was the same as that observed previously with crystalline vit. B_{12} administered in the same dosage. There was a prompt diminution in urinary phosphate excretion which occurred before any change in reticulocyte count. This was more marked in A.B. and was accompanied by a drop in serum phosphorus and serum potassium. Following the initial decrease there was a marked increase in urinary phosphorus parallel with the reticulocyte rise. Serum alkaline phosphatase in A.B. was 2.7 to 2.8 Bodansky units per 100 cc before and after treatment except for a value of 3.2 units on the day when serum and urine phosphorus were greatest. An increase in urinary uric acid-creatinine ratio accompanied reticulocytosis. Similar changes occurring after vit. B_{12} in pernicious anemia patients were interpreted by us as being due to increased utilization of phosphate accompanying a profound stimulation of nucleoprotein metabolism(8).

Summary. The thiocyanate analog of vit. B_{12} (thiocyanato-cobalamin) has been shown to be fully active hematologically in pernicious anemia. In two patients the early biochemical and hematologic responses were the same as observed with same dosages of vit. B_{12}. Further evaluation of the neurological response to this analog is necessary.

Received May 24, 1951. P.S.E.B.M., 1951, v77.

Ergothioneine Depletion in Rabbit Erythrocytes and Its Effect on Methemoglobin Formation and Reversion. (18799)

S. S. SPICER, J. G. WOOLEY, AND VIRGINIA KESSLER.

From the National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, Public Health Service, Federal Security Agency, Bethesda, Md.

Recent studies on methemoglobin (MHb) formation and reduction in nitrite-treated rabbit erythrocytes revealed an acceleration in both the appearance and reversion of MHb in red cells of animals raised on a purified diet(1). Liver paste added to this diet retarded the MHb formation rate but various crude supplements such as liver, yeast, wheat germ, and kale failed to alter the MHb reversion rate. Supplements of ergothioneine, glutathione, and vit. B_{12} have since been tested and it has been found that the ergothioneine retards MHb formation or reduction to a rate characteristic of corpuscles of animals raised on a stock diet. Moreover, the blood ergothioneine level varies inversely with the MHb rates, being low in rabbits on the purified diet and high in those fed the stock diet or treated with ergothioneine.

Procedure. Fifty male New Zealand strain albino rabbits weighing 1600-1800 g were placed on the purified diet designated as No. 672, while 12 animals were put on the stock diet composed of Purina rabbit pellets(1). Diet 672 contained sucrose, casein, cellophane, Wesson oil, O.M. salt mixture and vitamins as described previously. After 5 months the test rabbits were separated into 5 equal groups. Equal blood samples of the individuals in each group were pooled, and the rate of MHb formation was determined in the washed substrate-free erythrocytes following addition of a standard dose of $NaNO_2$. This test and the determination of the rate of reversion of MHb after addition of a standard $NaNO_2$ dose to corpuscles suspended in a medium containing lactate as substrate were carried out according to the procedure already outlined(1). Supplements of vit. B_{12}, ergothioneine and glutathione, were then given three groups (Table I). Three months later the MHb tests were repeated, and in addition the erythrocyte ergothioneine and glutathione content was measured using the most recent Hunter method for ergothioneine(2). In or-

1. Spicer, S. S., and Wooley, J. G., *Arch. Biochem.*, 1950, v27, 202.

TABLE I. Effect of Diet on Blood Ergothioneine and the Rate of MHb Formation and Reversion. E designates 6.7 mg % ergothioneine, G–SH 10 mg % glutathione, B_{12} 8.3 γ % vit. B_{12}, L 1% liver paste (Valentine's 1:20 concentrate). Flasks for MHb formation tests contained 2 ml washed corpuscles + 6 ml .85% NaCl + .15 ml 1% $NaNO_2$. Flasks for MHb reversion tests contained 2 ml washed cells + 2 ml M/4 sodium lactate + .3 ml 1% $NaNO_2$.

Duration of exp. (mo.)	Supplement to diet 672			MHb formation: g % MHb 7 min. after addition of $NaNO_2$			MHb reversion: time in hr for MHb to return to 3 g %			mg ergothioneine/ 100 ml of erythrocytes*	
	5	8†	10†	5	8	10	5	8	10	8	10
Purified diet	0	B_{12}	0	4.8	5.4	4.6	1.83	2.17	1.83	0	0
	0	E	0	5.2	1.8	1.7	1.83	2.67	2.58	23.1	14
	0	G–SH	B_{12}	4.7	5	5.2	1.75	1.75	1.50	0	0
	0	0	L	4.8	5.4	3.8	1.58	1.75	1.75	0	4
	0	0	E	—‡	4.7	3.5		1.83	2.25	0	9.6
Stock diet				.7	1.8	1	2.08	2.58	2.83	7	11

* A faint yellow color equivalent to 1-2 mg % ergothioneine was encountered in purified diet cell filtrates but only characteristic pink color was interpreted as representing ergothioneine.

† Duration indicated by 8 is from 5 mo. through 8 mo., that by 10 is 8 mo. through 10 mo.

‡ Not tested at this time.

der to check the findings, an altered supplemental regime was instituted for a final two months after which the MHb and ergothioneine determinations were repeated.

