and/or biotin may be closely related to cystine and methionine in normal hair formation.

Another type of hair loss, referred to as the "spectacle eye" condition, has been associated with a biotin deficiency by Neilsen and Elvehjem[10] and to an inositol deficiency by Pavcek and Baum.[11] It seems possible that these two factors may be closely related.

The commercial soybean oil meal rations supported better growth than did the raw soybean oil meal rations. Cystine and methionine supplements stimulated growth especially when added to the raw soybean oil meal rations. These findings support the observations reported by earlier investigators.[12,13] Biotin supplements had a slight stimulatory effect on growth when added to the raw soybean oil meal basal ration. Cunha[14] reported earlier that biotin supplements stimulated growth in rats fed a diet consisting largely of corn, soybean oil meal and alfalfa.

Further investigations now in progress suggest that the situation may become more complicated under certain conditions. While low levels (1-4 μg) of biotin have been shown to prevent the loss of hair, higher levels (12 μg) of this vitamin may actually accentuate the condition. The hair loss resulting from feeding such a high level of biotin was prevented by supplementation with adequate inositol.

Summary. Rats fed certain soybean oil meal rations developed a characteristic hair loss which was prevented by supplementation of inositol and/or biotin. The condition did not develop if the rations contained added cystine or methionine. Inositol supplementation resulted in no marked changes in rate of growth. However, supplements of cystine and methionine markedly increased the rate of growth when added to the raw soybean oil meal basal ration. Biotin supplements had a slight stimulatory effect on growth when added to the same ration. It is believed that soybean oil meal in some manner increases the dietary requirement for biotin and/or inositol. Possible mechanisms for such action may be an alteration of the intestinal flora, some absorptive disturbance, a vitamin imbalance or the presence of antivitamins in the soybean oil meal.

We are indebted to Merck and Co., Rahway, N.J., for synthetic vitamins; and to Abbott Laboratories, North Chicago, Ill., for halibut liver oil.

10 Nielsen, E., and Elvehjem, C. A., PROC. SOC. EXP. BIOL. AND MED., 1941, **48**, 349.

11 Pavcek, P. L., and Baum, H. M., *Science*, 1941, **93**, 502.

12 Hayward, J. W., Steenbock, H., and Bohstedt, G., *J. Nutrition*, 1936, **11**, 219.

13 Hayward, J. W., and Hafner, F. H., *Poultry Sci.*, 1941, **20**, 139.

14 Cunha, T. J., Ph.D. Thesis, University of Wisconsin, 1944.

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Effect of Low Protein Diets upon Creatine Excretion of the Rat.

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In a previous study[1] qualitative tests upon the urine of rats after a period on a low protein and low choline intake suggested an increased excretion of creatine by these animals. Roberts and Eckstein[2] have shown that the creatine content of the gastrocnemius muscle of young rats is not lowered when a diet deficient in choline and methionine is fed for 3 weeks. A similar finding in chicks was reported by Almquist and coworkers[3] who found a deficiency in dietary choline did not appreciably diminish the muscle creatine.

1 Tidwell, H. C., and Treadwell, C. R., *J. Biol. Chem.*, 1946, **162**, 155.

2 Roberts, E., and Eckstein, H. C., *J. Biol. Chem.*, 1944, **154**, 377.

3 Almquist, H. J., Kratzer, F. H., and Mecchi, E., *J. Biol. Chem.*, 1943, **148**, 17.

TABLE I.
Weight changes and Creatine and Creatinine Excretion of Rats on 5% (Diet 1) and 25% (Diet 2) Protein Diets.

Diet No.		Body wt in grams and % of change. Control	4th da.	8th da.	12th da.	16th da.	20th da.
1	(g)	236	231	226	224	225	226
	(%)	—0.0	—2.1	—4.2	—5.1	—4.7	—4.2
2	(g)	232	239	245	255	260	266
	(%)	+0.0	+3.0	+5.6	+9.9	+12.1	+14.7
		Creatinine excreted—mg/100 g rat.*					
1		3.69 ±.10	3.82 ±.10	3.50 ±.16	3.53 ±.12	3.71 ±.08	3.73 ±.06
2		3.48 ±.12	3.45 ±.10	3.48 ±.10	3.49 ±.08	3.77 ±.08	3.66 ±.09
		Creatine excreted—mg/100 g rat.*					
1		0.61 ±.15	0.40 ±.05	1.18 ±.18	1.32 ±.48	1.19 ±.32	0.62 ±.09
2		0.56 ±.12	0.50 ±.06	0.51 ±.13	0.71 ±.14	0.59 ±.14	0.57 ±.04

* Including the standard error of the mean calculated as follows:

$$\sqrt{\Sigma d^2 / n - 1} / \sqrt{n}$$

These observations along with those that indicate that the physiologically labile methyl groups are preferentially used for growth rather than lipotropism[4] suggest no sparing of these groups as regards the synthesis of creatine.

