4. Rebouças, G., Isselbacher, K. J., *J. Clin. Invest.*, 1961, v40, 1355.
5. Lieber, C. S., Schmid, R., *ibid.*, 1961, v40, 394.
6. Quick, A. J., *Am. J. Clin. Path.*, 1940, v10, 222.
7. ———, *Am. J. Med. Sci.*, 1933, v185, 630.
8. de Vries, A., Alexander, B., *J. Clin. Invest.*, 1948, v27, 665.
9. Schachter, D., Taggart, J. V., *J. Biol. Chem.*, 1954, v208, 263.
10. Harper, H. A., *Review of Physiological Chemistry*, Lange Medical Publications, 9th Ed., Los Altos, Calif., 1963, pp184-186.

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Creatine Synthesis in Perfused Liver of Vitamin E-Deficient Rats.* (29398)

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An intensive creatinuria and a decrease in the amount of creatine in skeletal muscle are among the manifestations of metabolic disorder observed in vit. E deficiency(1,2). Results of previous experiments(3) have suggested that a lowered uptake of creatine by skeletal muscle without accompanying changes in rate of creatine synthesis by the liver is responsible for the disturbance in creatine metabolism in vit. E-deficient rats. The view that creatine synthesis is unchanged in vit. E-deficient animals is supported by the observations of van Pilsum *et al*(4) on the changes in excretion and body content of creatine in vit. E-deficient rabbits. However, others(2,5,6) have suggested that creatinuria in vit. E deficiency results from an increased synthesis of creatine by the liver and an increased release of creatine by skeletal muscle. The present investigation tests the hypothesis that creatine synthesis is not altered in vit. E deficiency by comparing the rate of creatine synthesis in isolated, perfused livers of rats fed a vit. E-depleted diet with that of rats fed a normal stock diet and, also, of rats fed a vit. E-depleted diet supplemented with α-tocopheryl acetate.

Methods. Six male rats of the Wistar strain (obtained from Carworth Co.) after weaning were placed on a diet deficient in vit. E(7) for a period of 4 to 14 months. At the time of hepatectomy, the deficient rats had developed intensive creatinuria and creatinemia. Three rats of the same strain maintained on the same high fat diet for 16-35 months but supplemented with vit. E (50 mg α-tocopheryl acetate/week) served as controls. Observations made on male rats used in another study(10) which were fed a stock Purina fox chow diet, are included in this study as a second control.

The liver was removed from each rat and perfused with whole heparinized rat blood diluted with one-third of its volume of Ringer's solution containing 250 mg of glucose and 4 μc (0.185 mg) of glycocyamine-2-C^{14} (purchased from New England Nuclear Corp.). Blood donors were normal adult Sprague-Dawley rats, fasted 18 to 20 hours before bleeding. Details of the perfusion technique and apparatus have been described(8, 9). During the first hour of the experiment (initial phase), the synthesis of creatine was determined at glycocyamine concentrations approximating the levels ordinarily found in the blood. After one hour, 20 mg of nonradioactive glycocyamine and 35 mg of L-methionine were added to the perfusate and the perfusion continued for 5 hours (final phase of experiment). Thus, in the latter phase, synthesis of creatine was determined at high precursor concentrations normally not found in the blood. At regular intervals

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TABLE I. Creatine Synthesis in Perfused Rat Liver.

Treatment	Duration of diet (mo)	Liver wt (g)	Body wt (g)	Rate of creatine synthesis (μg/hr) computed by: Change of its specific activity: Initial phase	Change of its specific activity: Final phase	Increase in perfusate of creatine: Final phase	Decrease in perfusate of glycocyamine: Final phase
Stock diet		5.85	221	750	820		
" "		10.8	281	730	625	830	610
" "		5.21	222	960	630	430	355
" "		13.1	416	1200	770	810	565
Vit. E deficient diet	16	11.09	562	1150	1310	890	930
supplemented with	16	8.02	417	970	1140	1105	940
α-tocopheryl acetate	35	8.08	419	900	920	825	945
Vit. E deficient	9	9.67	219	800	490	570	760
" "	10	13.3	254	950	520	400	560
" "	11	7.16	186	780	640	540	550
" "	14	11.3	261	960	590	400	920
" "	4	8.90	211	705	490	550	425
" "	5	6.34	166	680	480	715	490

throughout the experiment, 2- to 3-ml samples of blood were withdrawn from the perfusate and concentrations and specific activities of creatine and glycocyamine determined (10).

