Of ten mice given 4.0 grams per kilogram five died; the blood levels in two at death were found to be 77 and 106 mgm per cent. It appears that the acetyl-derivative of sulfanilamidopyridine is of the same order of toxicity as the unconjugated compound if blood concentrations are taken into consideration.

In conclusion, we wish to emphasize that sulfanilamidopyridine on the basis of blood concentration values appears to be more toxic than sulfanilamide. Until more is known about the drug, it should not be used in conditions where sulfanilamide has been shown to be effective.

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THE APPLICATION OF THE NITROGEN ISOTOPE N^{15} FOR THE STUDY OF PROTEIN METABOLISM

THE production of nitrogen with an increased concentration of the isotope of atomic weight 15 (N^{15}) by Urey and his collaborators has opened the possibilities of investigating the metabolism of amino acids, proteins and other nitrogenous compounds in normal healthy animals. The principles underlying the procedure are similar to those which have been successfully employed in the investigation of the fate of fats, steroids and other compounds, with deuterium as a tracer. The substance to be investigated is synthesized in the laboratory in such a manner that one of its atoms contains an increased concentration of isotope, in the present case[1] by starting the synthesis with ammonia N^{15}. The rarity and value of the isotopic ammonia compelled the development of methods for amino acid synthesis which should lead to complete recovery of the isotope. The methods employed were modifications of the phthalimide synthesis of Gabriel and of the catalytic reduction of α-keto acids in the presence of ammonia according to Knoop. The following isotopic and racemic amino acids are now available for biological investigations, all of which contain nitrogen with more than 2 per cent. N^{15} as compared with the normal abundance of 0.368 per cent.: glycine, alanine, nor-leucine, tyrosine, phenylalanine, glutamic acid, aspartic acid, lysine and leucine. The latter compound also contains, besides the nitrogen isotope, stably (carbon) bound deuterium, and has been resolved in the laboratory into its optical isomers.

The biological application of such substances requires a highly sensitive micro method for the analysis of the N^{15} content in the nitrogen of organic compounds. The only practical procedure requires the use of a mass spectrometer. The nitrogen of the compound is converted to ammonia, elementary nitrogen is liberated from it in a high vacuum system and this admitted to the vacuum tube containing the mass spectrometer proper. In this tube the gas is ionized and under the influence of electric and magnetic fields is dispersed into a spectrum of the component masses. The instrument constructed in our laboratory requires less than 1 mg of nitrogen for an analysis and has a sensitivity of 0.003 per cent. N^{15} when normal nitrogen is analyzed. Any of the amino acids listed above can thus be mixed *in vivo* or *in vitro* with several hundred times its weight of the normal analogue before the analytical methods miss the isotope label.

The concentration of N^{15} in the nitrogen of ordinary casein as well as of ten different natural amino acids was determined and found to be the same as in the nitrogen of air, a finding which indicates that both isotopes of nitrogen are treated indiscriminately in anabolic and catabolic processes.

The nitrogen in organic linkage, as for example in amino acids, is stably bound and does not exchange with the nitrogen of other nitrogenous compounds with which it is brought in contact. This was established by the investigation of ten different systems, each of which contained one normal and one isotopic compound. Whenever, in experiments either *in vivo* or *in vitro,* compounds are observed which contain more than the normal abundance of isotopic nitrogen, the formation of such compounds must therefore be ascribed to chemical reactions.

An investigation on hippuric acid formation has already been reported[2]; other experiments on protein metabolism have now been carried out. The first is concerned with the much discussed question as to whether the animal organism can utilize the nitrogen of dietary ammonia for amino acid formation. Rats were given an ordinary stock diet to which were added benzoic acid and isotopic nitrogen as ammonium citrate. The experimental conditions under which the animals were kept were so chosen that most of the glycine excreted in the urine as hippuric acid was newly formed. This contained a small but significant amount of isotope, indicating that a small part of the glycine had been formed from ammonia nitrogen.

Another experiment carried out with immature rats was still more illuminating. The animals were given a protein-low diet to which isotopic ammonium citrate had been added. After five days they were killed, the carcasses were hydrolyzed and the following compounds were isolated in pure form; glycine, glutamic acid, aspartic acid, proline, histidine, lysine, arginine and creatine. All, with the exception of lysine, con-

[1] The authors are highly indebted to Professor H. C. Urey for the valuable gift of nitrogen isotope.

[2] R. Schoenheimer, D. Rittenberg, M. Fox, A. S. Keston and S. Ratner, *Jour. Am. Chem. Soc.,* 59: 1768, 1937.

tained a small amount of the nitrogen isotope. Both experiments must be taken as proof that at least a small amount of creatine and amino acids can be formed with ammonia as a nitrogen donor and that dietary ammonia may be utilized for this process.

In another experiment the fate of one dietary amino acid, tyrosine, was followed in a full-grown adult rat kept in nitrogen equilibrium on a normal diet, the protein of which consisted of casein. To this was added an amount of isotopic *dl*-tyrosine corresponding to only 14.4 mg nitrogen addition per day. The animal was kept on this diet for ten days. It excreted an amount of total nitrogen equivalent to that in the total diet, but about half of the isotope was retained by the tissues. The retention must have been accompanied by the liberation (for excretion) of an equivalent amount of nitrogen. The liver and the remaining carcass were worked up separately to locate the isotope. Almost all of it was recovered in the proteins, while the non-protein-nitrogen revealed only traces. Both liver and carcass proteins were hydrolyzed, and pure tyrosine was isolated. The samples contained a high concentration of isotope, indicating an extensive deposition of the dietary tyrosine in the body proteins. However, the isotope content in tyrosine accounted for only about one quarter of the total isotope content in the proteins. Amino acids, other than tyrosine, must thus have taken up nitrogen originally present in tyrosine. This could be proved. The following other amino acids were isolated: arginine, lysine, histidine and the mixture of the dicarboxylic acids, glutamic and aspartic acid. With the exception of lysine, all of them contained a significant amount of isotopic nitrogen. As the dicarboxylic acids contain only 1 nitrogen atom per molecule, the position in the molecule of the newly introduced nitrogen is certain. The position of the isotope in the arginine and histidine, both of which contain more than one nitrogen atom per molecule, had to be investigated. The arginine isolated was split into ornithine and urea. All the isotope was found in urea moiety, while the ornithine contained normal nitrogen. The α-amino group of histidine was removed by converting the amino acid into imidazole lactic acid. The latter contained normal nitrogen; all the isotope must have been in the α-amino group of the original histidine.

The experiment shows that in a normal full-grown and healthy animal, kept on a normal diet, the nitrogen of at least one of the dietary amino acids, tyrosine, is only partly excreted in the urine, while the rest is retained in the protein of the animal, with a corresponding excretion of tissue nitrogen. Only a fraction of the nitrogen deposited remains attached to the original carbon chain of the amino acid, with which it was given, the bulk being utilized in the formation of some other amino acids. Degradation of some of the isolated amino acids has given some insight into the processes which must have been responsible for their formation:

(1) The dicarboxylic acids containing only one nitrogen atom were either newly synthesized from substances with different carbon chains or underwent deamination followed by amination of the remaining keto acid. Whichever of these two processes was responsible, its occurrence was not suppressed by the abundance of these substances in the dietary protein, casein.

(2) Arginine was formed from ornithine, probably in the course of urea formation, according to the theory of Krebs.

(3) Histidine was successively deaminated and reaminated at the α-carbon atom alone.

(4) Ornithine and lysine are apparently not subject to such processes.

All these reactions had occurred with constituents of the proteins of a normal animal and reveal an extensive chemical activity of its proteins.

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