those mentioned above; but their operation may not be ruled out. If, as Fisher and Pencharz[1] have found, a larger proportion of carbohydrate is oxidized by hypophysectomized rats following glucose feeding, this process also would be expected to decrease the amounts of carbohydrate stored in the liver under these conditions.

Although normal high carbohydrate levels in blood and muscle and moderately high liver glycogen values (maximum average 49% of normal) were obtained by feeding standard carbohydrate meals, these levels differed from the normal in that they were not maintained for any length of time, falling even while absorption was still continuing. The levels were followed for 36 hours after the initial feedings of one gm. of starch to normal and 1 and 2 gm. to hypophysectomized rats, determinations being made at 4, 8, 12, 24 and 36 hours, and 6-10 animals being used for each point on the several curves; the results amply confirm the conclusion previously drawn from work on fasted animals that hypophysectomized rats lose body carbohydrates at a much greater rate than do normal animals.

8589 C

Cataract- and Dermatitis-Producing Nutritional Factors.

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Cataract has been produced experimentally by parathyroidectomy, pancreatectomy, vitamin G-low diets,[1] lactose-rich[2] and galactose-containing[3] diets. In human subjects dinitrophenol has been reported as likewise cataract-producing. The difficulty of reconciling these diverse precipitating conditions is great, and in fact may not be possible. However, it is interesting to speculate upon the possible interrelation of the 2 obviously nutritional factors, vitamin G (B_2) and lactose.

The delay in, or interference with, complete absorption accompanying the presence of large amounts of lactose or galactose in the

[1] Fisher and Pencharz, PROC. SOC. EXP. BIOL. AND MED., 1936, **34**, 106.

[1] Day, P. L., and Langston, W. C., *J. Nut.*, 1934, **7**, 97.

[2] Mitchell, H. S., and Dodge, W. M., *J. Nut.*, 1935, **9**, 37.

[3] Mitchell, H. S., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 971; Yudkin, A. M., and Arnold, C. H., *ibid.*, 1935, **32**, 836.

intestine and the frequent gastro-intestinal absorption difficulties in clinical conditions associated with vitamin G deficiency, for example pellagra, pernicious anemia and sprue, offer a possible common avenue of attack upon the problem. The present study was planned to test the bearing of such absorption-disturbances upon the production of cataract, of dermatitis and of growth-abnormality.

The diets used are of the usual vitamin-testing type containing casein purified by repeated extraction with 60% alcohol and varying chiefly in the carbohydrate fraction and in the supplement. The formula of the diets follows:

	Cornstarch	Lactose
Casein	18	15
Salt Mixture	4	4
Butter Fat	8	
Crisco		9
Codliver Oil	2	2
Raw Cornstarch	68	
Lactose		70

As source of vitamin B a concentrated rice-polish extract (tikitiki) is fed in the amount of 0.06 gm. daily to each rat. This extract is not wholly lacking in the G factors but contains relatively small amounts of them. When fed to 5 rats during a preliminary test on cornstarch-containing diet at a level 10 times (0.6 gm.) that employed in this experiment, definite and in some cases marked dermatitis was developed although growth was promoted at the rate of 8 gm. per week, slightly better than that seen in a parallel series of animals receiving daily 0.075 gm. of a potent brewery-yeast. The latter animals, however, showed practically no dermatitis.

That the amount of vitamin B afforded by 0.06 gm. daily of this extract was sufficient for the animals in this experiment was shown by the 4-week failure in growth-improvement of 4 rats given additional and excessive doses of crystalline B_1 when they began to fail on the cornstarch-containing diet from lack of the G factors. The average weekly gain of this small group was 2 to 3 gm., the same as that of the larger group receiving no additional B.

Further to decrease the possible vitamin G-content of the carbohydrates used, the cornstarch and lactose were spread out in thin layers and exposed to bright diffused daylight and for part of the time to direct sunlight for 8 to 10 days. This was done because of the well established light lability of the flavins.[4]

Certain animals from each litter were given 0.5 gm. untreated

[4] György, P., *Biochem. J.*, 1935, **29**, 767.

Northwestern brewery-yeast instead of the tiki-tiki in addition to the basal diet. These groups served as presumably normal controls receiving adequate amounts of the B and G factors.

Comparison of 4 groups was thus possible, (1) those receiving the basal diet containing raw cornstarch and the yeast-supplement, (2) those receiving the same diet but with tiki-tiki instead of the yeast, (3) those receiving the basal diet containing lactose and the yeast-supplement, and (4) those receiving the lactose-diet with tiki-tiki instead of yeast. All animals were taken at weaning and the litters divided among the experimental groups. They were examined regularly twice a week for the appearance of cloudiness, rings or striæ in the eye lens and for scaliness, scabs, loss of hair, or other evidences of abnormality. Three different lots of animals were used, but the results were so similar that they are reported together in Table I. For convenience these groups are designated as

TABLE I.

Relation of Lactose and Vitamin G (B_2) to Cataract- and Dermatitis-Production in Rats.

Diet	Lactose G-low	Lactose G-rich	Cornstarch G-low	Cornstarch G-rich
No. of rats	10*	14	12	12
Duration of exp., wks.	12-15	12-15	12-15	12-15
Aver. weekly gain, gm.	8	10	3	17
Incidence of cataract, %	100	85	0	0
Time of appearance of cataract, days	21-35	60-84	—	—
Dermatitis	none	none	severe	none

*3 rats died after 5 weeks.

lactose-G-low, lactose-G-rich, cornstarch-G-low and cornstarch-G-rich.

