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PROPOSAL FOR THE INVESTIGATION OF PROTEIN SYNTHESIS  
BY USE OF THE ISOTOPE N-15

at  
TULANE UNIVERSITY

The current proposal is to continue and enlarge primarily the physical aspects of an investigation in protein metabolism by the use of stable isotopes in the Biophysics Laboratory of Tulane University.

PRESENT STATUS OF THE PROBLEM

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In a cooperative venture with the Department of Medicine, Surgery and Physiology, of the Tulane University Medical School, a preliminary investigation into protein metabolism in man has been made. The physical procedure, as well as the fundamental interpretation of such results as were forthcoming, have been the responsibility of the Biophysics Laboratory. Human subjects, kept in nitrogen equilibrium, that is, with an equal input and output of nitrogen, were fed a single test meal containing an amino acid, glycine, labelled with an excess of N-15. The subsequent excretion of N-15 was then followed over a three- to five-day period. The measured dilution of the N-15 excreted was then compared with the N-15 dilution predicted on the following simple assumptions.

Let:  $N_1$  = amount of Nitrogen ingested

$N_e$  = amount of Nitrogen excreted

$N_x$  = amount of Nitrogen in the body exchangeable with  
ingested Nitrogen

$N_{15}$  = amount of ingested N-15

$N_{e15}$  = amount of N-15 excreted

$p$  = a factor which is a function of time and the measure of  
the proportion of previously ingested nitrogen which has  
entered the cycle characterized by the  $N_x$  to the total amount of nitrogen ingested over the period of observation.

Then one may write Equation 1:

$$N_{e15} = pN_{i15} \frac{N_{e15}}{pN_{i15} + N_x} + N_{i15} \frac{N_{e15}}{pN_{i15} + N_x}$$

and the term from the right hand side of the equation

$$\frac{pN_{e15}}{pN_{i15} + N_x} \text{ equals a dilution factor,}$$

or 
$$D_f = \frac{N_{e15}}{N_{i15}} = \frac{pN_{e15}}{pN_{i15} + N_x} \text{ Equation 2.}$$

The dilution factor may be obtained from an analysis of the samples of excreted nitrogen and the ingested nitrogen with the mass spectrometer. One may then solve Equation 2. for  $\frac{N_x}{p}$ .

$$\frac{N_x}{p} = \frac{N_{e15}}{D_f} - N_{i15} \text{ Equation 3.}$$

Thus, it has been possible to measure the quotient  $\frac{N_x}{p}$ .

$N_x$  may then be defined as the amount of body nitrogen into which a fraction "p" of the test glycine has been assimilated. The factor p is a function of time and represents the portion of the test meal that, within the time of observation, has entered into the metabolic process studied.

Assuming p to be constant, the  $N_x$  has been studied as a function of time and over the three days which it has so far been possible to study it, it increases logarithmically with time, that is:

$$N_x(t) = N_{x0} e^{+nt}$$

where  $n$  may now be defined as the assimilation coefficient for the metabolic processes investigated.

In patients with adrenal disturbances subject to endocrine therapy, significant variation in  $N_x$  and  $n$  have already been discovered.

PROPOSAL FOR CONTINUATION OF WORK

1. A modified experiment is to be performed for the elimination of the factor  $p$ . In the previous work, the metabolic nitrogen into which the test meal was assimilated was termed  $N_x$ . Actually measured was the factor  $\frac{N_x}{p}$  where  $p$  designated the fraction of test meal nitrogen that had entered the  $N_x$  "pool" in the time of observation. The factor  $p$  is therefore dependent on time in such a way that if there is no time difference in the isotopic ratio of the nitrogen taken in the diet,  $p$  becomes unity and may be eliminated.

To do this, requires that the subject be kept on a diet in which the N-14 --N-15 ratio is constant with a fairly high excess of N-15, about 10 atom-percent. Each experiment would require from three to five days for the subject to reach equilibrium. By such an experiment variation in  $p$  may be distinguished from variation in  $N_x$  with time and the true value of  $n$ , an assimilation coefficient, be more nearly determined.

2. The change of  $N_x$  with time over short periods of observation (three to five days) was found to be represented by:

$$N_x(t) = N_{x0} e^{-nt}$$

But obviously, the maximum value of  $N_x$  is something less than the total weight of Nitrogen in the subject. There must be a second term in the

left side of the above equation which would describe the dissimilation or breakdown of nitrogen. This could only be followed isotopically when enough tracer was introduced to go through the whole metabolic cycle without too great dilution. For this purpose, the continuous feeding experiment described above should be adequate.

