ture and secondarily by the double bond. Possible peroxide formation would seem to account for only a small portion of the cholesterol effect. 2. Active pneumolysin is inhibited promptly, while inactive (air-oxidized) lysin is affected slowly if at all by cholesterol. Likewise, active lysin is adsorbed by red cells, while the inactive form is not. That is, the state of oxidation of the lysin conditions its reactivity with the sterol and with its adsorption on red cells. 3. The free thiol grouping on the active lysin molecule remains free after treatment with excess cholesterol. The lytic activity, therefore, is associated with at least 2 functional groupings, one reversibly oxidizable, and the other more or less specific for a certain sterol grouping and configuration.

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Synthesis of Protein and Amino Acids in Mice with the Aid of Deuterium.

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Schoenheimer and Rittenberg[1] have proposed that if non-labile deuterium is found in the molecule of a fatty acid isolated from tissues of mice which were receiving heavy water, this finding is then indicative of the synthesis of the fatty acid in the animal body. In connection with the study of the synthesis of protein and amino acids in animals, we were interested to ascertain whether or not a similar criterion, as used by Schoenheimer and Rittenberg, is applicable to protein and amino acid synthesis. Barbour, *et al.*,[2] on the assumption that one hour after a single injection of D_2O into mice a maximum of exchangeable deuterium will be fixed in the tissue, conclude that inasmuch as "mice drinking 15% D_2O for 2 months have 3 times the concentration of deuterium (relative to body water deuterium) in the tissue as mice receiving a single injection of D_2O," the difference in the deuterium content of the tissue indicates the fixation of deuterium in tissue in stable form. It occurred to us that the isolation of tissue protein and of amino acids derived therefrom and the analysis of the isolated products from which all deuterium in labile position has been removed would constitute more direct evidence for the fixation of deuterium in the

1 Schoenheimer, R., and Rittenberg, D., *J. Biol. Chem.*, 1935, **111**, 163.

2 Smith, P. K., Trace, J., and Barbour, H. G., *J. Biol. Chem.*, 1936, **116**, 371.

protein in stable form than the one offered by Barbour.[2] Twenty adult and 20 growing mice were used in a preliminary study. The animals were kept, each group separately, in metabolism cages and 2% D_2O and food consisting of 70 parts of oats, 15 of yeast powder and 15 of "Klim" milk powder were allowed *ad libitum*. A record of food and water consumption of the young mice was taken daily. The young mice were weighed every 3 to 5 days, while the weight of the adult mice was taken at the beginning and at the end of the experiment. Beginning on the 3rd day of maintenance on the 2% D_2O, a mouse was withdrawn from the group of growing mice, killed by a blow, and the body fluid and protein isolated. This was done thereafter every 3-5 days, in order to ascertain the changes in the deuterium content of the body fluid and of the protein with growth. After 10 days, the group of adult mice was killed, body fluid removed by distillation *in vacuo* with the aid of dry ice, and the protein isolated in the same manner as in the case of growing animals. The method of Addis, *et al.*, was employed.[3] The protein thus isolated was then digested with HCl or H_2SO_4 in the usual manner and various amino acids were isolated and identified by analysis. The deuterium content of the body fluids, protein and the amino acids was estimated as follows: Samples of protein or of amino acids to be analyzed, after treatment to ensure complete removal of deuterium in labile positions by repeated precipitation from acid and alkali, were dried *in vacuo* and then burned in the regular manner according to Pregl. The combustion water was collected by condensation in a small glass tube packed in dry ice. To ensure complete removal of excess deuterium from the furnace, about 30 mg. of urea were burned in 2 portions and these flushings combined with water first obtained. This combustion procedure has been tested by analysis of known compounds and found quantitatively correct. The water so obtained was diluted by weighed addition of tap water to bring the total to about 60-70 mg. Then this water was heated in a sealed tube at 140°C. with solid NaOH and $KMnO_4$ for 18 hours, then distilled *in vacuo* repeatedly. The density of the purified water was determined with a small diver (weight, 2 mg.), regulated by temperature and sensitive to a temperature difference of 0.01°C. or 0.002% D_2O. Divers were made of quartz and were calibrated by reference to purified tap water. The diver was observed by means of a cathetometer, the containing vessel being immersed in a glass-walled thermostat which maintained constant temperature within 0.01°C.

[3] Addis, T., Poo, W. J., Lew, W., and Yuen, D. W., *J. Biol. Chem.*, 1936, **118**, 497.

TABLE I.
The Deuterium Content of Protein, Amino Acids and Body Fluid of Mice which received 2% D_2O.

Animal	Adult		Non-Adult			
Days on D_2O	0	10	3	6	9	14
Weight, gm.			9.0	11.3	13.1	15.1
Body fluid, *% D_2O		1.03	0.74	0.96	1.00	1.20
Protein, *% D_2O	0.002	0.100	0.105	0.118	0.179	0.200
l-cystine, *% D_2O		0.230				
Tyrosine, *% D_2O		0.290				

*% D_2O represents excess of D_2O with reference to tap water.

Some of the typical results are shown in Table I. The data seem to indicate that the protein of both the adult and growing mice receiving D_2O contains deuterium in a non-labile form. The isolation of deuterium-containing amino acids from the protein of adult mice further confirms this observation. The protein similarly isolated from control mice receiving distilled water and the same diet was found to contain deuterium in concentrations not exceeding those usually found in tap water. The deuterium content of the protein isolated from the growing mice ran parallel to the gain in weight of mice, suggesting synthesis of protein with growth. The isolation of the known amino acids from the protein of the growing mice which received D_2O is now in progress. We hope by further study of the manner in which deuterium could possibly enter the molecule of an amino acid to throw additional light on the rôle of amino acids in the protein synthesis from the standpoint of their indispensability in nutrition. Should the assumption that the presence of deuterium in an amino acid in non-labile form is an indication of the synthesis of this amino acid in the animal body be correct, our finding as regards l-cystine and tyrosine indicates that these amino acids can be synthesized by the adult mouse.