ing to 30 to 40 per cent. of the values given by titration procedures.

The results (Table I) show that within 2 hours an

TABLE I

RATS GIVEN 75 MG. GLYCINE (1.18 ATOM PER CENT. N^{15} EXCESS)

Liver	N^{15} excess atom per cent.	Dilution factor of labeled N	Dilution factor of labeled glycine
Cu glutathione	0.09	1:13	1:4.3
NPN	0.04	1:29	
Glycine (protein)	0.019	1:62	1:62
Intestine:			
Cu glutathione	0.038	1:31	1:10.3
NPN	0.014	1:84	
Glycine (protein)	0.017	1:70	1:70

appreciable part (7.6 per cent.) of the nitrogen of the liver GSH was derived from the administered glycine. By contrast the nitrogen of the protein glycine and of the NPN of the liver represented only 1.6 and 3.4 per cent. respectively. A similar relationship was found in the intestine. In view of the short period of the experiment and of the finding by Braunstein and Kritzmann,[4] Bach[5] and others that the speed of deamination of glycine is slow it is probable that all the labeled N is in the glycine portion of the GSH. If this assumption is correct the concentration of N^{15} in the glycine of the liver GSH would be 3×0.09 atom per cent. and the minimum rate of turnover of GSH would be 22.3 per cent. (intestine 9.6 per cent.) in two hours (Table I, column 4). By contrast in the same period only 1.6 per cent. (intestine 1.5 per cent.) of the protein-glycine of the liver was exchanged. This experiment establishes the fact that GSH is a very unstable and reactive compound in the living organism.

Rittenberg and Schoenheimer[6] administered benzoic acid and glycine labeled with N^{15} to rats. One third of the glycine excreted as hippuric acid during the following 24 hours came from the glycine administered. To obtain further information concerning the synthesis of hippuric acid in relation to the turnover of GSH, benzoic acid and glycine containing 1.98 per cent. of N^{15} excess were injected subcutaneously into rats. After five hours GSH was isolated from the liver. The GSH contained 0.151 atom per cent. N^{15} excess. Assuming again that it represents an uptake of labeled glycine by the GSH the minimum rate of turnover of the GSH from the liver would be 23 per cent.

Hippuric acid isolated from the urine collected during the five-hour period contained 1.08 per cent. N^{15} excess, indicating that 55 per cent. of the glycine of the hippuric acid was derived from administered glycine. Since the liver GSH glycine contained less N^{15} (0.453 atom per cent. excess) at the time it was measured than did the excreted hippuric acid (1.08 atom per cent. excess) the experiment offers no support for the hypothesis that GSH furnishes glycine for hippuric acid formation.

Further work is in progress to determine whether the rapid turnover of GSH is indicative of its role as an intermediary in the metabolism of proteins.

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SYNTHETIC ALPHA-TOCOPHEROL AND NUTRITIONAL MUSCULAR DYSTROPHY[1]

RECENTLY Goettsch and Ritzman[2] found that alpha-tocopherol prevented the development of muscular dystrophy in young rats when alpha-tocopherol was fed from the tenth to the twenty-fifth day after birth. Control rats under similar conditions but without the supplement of alpha-tocopherol showed symptoms of muscular dystrophy at the end of the above test period. Their own criticism of the results was that it was conceivable that the alpha-tocopherol employed might have contained impurities that were active in preventing the muscular disease. The alpha-tocopherol was a natural product isolated according to the method of Evans, Emerson and Emerson.[3]

Mackenzie and McCollum[4] later showed that the natural alpha-tocopherol was effective in curing muscular dystrophy of rabbits on the Goettsch and Pappenheimer diet no. 13 plus 10 per cent. of ether extracted wheat germ.

In the present work with muscular dystrophy of rabbits, the writer has used a diet consisting of ground U. S. No. 3 grade alfalfa hay ad libitum, and 20 grams of a grain mixture daily per rabbit. The grain mixture is made up of 40 parts of whole yellow corn meal, 30 parts of wheat bran, 20 parts of soybean meal and 10 parts of linseed meal. One part of sodium chloride is added to the mixture. Each animal received daily 1 cc of a good grade cod liver oil, either placed upon

[1] This research was supported by an appropriation from Bankhead-Jones funds (the Bankhead-Jones Act of June 29, 1935).

[2] M. Goettsch and J. Ritzman, *Jour. of Nutrition*, 17: 371, 1939.

[3] H. M. Evans, O. H. Emerson and G. A. Emerson, *Jour. of Biol. Chem.*, 113: 319, 1936.

[4] C. G. Mackenzie and E. V. McCollum, SCIENCE, 2312, 370, 1939.

[4] D. F. Braunstein and M. G. Kritzmann, *Biokhimiya*, 3: 590, 1938, C.A. 33, 2916, 1939.