The rate of synthesis of creatine during the initial phase of experiment was estimated from the increase in specific activity of creatine in the perfusate. In contrast to our previous studies(10), calculations of rate of synthesis of creatine from the increase in concentration of creatine in the perfusate have been omitted. This was necessary since, in several experiments, large errors were encountered in determination of small differences in creatine concentration of the perfusate as a function of time. During the final phase of the experiment, creatine synthesis was estimated from the increase in concentration of creatine, the decrease in concentration of glycocyamine, and the decrease in specific activity of creatine. Details of experimental procedure and methods of calculation of results have been described(10).

Results and discussion. Data on the synthesis of creatine in the isolated, perfused livers of rats fed a vit. E-depleted diet, a vit. E-depleted diet supplemented with α-tocopheryl acetate and a standard stock diet are presented in detail in Tables I and II. Table II presents results of calculations based on the data of Table I which have been made to interpret the data as broadly as possible.

As shown in Table II, the rats fed a vit. E-depleted diet supplemented with α-tocopheryl acetate exhibited a somewhat higher rate of synthesis of creatine during the final phase when compared to the vit. E-deficient animals if the data are expressed on the basis of total liver or per gram liver weight. However, if comparison is made on the basis of body weight, there is no statistically significant difference in creatine synthesis (final phase) among the 3 groups of rats. If one chooses to emphasize creatine synthesis computed on the basis of body weight, the apparently lower synthesis (initial phase) for the vit. E-deficient group supplemented with α-tocopheryl acetate must be interpreted in the light of the obvious gross increase in body fat which the animals on this diet accumulate. The apparent creatine synthesis per 100 g body weight (initial phase) in the vit. E-deficient rats would be less than that found for rats fed a stock diet if the former accumulated as much body fat as the latter.

In any case, or on any basis (total liver, per gram liver, or per 100 g body weight), the limited data presented here will not support the view that there is enhanced hepatic creatine biosynthesis in vit. E-deficiency. Accordingly, these findings may be taken to support the hypothesis that the creatinuria

TABLE II. Lack of Effect of Vitamin E-Depletion on Creatine Synthesis in the Isolated, Perfused Rat Liver.

Treatment	Body wt (g)	Liver wt (g)	Rate of creatine synthesis* computed on the basis of: μg/hr		μg/hr/100 g body wt		μg/hr/g liver wt	
			Initial phase	Final phase	Initial phase	Final phase	Initial phase	Final phase
Stock diet (4)†	285 ± 46‡	8.7 ± 1.9	910 ±110	674 ± 73	330 ± 38	260 ± 52	118 ±25	87 ± 18
Vit. E-deficient diet supplemented with α-tocopheryl acetate (3)	466 ± 47	9.1 ± 1.0	1007 ± 75	1001 ± 52	217 ± 8	218 ± 20	112 ± 5	112 ± 11
Vit. E-deficient diet (6)	216 ± 15	9.4 ± 1.0	812 ± 46	561 ± 25	378 ± 13	266 ± 22	89 ± 6	63 ± 8

* For each treatment group the mean and S.E. for creatine synthesis (initial phase) was calculated from the individual values listed in Table I. The mean and S.E. for creatine synthesis (final phase) was calculated by pooling all individual values listed in Table I which were computed by increase in perfusate creatine, by change of creatine specific activity and by decrease in perfusate glycocyamine.

† Numbers in parentheses represent No. of animals in group.

‡ $\text{S.E.} = \sqrt{\frac{\Sigma(\Delta x)^2}{n(n-1)}}$.

observed in vit. E-deficiency is not referable to an increase in hepatic synthesis of creatine.

An imbalance between the synthesis of creatine in the liver and rate of utilization of creatine by skeletal muscle, which represents the largest fraction of the body pool of creatine, very likely exists in the vit. E-deficient rats. This imbalance probably results from an increased release of creatine by muscle(11) or from a decrease in utilization of creatine by skeletal muscle rather than from increased synthesis of creatine by the liver.

It is of interest that a mechanism similar to that proposed by Gerber *et al*(3), namely, that the creatinuria observed in vit. E-deficiency is referable to a decrease in utilization of creatine by skeletal muscle, has been suggested by Roche *et al*(12) as the cause of creatinuria in progressive muscular dystrophy of man.