3. It is desirable to measure  $N_x$  and n not only for glycine, but other amino acids as well. These may be obtained in the biologically active isomeric form by using yeast as a biological synthesizing medium in which the only source of nitrogen available to the yeast would be an ammonium salt enriched with N-15. Here the improved sensitivity which we expect from the new design of a mass spectrometer (described under facilities for investigation) is important.

4. The limit of the sensitivity of a technique involving the new spectrometer will be the natural variation and the relative abundance of the stable isotopes of nitrogen. Various figures for this variation are currently recorded. It has been our experience that at least a portion of this variation is due to the formation of methylamine in the digestion process. We should like, therefore, to undertake a more extensive investigation into the procedures for the digestion of protein in the preparation of nitrogen with the object of circumventing such procedures as may cause apparent variations in the N-15 concentration.

Since there is no hazard in the isotope procedure itself, we plan to use laboratory personnel as normal experimental subjects. We wish to continue these investigations in man, for quite apart from any fundamental information that we may gain from such studies, there are purely empirical

observations that may be of interest clinically. We should like to coordinate our work of a fundamental nature with clinical problems that will continue to be investigated in the metabolic unit at the Tulane University Medical School, and with problems of hormonal regulation of protein synthesis, which we can do in cooperation with the Ochsner Foundation Hospital.

It is realized that the simple assumptions and formulations made in the preceding equations are rough approximations of a static character. As observation progresses, we should like to formulate the proper differential equations to describe the metabolic processes under investigation.

#### EQUIPMENT AND FACILITIES AVAILABLE FOR CONTINUATION OF THIS INVESTIGATION

The Biophysics Laboratory has had in operation for approximately one year a Nier type mass spectrometer, specially designed for determining relative abundances of the stable isotopes. This spectrometer has been extremely satisfactory and has been in almost continuous operation; only about ten hours a month are lost from running time for maintenance. It is anticipated that half the available time on this spectrometer will be spent on the continuation of the purely clinical possibilities opened up by the investigation outlined above. The other half time would be available for the pursuit of more fundamental inquiries into the mechanism of protein synthesis, according to the leads provided by the pilot investigations described above.

In addition, a new and improved type of mass spectrometer, designed and in construction under contract with ONR, is nearing completion. It is anticipated that this instrument will be ready for use about July 1. The new spectrometer, of the shaped field type, designed to focus for divergence in all planes, should provide not only increased resolution but an

increased intensity of the ion beam. It has also been designed to have about twice the inherent stability of the present spectrometer.

The Biophysics Laboratory also has the usual facilities for the digestion of proteins and the liberation of nitrogen required for analyses with the mass spectrometer. In addition the Laboratory has an ultra-centrifuge and electrophoresis equipment useful for the separation of small amounts of proteins from complex mixtures. The Laboratory has quite complete facilities for investigations with radioactive material.

The Laboratory has its own shop facilities and mechanic, complete provisions for electric and electronic instrumentation and so forth.

#### PERSONNEL

Upon completion of the spectrometer (June 30, 1949) the personnel now engaged in the design and construction of it will be available for the work here proposed. This includes the Director of the Biophysics Laboratory, Dr. Robert Nisbet, about 1/3 time; Walter C. Byrne, Research Associate in Biophysics, 1/2 time; the full time of one biophysics technician whose principal training has been in physics. Assistance and consultation in purely biochemical aspects is available through the Department of Biochemistry of Tulane University Medical School.

In the endocrinological aspects of the metabolic problems to be attacked the advice and direction of Dr. William Parson, now of the Ochsner Clinic and Tulane Medical School with whom work is currently in progress, will be continued even though he goes to the Medical Department of the University of Virginia in June 1949. Plans for close coordination in the investigations of endocrine factors in nitrogen metabolism between his group and the Biophysics group at Tulane have been worked out.

REQUIREMENTS FOR THE INVESTIGATION

Personnel: 1 Research Associate (Physics) 1/2 time ....\$2250 a yr.  
 1 Technician (Physics) full time .....\$2500 a yr.  
 1 Physical Director, 1/3 time .....\$2500 a yr.

Materials: One year's supply of N-15 .....\$3000  
 Chemicals and chemistry lab. supplies .....\$1000  
 Mechanical and electronic instrumentation  
 and supplies .....\$1000  
 Travel, communication, publication .....\$ 500  
 Overhead, 20% of salary .....\$1450

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Total \$14,200