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.

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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

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mixture (KH_2PO_4 and Na_2HPO_4). To this is added 1.5% purified agar, prepared according to the method of Robbins.(6) Addition of nicotinic acid to such a medium allows rapid growth of the fungus. Nicotinic acid may be substituted for by equal amounts of nicotinic amide, and by comparatively large amounts of the amino acid l-tryptophane.

All vitamins and amino acids were prepared in water solutions, sterilized by filtration, and added to the melted, partially cooled basal agar. Except for nicotinic acid and its amide, none of the other vitamins tested: thiamine, pyridoxine, para-amino-benzoic acid, i-inositol, choline, pantothenate, and folic acid showed any stimulatory effect when added singly or in combinations to the basal agar. The vitamins were added in amounts of 0.1 mg to 5 ml of the basal agar except for biotin where 0.05 mg was added. Twenty-two amino acids: glycine, dl-alpha alanine, beta alanine, dl-serine, dl-threonine, dl-valine, l-leucine, dl-norleucine, dl-aspartic acid, l-glutamic acid, l-lysine, l-arginine, l-histidine, l-cystine, dl-cysteine, dl-methionine, l-proline, dl-tryptophane, l-tyrosine, dl-phenylalanine, and l-hydroxyproline were tested singly and in combination in M/1000 dilution in the basal agar (with the exception of cystine which is highly insoluble, and was tested in more dilute solution). Of these only tryptophane showed any stimulatory effect.

The minimum effective concentration of nicotinic acid or amide was determined by diluting these substances serially (by halving) in tubes containing 5 ml of NH_4NO_3 basal broth (prepared similarly to the NH_4NO_3 basal agar described above except that no agar is added) and inoculating with washed fragments of a culture of *Trichophyton equinum* grown in a 1.0% peptone nutrient broth. Both nicotinic acid and nicotinic amide were active to a dilution containing 0.25 μg in 5 ml NH_4NO_3 broth. No growth occurred in higher dilution or in control tubes which contained no vitamin. By a similar titration it was determined that l-tryptophane was active to a dilution containing 625 μg in 5 ml NH_4NO_3 broth. dl-tryptophane showed only half this activity indicating that only the l form was active.

This substitution of the amino acid for nicotinic acid suggests the possibility of tryptophane as a precursor of this vitamin. In view of these findings, it was of interest to determine whether the strain of *T. equinum,* a naturally occurring nicotinicless fungus, could be shown to have the ability to convert tryptophane to nicotinic acid.

A series of 150 ml Erlenmeyer flasks each containing 50 ml NH_4NO_3 broth (described above), and a similar series containing 50 ml of the basal broth prepared without NH_4NO_3 were inoculated with small fragments of the fungus which had been grown for 7 generations on slants containing 1000 μg l-tryptophane in 5 ml NH_4NO_3 basal agar. Varying amounts of l-tryptophane were added to the flasks in duplicate series. One series was autoclaved immediately and served as control. The fungus grew in all of the non-autoclaved flasks containing tryptophane, but no growth occurred in control flasks of NH_4NO_3 basal broth. The amount of growth was roughly proportional to the amount of tryptophane present although in flasks where the NH_4NO_3 had been omitted and tryptophane was the only source of nitrogen, the amount of growth was much less.

After 6 weeks the entire growth and culture medium of each flask was hydrolyzed and biologically assayed for nicotinic acid with the *Lactobacillus casei.*‡ The results indicated

TABLE I.

Media* 50 ml basal broth +	Nicotinic acid assay‡ Tests/μg	Controls/μg†
25 mg l-tryptophane	2.32	—
5 mg l-tryptophane	2.02	.0085
5 mg l-tryptophane + 100 mg NH_4NO_3	1.18	.008

*Basal broth—5% dextrose (C.P.) + 0.01% $MgSO_4$ (C.P.) in H_2O solution of M/15 phosphate buffers (KH_2PO_4 and Na_2HPO_4) at pH 7.

All flasks inoculated with *T. equinum* and maintained at room temperature 6 weeks before testing for nicotinic acid.

† Controls—media autoclaved immediately following inoculation with *T. equinum.*

‡ Nicotinic acid—determined by biological assay with *L. casei.*

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‡ The biological assay was very kindly performed by Dr. Ray F. Dawson of the Botany Department of Columbia University.

that nicotinic acid had been produced by the fungus in flasks containing tryptophane. The similar flasks which had been autoclaved immediately after inoculation with the fungus showed only negligible traces of nicotinic acid. (Table I). It is interesting to note that although twice to 3 times as much growth occurred in the tryptophane flasks which also contained NH_4NO_3, less nicotinic acid could be detected in these flasks. It seems possible that in the presence of readily available nitrogen, much of the nicotinic acid produced from the tryptophane was utilized as readily as it was formed.

Discussion. Krehl *et al.*(7) in their studies of the pellagragenic effect of corn were the first to show a possible interchangeable role of nicotinic acid and tryptophane. Later experiments by Rosen *et al.*(8) and Sarett and Goldsmith(9) indicated that tryptophane may be an important precursor of nicotinic acid in rats as well as in humans, and may explain the anti-pellagragenic effect of certain foods such as milk which are low in nicotinic acid but rich in protein. Bonner and Beadle(10) have described 3 x-ray mutants of *Neurospora* which require nicotinic acid for growth, and in a later communication, Beadle, Mitchell, and Nyc(11) have shown that one of the nicotinicless mutant strains, No. 65001, was able to grow when supplied with either tryptophane or nicotinic acid. By means of another nicotinicless mutant strain which could not utilize tryptophane, they further showed that strain No. 65001 actually had the ability to convert tryptophane to nicotinic acid. Nicotinic acid could also be produced by this strain from dl-kynurenine, a substance which has been known as a product of tryptophane metabolism in animals and bacteria, and is a possible intermediate in the conversion of tryptophane to nicotinic acid. On the basis of the growth responses of this *Neurospora* mutant strain to tryptophane, nicotinic acid, and kynurenine, it was postulated that nicotinic acid is normally formed from tryptophane with kynurenine as an intermediate.

According to Schopfer,(12) no naturally occurring fungi have been shown to require nicotinic acid. Whether the strain studied is a normally occurring type or an unusual mutant form is not known, and it is doubtful that the requirement for nicotinic acid by this strain of *T. equinum* is a characteristic which would be of value in species determination. The only other strain of *T. equinum* available for testing was No. 9870 from the American Type Culture Collection. This strain did not require nicotinic acid, but did require thiamine and i-inositol. In this respect it resembles *T. faviforme*, a common agent of ringworm in both cattle and horses. Since the separation of *T. equinum* from *T. faviforme* depends on the insecure grounds of its more frequent isolation from horses than from cattle and its ability to produce more aerial mycelium on the surface of colonies on Sabouraud's dextrose agar, it seems highly probable that *T. equinum* is synonymous with *T. faviforme*. Further strains must be obtained and studied in order to determine whether the requirement for nicotinic acid is present in any other strain of this group.

The transformation of tryptophane to nicotinic acid by the strain *T. equinum* (Gedoelst) gives further evidence for the hypothesis that, in both plants and animals, tryptophane is a precursor in the formation of nicotinic acid, essential to the growth of these forms.

Summary. A strain of *Trichophyton equinum* has been shown to have a requirement for nicotinic acid. Nicotinic acid may be substituted for by equal amounts of nicotinic amide and comparatively large amounts of l-tryptophane. The fungus has been shown to have the ability to convert tryptophane to nicotinic acid, thus giving further evidence in support of the hypothesis that tryptophane may be a precursor of nicotinic acid.

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