours.

Both of these observations suggest that glutamine may have a function in the catabolism of amino acids, regardless of whether the source is dietary or from the body tissues. Whether this function is a direct one involving the utilization of glutamic acid or more indirect with glutamine acting as a reservoir for the ammonia released by the deamination of amino acids is not known. Preliminary studies in these and other laboratories do not indicate a direct conversion of administered glutamic acid to glutamine, thus suggesting that this amide may not be too closely involved in the metabolism of the corresponding dicarboxylic amino acid.

*Summary.* The variation of glutamine content of rat tissues with protein level or period of inanition has been studied. Glutamine levels showed a marked increase as the protein level of the diet decreased. An increased glutamine level was also observed with inanition. These observations indicate the possible importance of glutamine in protein metabolism.

Received November 10, 1949. P.S.E.B.M., 1949, v72.

## Conversion of Tryptophane to Nicotinic Acid by *Trichophyton equinum.** (17532)

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A strain of *Trichophyton equinum,* one of the faviform trichophytons which commonly causes ringworm in horses, was found to require nicotinic acid.(1) *T. equinum* (Gedoelst) is a member of the group of large-spored, ectothrix trichophytons of animal origin which may infect man. They are characterized by their poor growth on the usual sugar-peptone media on which they produce slow-growing, glabrous, or slightly downy colonies composed largely of irregular hyphae and chlamydospores. By the use of natural carbohydrate media, Langeron and Milochevitch(2) and later Lebasque(3) showed that the growth and production of spores by these strains could be stimulated by better nutrition, and it was later shown(4,5,1) that many of the species in this group required certain vitamins and other growth factors present in natural substances. The requirement in most instances has been for thiamine and inositol, and in one case for pyridoxine as well. This Baudet strain of *T. equinum* obtained from the Culture Bureau for Fungi, Delft, Holland is the only strain known to show a requirement for nicotinic acid.

*Experimental.* No growth of this strain could be obtained on a vitamin-free basal agar prepared as follows: 50 g dextrose (C.P.), 2.0 g $NH_4NO_3$ (C.P.), and 0.5 g $MgSO_4$ (C.P.) dissolved in one liter of distilled water buffered to pH 7 with Sorenson's phosphate

* This study was made with the help of a grant-in-aid from the United States Public Health Service.

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