The lactose-fed controls grew at about 60% the rate of the cornstarch-fed controls, a condition found previously by Koehler and Allen[5] in a comparison of regrowth of starved rats on lactose as compared with sucrose and glucose, and by Mitchell[6] on young rats. Occasional attacks of diarrhea were seen in all the lactose-fed rats, particularly during the first weeks of feeding. Usually these attacks lasted only a day or two. The feces were never of the refective type. Coprophagy has not been noted in any of the animals, all of which have been kept on wide mesh screens. The work of Guerrant, Dutcher, and Tomey[7] on the effect of lactose in

5 Koehler, A. E., and Allen, S. E., *J. Nut.*, 1934, **8**, 377.

6 Mitchell, H. S., *Am. J. Physiol.*, 1927, **79**, 542.

7 Guerrant, N. B., Dutcher, R. A., and Tomey, L. F., *J. Biol. Chem.*, 1935, **110**, 233.

diets deficient in the whole vitamin B complex (including G) indicated only a slight resistance to the progress of the deficiency when the feces of the rats on such a diet were fed to them. The overwhelming influence of the lack of vitamin B in that case undoubtedly obscured whatever effect might have been produced upon the G-deficiencies.

The vitamin G low-cornstarch rats grew very little and developed severe cutaneous lesions, alopecia, scabs, and incrustations about the eyes and ears. On the other hand, the vitamin-G-low lactose rats grew nearly as well as their controls and showed normal skin- and fur-condition at all times. There was an exception in the case of 3 rats of one group which grew poorly, had more or less continual diarrhea, and died after 5 weeks, although no dermatitis occurred.

Mature bilateral cataracts occurred in all of the lactose-G-low animals including those which died at 5 weeks. The time of occurrence of the cataracts varied from 21 to 35 days. In the yeast- and lactose-fed group, 85% of the rats developed cataract but after a longer time, 60 to 84 days. None of the cornstarch-fed animals have shown cataract.

Obviously the same deficiency cannot account for the dermatitis and the cataracts here reported. Whatever nutritional failure is caused by the lactose involves no dermatitis and indeed prevents it.

There is a possibility that the lactose prevents the absorption or allows the destruction in the intestine of the cataract-preventing factor. To test this, 4 rats fed the lactose-G-low diet were given a pure lacto-flavin preparation supplied by Dr. S. Lepkovsky. Two of these animals were fed 20 gamma of this preparation daily by mouth and 2 given the same amount by subcutaneous injection. All 4 of these animals developed cataract at about the same rate. The rate of increased body-growth has been different, however. The 2 rats fed with flavin have gained 85 gm. in 12 weeks while the 2 injected have gained 112 gm. in the same time. This would seem to indicate some intestinal loss. Since the rats were on the deficient diet for 2 weeks and may have had incipient cataract before the flavin-administration began, it cannot be said definitely that the flavin failed to prevent cataract.

Four rats fed the G-low cornstarch-diet were also fed or injected for 6 weeks with flavin with complete cure of all symptoms of dermatitis and with no differences in growth, the average gain being 12 gm. per week in both cases. Evidently no absorptive loss of flavin occurs on these diets. The dermatitis produced by this par-

ticular vitamin G-deficient diet is completely cured by lacto-flavin, as contrasted with the negative results of flavin-administration for the cure of so-called rat-pellagra by György,[8] Chick, Copping, and Edgar,[9] and others.

8590 C

X-Ray Diffraction Patterns from Reprecipitated Connective Tissue.

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For a number of years[1] it has been known that tendon gives excellent crystalline reflections. Recently[2] large spacings have been observed which provide strong evidence for the view that collagenous structures may be fibrous arrays of true crystals composed of very large molecules.

Most forms of tendon from adult animals cannot be put into solution without obvious chemical disintegration. The work of Nageotte and of others[3] has, however, demonstrated that optically active fibrous precipitates can be obtained from weakly acid solutions made from the tail tendon of adult rats and from many connective tissues of foetal animals. The optical appearance of these precipitates suggests that they are at least similar to the tissue from which they were dissolved. We have carried out a series of experiments to determine: (a) whether reprecipitated tendon is really crystalline and (b) whether its X-ray diffraction pattern is like that of the original tissue.

Reprecipitated connective tissue was obtained according to the procedures outlined by Nageotte. Pieces of freshly excised tail tendon from adult rats were placed in dilute (1 to 25,000) acetic

8 György, P., *Biochem. J.*, 1935, **29**, 741.

9 Chick, H. J., Copping, A. M., and Edgar, C. E., *Biochem. J.*, 1935, **29**, 722.

1 See for example, Katz, J., Die Röntgenspektrographie als Untersuchungsmethode, Berlin, 1934, p. 189.

2 Wyckoff, R. W. G., Corey, R. B., and Biscoe, J., *Science*, 1935, **82**, 175; Clark, G. L., Parker, E. A., Schaad, J. A., and Warren, W. J., *J. Am. Chem. Soc.*, 1935, **57**, 1509

3 Nageotte, J., and Guyon, L., *Compt. rend. Assoc. Anatomistes, Bruxelles*, 1934, 25-28 Mars.