This study was designed to obtain more information regarding total creatine formation in these rats as measured by its excretion rather than the creatine content of an isolated tissue. Such information would indicate whether the labile methyl groups are conserved in the body when there is an inadequate supply for lipotropic action, and whether the needs for creatine formation take precedence over the needs for lipotropism.

Methods. After 3 weeks on the stock diet (Rockland rat diet), 20 male white rats were divided into 2 equal groups, each averaging about 235 g. They were maintained for 21 days on the special diets containing 5 or 25% casein, 40% Crisco, 5% salt mixture,[5] 2% Cellu flour, and sufficient starch to complete the diet. In addition each rat received one yeast tablet (400 mg) and 2 drops of cod liver oil daily. This diet has been used repeatedly in this laboratory for the production of fatty livers. It seemed possible that the methyl groups required for the synthesis of creatine and choline might be conserved during the acute need for lipotropic substances. If so, this should be reflected in the creatine and creatinine excretion.

The urine was collected under light mineral oil over 24-hour periods while on the stock diet and every 4th day through the 21-day period on the special diets. Intraperitoneal injections of 5 cc of 0.9% saline were given each rat twice daily on the days the urine was collected so as to obtain more suitable urine volumes. The creatine and the creatinine excreted were determined according to the method of Folin[6] with color intensities measured photoelectrically.

Results. More creatinine was excreted per 100 g body weight on the 4th day by the animals receiving the low protein diet and thereafter the amount excreted remained the same during the test period for the animals

[4] a. Treadwell, C. R., Groothuis, M., and Eckstein, H. C., *J. Biol. Chem.*, 1941, **142**, 653; b. Treadwell, C. R., Tidwell, H. C., and Gast, J. H., *J. Biol. Chem.*, 1944, **156**, 237.

[5] Osborne, T. B., and Mendel, L. B., *J. Biol. Chem.*, 1919, **37**, 572.

[6] Folin, O., *J. Biol. Chem.*, 1914, **17**, 469.

on both diets (Table I). A significantly greater* amount of creatine was excreted by the animals on the diets deficient in methionine and choline on the 8th and possibly the 12th days only. This increased excretion of creatinine at the start, and of the creatine later, appeared to be associated with the time when the animals were losing weight. Generally an increased creatine excretion was associated with a weight loss in the individual animals whenever it occurred during the experimental period. The differences in creatine and creatinine excreted by the 2 groups were not significant during the latter part of the period when the animals on the low protein diet maintained their weight or gained slightly.

The excretion of similar or greater amounts of creatine or creatinine by the animals on the diet deficient in methionine and choline for lipotropism, gives no indication of a deficiency of methyl groups for creatine formation. This again suggests that there is a preferential use of labile methyl groups for creatine formation associated with the demand for growth. Apparently there is no conservation of the deficient supply of methyl groups during the time fatty livers are developing in these animals, but actually a wastage due to the increased creatine or creatinine excreted when the fed animals are losing weight. The general constancy of the creatinine excretion is in line with the finding of Borsook and Dubnoff[7] that under physiological conditions the phosphocreatine spontaneously yields 2% of free creatinine and that its excretion does not characterize any active process in the tissues. The increased creatinine excretion during the early part of the test period was probably due to a readjustment of the body fluids to the new dietary conditions. The loss of weight involving muscle tissue would explain the increased creatinuria.

Summary. No diminution in the formation of creatine, as measured by its urinary excretion, was found in animals on a diet deficient in labile methyl groups. The available methionine was preferentially used for growth and creatine formation. The increased excretion of creatine by these animals, apparently associated with weight loss, causes a waste of needed methyl groups instead of their conservation.

Grateful acknowledgment is made to Dr. C. R. Treadwell for his generous advice.

* Apparent differences were analyzed for significance by the *t* method of Fisher, R. A., *Statistical Methods for Research Workers*, 7th edition, Edinburgh, 1938. Only those showing a *P* value of 0.01 or less were considered significant.

7 Borsook, H., and Dubnoff, J. W., *Annual Review of Biochemistry*, 1943, **12**, 187.

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Urinary Secretion of Acetone Bodies in Diabetic Ketosis.

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It is generally recognized[1] that the urine from a patient with diabetic coma, occasionally, may give a negative test for diacetic acid with ferric chloride, and only a mild reaction for acetone bodies with nitroprusside. The studies of Widmark,[2] and Briggs and Shaffer[3] indicated that acetone passes into the urine and expired air by the simple process of diffusion. If this is true, then the concentrations of this fraction of the acetone bodies in the blood and urine should always

1 Joslin, E. P., Root, H. W., White, Priscilla, and Marble, A., *The Treatment of Diabetes Mellitus*, seventh ed., Lea and Febiger Co., Philadelphia, 1940.

2 Widmark, E. M. P., *Biochem. J.*, 1920, **14**, 364.

3 Briggs, A. P., and Shaffer, P. A., *J. Biol. Chem.*, 1921, **48**, 413.