Summary. The synthesis of creatine from glycocyamine was studied in the perfused livers of rats fed a stock diet, a diet deficient in vit. E and a diet deficient in vit. E but supplemented with α-tocopheryl acetate. No significant differences were found when creatine synthesis was compared in the 3 groups of animals. It is postulated that the inability of skeletal muscle to utilize creatine, rather than increased synthesis of creatine by the liver, is primarily responsible for the creatinuria observed in vit. E deficiency.

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1. Morgulis, S., Spencer, H. C., *J. Nutrition*, 1936, v12, 191.

2. Dinning, J. S., *ibid.*, 1955, v55, 209.

3. Gerber, G. B., Gerber, G., Koszalka, T. R., Emmel, V. M., *Am. J. Physiol.*, 1962, v202, 453.

4. van Pilsum, J. F., Wahman, R. E., *J. Biol. Chem.*, 1960, v235, 2092.

5. Dinning, J. S., Fitch, C. D., PROC. SOC. EXP. BIOL. AND MED., 1958, v97, 109.

6. Heinrich, M. R., Matill, H. A., *J. Biol. Chem.*, 1949, v178, 911.

7. Emmel, V. M., *J. Nutrition*, 1957, v61, 51.

8. Miller, L. L., Bly, C. G., Watson, M. L., Bale,

W. F., *J. Exp. Med.*, 1951, v94, 431.

9. Green, M., Miller, L. L., *J. Biol. Chem.*, 1960, v235, 3202.

10. Gerber, G. B., Gerber, G., Koszalka, T. R., Miller, L. L., *ibid.*, 1962, v237, 2246.

11. Fitch, C. D., Coker, R., Dinning, J. S., *Am. J. Physiol.*, 1960, v198, 1232.

12. Roche, M., Benedict, J. D., Yu, T. F., Bien, J., Stetten, D., *Metabolism*, 1952, v1, 13.

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Hormone Synthesis by Normal Human Thyroid Cells in Tissue Culture.* (29399)

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The process of thyroid hormone synthesis is presumed to proceed via the concentration of iodide within the thyroid gland and its conversion into a form suitable for substitution onto either tyrosine or tyrosyl residues in peptide linkage to form monoiodotyrosine (MIT) and diiodotyrosine (DIT). Subsequently, these iodotyrosines couple to form the metabolically active thyroid hormones, thyroxine (T_4) and triiodothyronine (T_3). Which of these steps in the biosynthesis of thyroid hormone occur in the cell and which occur in the colloid is not known. We have previously shown that isolated normal human thyroid cells incubated in tissue culture are able to concentrate iodide and to incorporate the iodine into organically bound iodine(1). The location of iodination in the thyroid gland remains poorly defined. Results of studies utilizing I^{-131} and histologic radioautographs have been at variance. Several investigators have observed that the earliest localization of protein-bound radioactivity on radioautographs is in the apical portions of the epithelial cells adjacent to the follicular colloid in rats(2-4). Other studies have shown that some iodination of protein occurs within the cells, as evidenced by the appearance of cell particulate protein-bound iodine-^{131} (PBI^{-131}) in tissue slices(5-7). Wollman and Wodinsky(8), however, found by radioautography that PBI^{-131} occurred only in the colloid and not in the cells of mice. Meyer(9) observed by autoradiographic study of human thyroid tissue that the radioactivity was localized chiefly to the colloid. It is therefore not clear whether iodinated protein is normally produced in the cells and secreted rapidly as such into the colloid or whether it is produced in the colloid at the cell surface.

The present study was initiated to determine whether isolated normal human thyroid cells are capable of synthesizing iodotyrosines and iodothyronines.

Materials and methods. Normal thyroid tissue was obtained from radical neck dissections for cancer of the head and neck. Other normal thyroid tissue was obtained from lobectomies for solitary nodules of the thyroid gland. All patients (age 40-66 years) from whom thyroid tissue was obtained were females except for thyroid No. 20, which was obtained from a 40-year-old male. All tissue was examined histologically for verification of the presence of normal thyroid tissue. None of the tissues contained I^{-131} prior to these studies as determined by counting in a well-type scintillation counter.

The method for tissue culture of isolated thyroid cells was that of Irvine(10), with modifications as previously described(11). Further changes in our procedure will be described briefly. Thyroid tissue was obtained at surgery and immediately transported in cold sterile Delbeccos saline solution containing potassium penicillin G (100 units/ml) and streptomycin sulfate (100 μg/ml). Cells were obtained by trypsiniza-

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