Table 2. Components of diet purchased from General Biochemicals. In the control diet, 10 mg of thiamine was added to each 1000 g of the GBI diet.

Component	Amount
Sucrose	68%
Casein, vitamin-free (GBI)	18%
Vegetable oil (hydrogenated)	10%
Salt mixture U.S.P. XIV	4%
Vitamin supplement (g/100 lb)	
Vitamin A conc. (200,000)	4.500 g
Vitamin D conc. (400,000)	3.000 g
Alpha-tocopherol	10.215 g
Choline chloride	272.400 g
Niacin	27.240 g
Inocitol	13.620 g
2-Methyl-1, 4-naphthoquinone	0.1021 g
Pyridoxine HCl	0.9534 g
Riboflavin	0.9534 g
Calcium pantothenate	2.0430 g

aqueous solution of toluidine blue, and the other half was stained for lipids in a solution of sudan black B. A total of 95 biopsy specimens was studied.

To insure that the mice would possess hair follicles at the proper stage of hair growth throughout the course of the experiments, club hairs were plucked from the resting follicles. Plucking of club hairs initiated a new wave of hair growth that required approximately 19 days for completion (*2*) in the plucked area only. The mice were placed on the thiamine-deficient diet at 21 days after plucking. The hairs at this time had just completed their growth and entered the resting phase and would remain in the resting phase for about 1 month (*2*). After 7 or 14 days of the deficiency regimen, one half of the dorsum of each mouse was again plucked, and in this replucked area hair growth was again initiated. Thus, throughout the remainder of the experiment, one half of the dorsum of each mouse had growing follicles and the other half had resting follicles.

Mice that were fed the deficient diet tended to increase in weight throughout the first week and level off during the second. About the 14th day, a progressive loss of weight began, and death occurred between the 21st and 25th days. Concomitant with the decrease in weight in the third week, the hair coat lost its smoothness, and the hairs felt dry to the touch. Although the pair-fed control animals lost much weight, their hair coat remained smooth and oily.

Biopsy specimens of skin from both deficient and pair-fed control animals showed essentially a normal appearance 7 days after the initiation of the deficiency regimen.

Skin that possessed growing or resting follicles, removed from both groups of mice at 14 days, exhibited a general atrophy that is primarily the result of a decrease in the size of the panniculus adiposus. The epidermis and its appendages were not appreciably reduced in size, and there was abundant lipid within the sebaceous gland.

Between the 21st and 25th days of the deficiency, a marked atrophy of the skin was seen, irrespective of whether the skin had growing or resting follicles. The panniculus adiposus had disappeared, the epidermis was reduced to a single thin layer of cells covered by a thin film of keratin, and the hair follicles, both growing and resting, were decreased in size. The sebaceous glands had atrophied, occasionally leaving a thin shell of flattened cells comprising the peripheral, basal, and undifferentiated cells of the gland. Lipid could not be demonstrated in these remnants of the glands (Fig. 1). Occasionally, however, a plug of lipid was seen in the duct of the gland and hair canal, which probably represents sebum previously synthesized but not extruded.

The pair-fed controls also showed a loss of the panniculus adiposus and a general decrease in size of the epidermis and its appendages. However, the sebaceous glands, although reduced in size, were intact, and intracellular lipid was demonstrable (Fig. 2).

One possible explanation for the loss of the sebaceous glands is that they are holocrine glands. In order to synthesize sebum, they need a constant supply of energy and materials to be used for mitosis and for the synthesis of the sebum. It is now established that the energy for mitosis of the cells of the epidermis and its appendages is produced to a great extent by the activity of the Krebs citric acid cycle (*3*). Since thiamine is an integral component of the Krebs cycle and of steps in intermediary metabolism leading into the cycle, a deficiency of this key component could conceivably disrupt or impair the function of the energy-producing mechanism. The loss of available energy would make continued mitosis impossible, and therefore, without continued cell replacement, the sebaceous gland would atrophy.

Fig. 1. Sudan black B preparation of skin 24 days after the initiation of the deficiency regimen. No sebaceous glands are present. × 50.

Fig. 2. Pair-fed control 22 days after initiation of deficiency regimen. Black areas are portions of the sebaceous glands stained for lipids with sudan black B. × 50.

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Congenital Galactosemia, a Single Enzymatic Block in Galactose Metabolism

The pathway of galactose to glucose-1-phosphate includes the following steps (compare Leloir, *1*, and Munch-Petersen *et al.*, *2*): (i) phosphorylation of galactose; (ii) incorporation into nucleotide; (iii) inversion of the 4-hydroxyl group; and (iv) release of glucose-1-phosphate. The following scheme summarizes these consecutive reactions (3):

$$\text{Gal}^* + \text{ATP} \rightharpoonup \text{Gal}^*\text{-1-P} + \text{ADP} \quad (1)$$

$$\text{Gal}^*\text{-1-P} + \text{UDPG} \rightleftharpoons \text{G-1-P} + \text{UDPGal}^* \quad (2)$$

$$\text{UDPGal}^* \rightleftharpoons \text{UDPG}^* \quad (3)$$

$$\text{UDPG}^* + \text{PP} \rightleftharpoons \text{UTP} + \text{G}^*\text{-1-P} \quad (4)$$

The enzymes that catalyze these four reactions are galactokinase, PGal-uridyl transferase, Gal-waldenase, and PP-uridyl transferase, respectively. Because the galactose was labeled with C^{14} (the asterisks indicate the C^{14}-labeling of the hexose moiety), the equations illustrate how this sugar is finally brought into the general carbohydrate metabolism.

Schwartz *et al.* (*4*) have described that galactose administration to infants who are afflicted with congenital galactosemia brings about a marked accumulation of a galactose-l-phosphate in the erythrocytes.