[5] S. J. Bach, *Biochem. Jour.*, 33: 90, 1939.

[6] D. Rittenberg and R. Schoenheimer, *Jour. Biol. Chem.*, 127: 329, 1939.

the grain or by mouth. Muscular dystrophy can be produced equally well when petroleum ether extracted No. 1 alfalfa hay replaces the No. 3 alfalfa hay.

The muscular dystrophy producing property of this diet is dependent upon the cod liver oil. This is in agreement with the report of Madsen, McCay and Maynard[5] that cod liver oil added to a synthetic diet increased the rate of development and early severity of the muscular dystrophy. The water-soluble factor associated with nutritional muscular dystrophy as indicated by Morgulis, Wilder and Eppstein[6] is not lacking in this diet.

In this laboratory feeding of alpha-tocopherol,[3] prepared from wheat germ oil[7] has cured muscular dystrophy of rabbits, confirming the findings of Mackenzie and McCollum. Recently 300 milligrams of synthetic alpha-tocopherol[8] were obtained. Six rabbits suffering from muscular dystrophy but still able to walk with difficulty were given doses of this synthetic material. Experience would indicate that each of these rabbits, if left untreated, would within 24 hours have been unable to stand or consume food.

The individual doses of the synthetic alpha-tocopherol in milligrams were as follows: 17, 18, 20, 26, 51 and 65. All animals were cured except the one receiving the 17 milligram dose. A seventh animal which received 30 milligrams also died, but it was in a much more advanced stage of collapse at the time of treatment.

It is apparent that 20 milligrams is near the lower limit as a single curative dose. An animal is considered as cured when it loses its stiffness and begins to gain in weight within 48 hours and continues to gain for 10 days or more. Although no attempt is made to establish the minimum curative dose with such limited amounts of material, it is definitely shown that synthetic alpha-tocopherol will cure muscular dystrophy in rabbits as produced experimentally under the conditions stated. The 5 cures become more significant when one considers that from several hundred dystrophic animals we have never observed spontaneous cure of an untreated animal.

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[5] L. L. Madsen, C. M. McCay and L. A. Maynard, *Proc. Soc. Exp. Biol. and Med.*, 30: 1434, 1933.

[6] S. Morgulis, V. M. Wilder and S. H. Eppstein, *Jour. of Nutrition*, 16: 219, 1938.

[7] The wheat germ oil from which the tocopherol was prepared was kindly supplied by the Archer-Daniel-Midland Company, Minneapolis, Minn.

[8] The synthetic alpha-tocopherol was furnished by Merck and Company through the courtesy of Dr. J. M. Carlisle.

SCIENTIFIC APPARATUS AND LABORATORY METHODS

STUDENT APPARATUS FOR ANALYSIS OF RESPIRATORY GASES

THE gas burette to be described was devised especially for medical students for whose instruction analyses of expired air are required of just sufficient accuracy to illustrate the general principles of respiratory exchange and metabolism. The usual complicated apparatus (*e.g.*, the Haldane-Henderson) is, we believe, poorly adapted to this type of instruction, for several reasons: (1) too much time is required to acquire proficiency with the method; (2) due to the excessive cost per unit insufficient apparatus is available; (3) the size of the equipment makes storage a problem; and (4) the difficulty of cleaning the apparatus when it becomes fouled, as so frequently happens, imposes a severe strain on the time and patience of the instructor.

By the simple method, here reported, an inexperienced operator may determine the CO_2 and O_2 of expired air with an error not often exceeding 0.1 volume per cent., corresponding to an error of about 5 per cent. in the calculated value of the metabolic rate. The time required for an analysis (30 to 60 minutes) may seem excessive, but students can actually make more determinations, during the limited period available for the work in respiration, than they can with the ordinary methods. It is suggested, in view of the low cost of the equipment, that each unit consist of two burettes, mounted together for simultaneous determinations. In this way the average time of an analysis will compare favorably with that required by the Haldane-Henderson method.

APPARATUS

The burette[1] is of Pyrex glass, with replaceable stopcock of the type which requires little or no lubricant. The graduations, which extend from the cock to 10 cc by 0.05 cc intervals, should be calibrated to within 0.005 cc for total and partial volumes. The burette should be carefully cleaned to allow complete drainage of the reagents. The leveling bulb should contain approximately 35 cc, and it may be conveniently suspended by a cord wrapped once over a horizontal rod, and a large rubber stopper may be used as counterweight. A thermometer reading to 0.2° C. should be available to each group of students.

REAGENTS

(1) Solution of NaCl (U.S.P.) 23.5 per cent. by weight, having a specific gravity of 1.18, to which is

[1] A suitable burette may be obtained from the Scientific Apparatus Company, Bloomfield, N. J., or from the Fisher Scientific Company, Pittsburgh, Pa.