XXXVII. FEEDING EXPERIMENTS WITH KYNURENIC ACID.

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In the endeavour to bring to light intermediate stages in the metabolic breakdown of individual amino-acids the method of feeding an animal with substances which, on chemical grounds, represent possible intermediate products has been widely employed. If a substance so administered can, under suitable circumstances, be shown to yield a characteristic end product common to it and to the original amino-acid its claim to be a normal intermediary becomes fairly well established. The light which was thrown upon the metabolism of tyrosine and phenylalanine when Otto Neubauer administered hydroxyphenyl-lactic acid, and hydroxyphenylpyruvic acid to alcaptonuric individuals is a familiar case in point.

A product appearing in the intermediate processes of metabolism is not necessarily a degradation product in the narrower sense of the term. It may be something other than a link in the chain of processes which yield energy alone. The substance may itself subserve some function in the body, or it may be a stage in the production of a substance with a function. If an amino-acid from protein were the normal precursor of a metabolite possessed of some particular functional significance, one of the effects of withdrawing that amino-acid from the food would clearly be an interference with the function concerned. In such a case it seems probable that in the absence of the amino-acid itself the animal would benefit if an intermediate product were administered. On these lines the metabolic importance of a substance known to arise from a given amino-acid may be tested by giving it in the food in place of the parent substance. Kynurenic acid, for instance, may be fed in place of tryptophane.

A supply of tryptophane is known to be essential to the animal, which seems unable to synthesise it. While it is of course required for the formation of tissue proteins, and therefore for growth, it is probable that it also yields products with more specialised functions [Hopkins and Willcock, 1906]. Is kynurenic acid such a product, or the precursor of such a product?

Since Ellinger showed that kynurenic acid arises from tryptophane the precise significance of the appearance of a quinoline derivative during the metabolism of the indole nucleus has become a matter of interest.

Kynurenic acid might be a product on the main line of the destructive breakdown of tryptophane; it might be the end product of some accessory, non-significant, process; it might be, on the other hand, the precursor of a substance with a special function.

Miss A. Homer [1915] believes that the first possibility should be rejected, as her experiments show that (in the dog) kynurenic acid given subcutaneously, or by the mouth, is almost quantitatively excreted in the urine, though when tryptophane itself is given the amount of kynurenic acid excreted is only a small fraction of what would correspond to the metabolised tryptophane. According to Miss Homer a certain amount of the latter is side-tracked into the form of the quinoline derivative, while the greater part is broken down on other lines, or converted into substances of significance to the body.

But the fact that an intermediate metabolite is excreted more or less quantitatively when administered in excess to an animal in a state of full nutrition does not disprove the possibility that it normally arises on a significant line of change. So long as sufficient is already being produced to subserve any supposed function the excess administered may well be excreted unchanged. Only when a gap, so to speak, is first produced in metabolism by wholly withdrawing the primary source of the substance can the effect of supplying the substance itself be properly observed.

In the experiments to be described in this paper, rats were fed upon a basal dietary containing all the amino-acids of protein except tryptophane. One set of animals was fed upon this dietary alone. To the food of a second set tryptophane was added, while a third set received kynurenic acid instead of tryptophane. The results seem to show that, in the case of the rat at any rate, kynurenic acid can in no sense replace tryptophane. It does not restore to any degree the power of maintenance which a dietary always loses when deprived of tryptophane. It produces no apparent betterment in the general health of the animal, and does not lengthen the survival period.

Methods used.

The basal dietary used in the experiments had the following composition:

Amino-acid mixture obtained from prolonged acid hydrolysis of caseinogen	250 g.
Potato starch	418 ,,
Cane sugar	207 ,,
Lard	125 ,,
Ash of oats	13·5 ,,
Ash of dog biscuit...	13·5 ,,

The fact that acid hydrolysis entirely destroys tryptophane without otherwise seriously interfering with the nutritive power of the mixture, makes the amino-acids from such hydrolysis a convenient source of tryptophane-free food. To prepare the dietary containing tryptophane 3·75 g. of the indole acid were added to the above mixture, while in the preparation of the third dietary 3·0 g. of kynurenic acid were added. Complete conversion of 3·75 g. tryptophane would yield 3·474 g. kynurenic acid. The latter was prepared from the urine of two dogs which had been given tryptophane by the mouth. It was carefully purified and melted at 89°. The necessary vitamine supply was given to the rats in the form of an alcoholic extract of the solids of fresh milk.