Results. At the end of the 5-months' induction period the rate of appearance and disappearance of MHb was found to be uniformly rapid for corpuscles of all the experimental groups as compared with the stock diet controls (Table I). After the various supplements to diet 672 had been consumed for 3 months the rate of formation and reversion of MHb became retarded in the cells of the group given ergothioneine, whereas no change occurred in the other groups. Ergothioneine determinations showed a very low or negligible amount in the washed erythrocytes of all the test groups except that which received the ergothioneine supplement. The cells of the latter had a higher level than those of stock diet controls.

The diets tested had no influence on the glutathione level, for the concentration of this substance was comparable in the washed cells of the several experimental groups and the controls. The animals were tested again 2 months after the supplements were altered. Some slowing of MHb formation and reversion was observed together with a corresponding increase in blood ergothioneine on adding this substance to the diet of a group previously unsupplemented. The group from which the ergothioneine supplement was withdrawn revealed little change either in the amount of the compound in the blood or the MHb tests. However, the level decreased to 4 mg % 4 months after the ergothioneine was withdrawn. Addition of liver extract to the diet of a previously untreated group caused the blood ergothioneine to increase. The anomalous results encountered in earlier studies with liver supplements recurred in this test—that is, a slowing of the rate of MHb formation without a corresponding change in the time required for MHb to disappear(1).

Certain incidental observations were made. Ergothioneine depletion was found to have no distinct influence on the hemoglobin concentration, hematocrit value, number of circulating erythrocytes, red cell fragility or oxygen dissociation curve. Following a standard dose of aniline, no difference in the rate of appearance of MHb was noted in the 6 groups of Table I. Washing erythrocytes decreased the ergothioneine content approximately 20% and storage at 4°C caused roughly a 50% decrease in a week.

Discussion. Dietary variation in the blood ergothioneine level of pigs and rats was reported by Eagles and Vars(3), and Potter and

2. Hunter, G., *Canadian J. Research*, 1949, v27 (E) 230.

3. Eagles, B. A., and Vars, H. M., *J. Biol. Chem.*, 1928, v80, 615.

Franke(4), respectively. However, Touster, using the bromine-labile sulfur method(5) as well as the Hunter method of ergothioneine assay, has failed to confirm the latter results. Hunter recently, unlike Eagles and Vars, did not find that maize meal contains an ergothioneine precursor(6). The definite dietary influence on blood ergothioneine reported here, while possibly a species characteristic, is quite likely at least in part also attributable to the fact that the animals were started on the experimental diet at weaning.

Absence of an ergothioneine protective action against oxidation of the hemoglobin could account for the occurrence of an unidentified hemoglobin derivative previously observed in erythrocytes of the purified diet rabbits(7). Likewise, ergothioneine differences may be implicated in the individual and age differences previously observed in MHb reversion(8).

Summary. 1. Erythrocytes of rabbits fed a purified diet are nearly or completely devoid of ergothioneine. 2. The amount of ergothioneine in the red cells of purified diet rabbits increases with a supplement of liver or ergothioneine. 3. MHb formation and reversion rates after $NaNO_2$ are related inversely to the ergothioneine blood levels. 4. The diets used do not influence the blood glutathione

The authors are indebted to Mr. Howard F. Brubach for valuable assistance in aspects of this work.

4. Potter, V. R., and Franke, K. W., *J. Nutrition,* 1935, v9, 1.

5. Touster, O., *J. Biol. Chem.,* 1951, v188, 371.

6. Hunter, G., *Biochem, J.,* 1951, v48, 265.

7. Spicer, S. S., Wooley, J. G., and Clark, A. M., *J. Biol. Chem.,* 1950, v182, 445.

8. Spicer, S. S., and Reynolds, H., *Am. J. Physiol.,* 1949, v159, 47.

Received May 26, 1951. P.S.E.B.M., 1951, v77.

Effect of Diet on Obesity of Yellow Mice in Inbred Lines.* (18800)

PAUL F. FENTON AND HERMAN B. CHASE.

From the Department of Biology, Brown University, Providence, R. I.

It has been well known for many years that obesity is associated with yellow coat color in mice. That continued inbreeding reduces the obesity is less widely known but has been the experience of several mouse geneticists who have developed inbred lines of this stock. In mice the gene (A^y) for yellow coat color is dominant over the other alleles (A^w, A, a^t, a) at this agouti locus, and the homozygote (A^y A^y) is lethal. Consequently yellow mice are always heterozygous for yellow and produce both yellow and non-yellow offspring. By continued inbreeding of a yellow stock (successive brother-sister matings with one or both parents yellow), complete homozygosity at all loci is approached except at the A locus. In this manner yellow and non-yellow littermates are obtained having the same genetic background and differing only in that the yellows have one A^y gene and the non-yellows have none. Danforth(1), by crossing yellows to certain unrelated lines, showed that it was possible to increase the expression of obesity in the F_1 hybrid yellows. This practice of obtaining F_1 obese yellows has been employed in other studies on obesity(2,3). In the experiments to be reported here, however, such hybrid yellows were not used.

Two inbred yellow sublines (one pink-eyed and the other not) have been developed by one of us (H.B.C.). In view of the failure of the yellows of these sublines (and certain ones

*This investigation was supported by grants from the American Cancer Society upon recommendation of the Committee on Growth of the National Research Council; The National Cancer Institute, U. S. Public Health Service; and the Anna Fuller Fund.

1. Danforth, C. H., *J. Hered.,* 1927, v18, 153.

2. Dickerson, E. G., and Gowen, J. W., *Records Gen. Soc. Am.,* 1946, v14, 44.

3. Dickie, M. M., and Woolley, G. W., *J. Hered.,* 19..., v37, 365.