The animals were kept in a room of uniform temperature. They were fed and weighed at uniform intervals, and all three sets received precisely similar treatment.

Discussion of Results.

The figures give the weight curves of the rats under experiment. The first three show those of individuals, and the fourth the average weights in the case of each experimental set. The animals were fed upon bread and milk up to the 28th day (marked by an arrow) and were then put upon the experimental dietaries.

Fig. 1 shows the characteristic loss of weight which immediately follows when tryptophane is absent, the animals of this set being upon the basal dietary without any addition.

Fig. 2 shows the effect of adding tryptophane to the basal dietary. Instead of loss of weight there is maintenance, and, as seen in the average

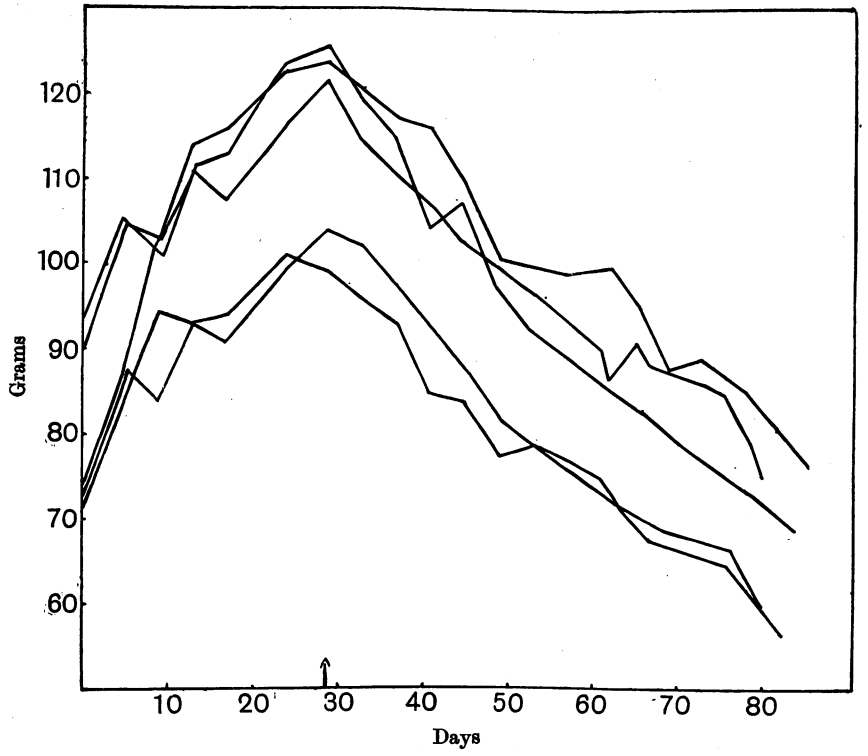

Fig. 1. Body weight curves of individual rats which from the 28th day received the basal diet alone.

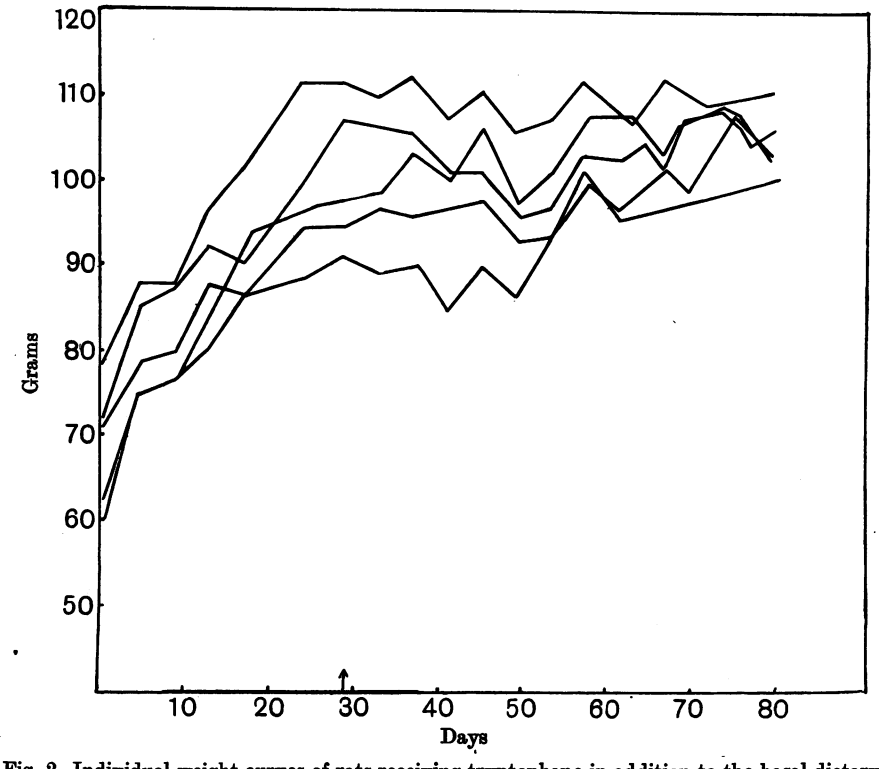

Fig. 2. Individual weight curves of rats receiving tryptophane in addition to the basal dietary.

weight curve of Fig. 4, a certain amount of growth. In a large number of experiments carried out in this laboratory, in other connections, quite normal growth has been obtained when the sole nitrogen supply was the amino-acid mixture from acid hydrolysis with added tryptophane, though a small addition of cystine has sometimes proved necessary. The somewhat less favourable result seen in my experiments was due, I think, to a deficiency in

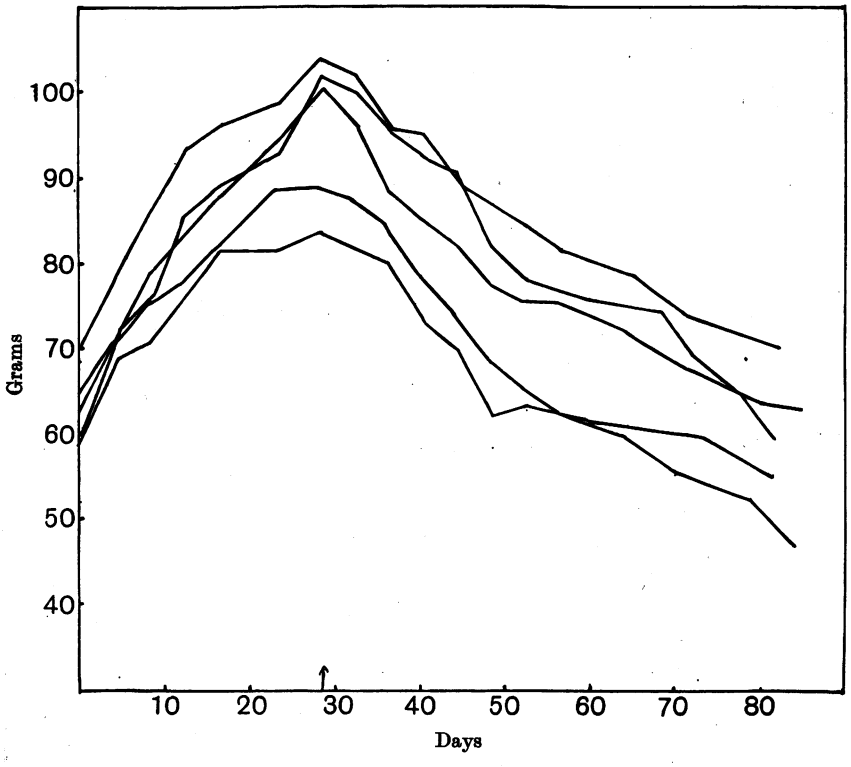

Fig. 3. Individual weight curves when kynurenic acid was added instead of tryptophane.

cystine. The protein used was caseinogen; the acid hydrolysis was prolonged (40 hours) and no cystine was added.

The effect of adding tryptophane to the basal dietary was striking enough however as will be seen from the curves. On the other hand the set of rats which received kynurenic acid instead of tryptophane showed in respect of preservation of weight no difference whatever from those on the basal dietary alone. The latter were somewhat heavier animals than the former, but it will be seen that the curves run parallel. Loss of weight occurred with equal rapidity in both. The general condition in these two sets was also strikingly

similar from week to week, and the survival period was identical, being on the average exactly fifty days in each set. In sharp contrast was the condition of the set receiving tryptophane. These rats appeared normal throughout, and were in perfect health at the end of the experiment.

The consumption of food was similar in each set and was quite satisfactory throughout. The experiments show that tryptophane cannot in any sense be replaced in the food by its quinoline metabolite. It was perhaps not to

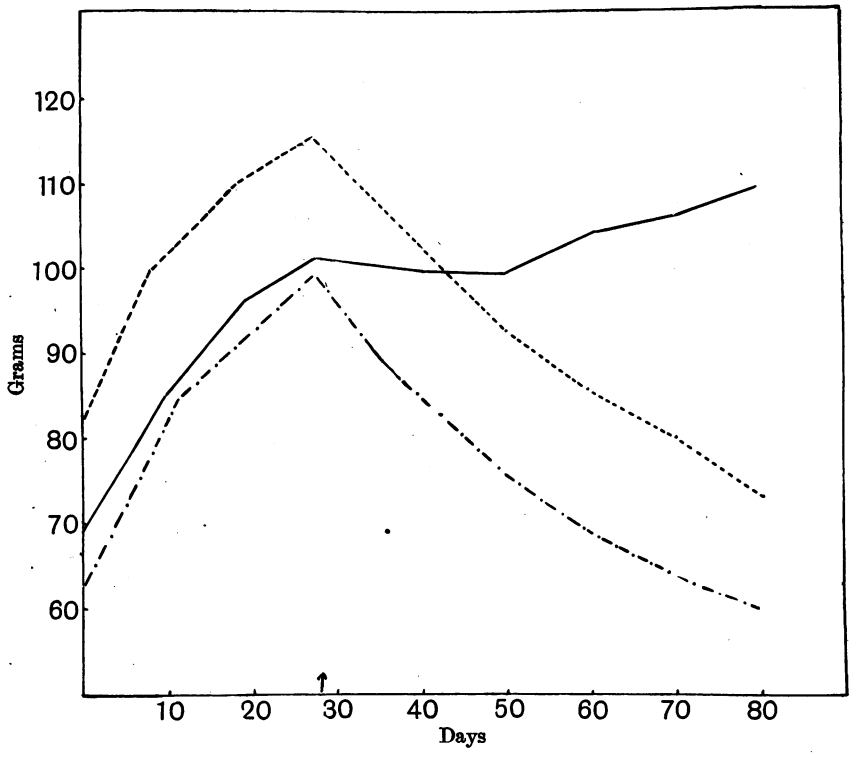

Fig. 4. Average weight curves. Dotted line, rats upon basal dietary alone. Continuous line, set upon the basal diet plus tryptophane. Dot and dash line, set taking kynurenic acid.

be expected that it could replace it as a factor in growth, as the reactions by which it is formed are not likely to be easily reversible; but, in the rat at any rate, it would appear to be without significance to the body[1].

[1] *Note by F. G. Hopkins.* Dr Asayama's results gain in significance if it be shown that kynurenic acid is a normal metabolite of the rat. The animal certainly excretes extremely little of the substance, but after feeding with tryptophane I have been able to separate small amounts of a substance from rats' urine which gives the reactions of kynurenic acid and melts at 88°–89°.

I have to thank Professor Hopkins for suggesting the experiments described, and for the interest he has taken in them. The administration of the dietaries was carried out under his direct supervision, and he has been responsible for the care of the animals.

Summary.

When tryptophane is absent from the food of rats no betterment of nutrition is observed if kynurenic acid be given. The quinoline derivative of tryptophane seems to possess no special significance in metabolism.

REFERENCES.

Homer (1915). *J. Biol. Chem.*, **22**, 391.
Hopkins and Willcock (1906). *J. Physiol.*, **35**, 88.