Most recently it has been found (*5*, *6*) that hemolyzates from infants with congenital galactosemia are devoid of the enzyme in Eq. 2, PGal-uridyl transferase. However, it was not possible at that

time to state whether the step of Eq. 3, the inversion, was also absent because neither normal nor galactosemic hemolyzates showed any detectable amounts of galacto-waldenase. The problem whether galactosemia is a one-enzyme or a multi-enzyme defect, or whether a different pathway of galactose metabolism is operating as compared with normal individuals, could therefore not be sufficiently clarified at that time. Meanwhile, E. Maxwell, working in our laboratory, found that purified galacto-waldenase from calf's liver requires diphosphopyridine nucleotide (DPN) as a cofactor (*7*). This finding prompted us to add DPN to human hemolyzates in order to see whether galacto-waldenase might be present, but not yet detected because of the absence of its coenzyme (DPN) (*8*).

As shown in Table 1, it is indeed possible to demonstrate galacto-waldenase in hemolyzates from both normal and galactosemic subjects if DPN is also added. It appears that the lack of galacto-waldenase activity in crude hemolyzates is not due primarily to the destruction of DPN but mainly to a greatly increased requirement of galacto-waldenase for the coenzyme (*9*).

It can be seen from Table 1 that the activity of galacto-waldenase is of the same order of magnitude in the hemolyzates from galactosemic subjects as it is in those from normal.

Table 2 summarizes the average activities found for the various enzymes involved in the galactose metabolism of human hemolyzates. The galactokinase activity was measured according to a new sensitive and specific method that has not yet been published (*10*).

Table 1. Activity of galacto-waldenase in hemolyzates, with and without added DPN; 0.07 μmole of UDPGal are incubated with 0.5 ml hemolyzate corresponding to 0.25 ml of erythrocytes, 50 μl of 1*M* glycine at *p*H 8.7. Incubation time 15 min, at 37°C; DPN either omitted or added in amounts corresponding to 1 μmole. Total volume 0.6 ml. The activity is expressed as μmoles UDPG formed and UDPGal consumed (compare Kalckar *et al.*, *6*).

Condition of subjects	Enzyme activity (μmoles/ml hr)	
	No DPN	DPN
Normal (avg. of 3 cases)	0.002	0.32
Galactosemic (avg. of 3 cases)	0.002	0.35

Table 2. Activity of the four hemolyzate enzymes that catalyze the reactions of Eqs. 1, 2, 3, and 4 of the reaction scheme. Activity is given in micromoles of reactants converted per milliliter of lyzed erythrocytes, per hour.

Condition of subjects	Galacto-kinase		PGal-uridyl transferase		Gal-waldenase		PP-uridyl transferase	
	Sub-jects (No.)	Activity (Avg.)	Sub-jects (No.)	Activity (Avg.)	Sub-jects (No.)	Activity (Avg.)	Sub-jects (No.)	Activity (Avg.)
Normal	3	0.10	15*	0.82	3	0.32	9	1.20
Galactosemic	3	0.08	10	0.02	3	0.35	8	1.85

* This figure also includes infants that were on galactose-free diets (*5*, *6*).

The lack of PGal-uridyl transferase and the presence of galactokinase in the hemolyzates of blood from galactosemic subjects is in full accordance with the fact that galactose-1-phosphate accumulates in the erythrocytes of such patients if galactose (or milk) is administered (*4*). The presence of the freely reversible step of Eq. 3 in hemolyzates from galactosemic subjects would explain why normal development is possible (compare Mason and Turner (*11*) in these patients on galactose-free diets at an age when appreciable amounts of brain galactolipids are synthesized. These observations provide additional evidence for the fact that congenital galactosemia represents a block that is confined exclusively to a single enzyme, PGal-uridyl transferase. Genetic studies (*12*) indicate that the disease is presumably of hereditary origin and that it seems to be the result of a single recessive gene or of a more complex genetic pattern.

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3. The following abbreviations are used: Gal, Galactose; Gal-l-P, galactose-1-phosphate; G-1-P, glucose-1-phosphate; UDPG, uridine diphosphoglucose; UDPGal, uridine diphosphogalactose; ATP, adenosine triphosphate; ADP, adenosine diphosphate; UTP, uridine triphosphate; PP, pyrophosphate.
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8. Bodil Waage-Jensen rendered valuable technical assistance as a trainee under the American-Scandinavian Foundation through a grant-in-aid that was generously made available to one of us (H.M.K.) by the Eli Lilly Laboratories.
9. Unpublished experiments show that hemolyzates, even after heat inactivation, bring about a marked increase in the requirements of galacto-waldenase for DPN. This applies to the enzyme present in erythrocytes as well as to purified liver galacto-waldenase. If the norite eluate (nucleotide fraction) from a hemolyzate filtrate was tested with purified liver galacto-waldenase, it was found that by this new highly sensitive assay (*7*) sufficient amounts of DPN were present to bring about an almost optimal rate of reaction. By this assay method, the amount of DPN present in filtrates of hemolyzates is estimated to be 0.05 to 0.1 μmoles/ml in accordance with Leder and Handler [in *Phosphorus Metabolism*, W. D. McElroy and B. Glass, Eds. (Johns Hopkins Univ. Press, Baltimore, 1951), vol. 1, p. 421]. However, in order for the galacto-waldenase to be activated in the presence of crude hemolyzates, a large excess of DPN must be added. In the series of experiments cited in Table 1, a 250-fold excess over the amount of DPN that gives an effect on purified galacto-waldenase was used.
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Correction

In the article "Absorption and metabolism of iron" [*Science* 123, 87 (20 Jan. 1955)], the wavelength of maximum absorption of the iron-siderophilin complex was incorrectly given as 520 millimicrons. On page 89, the first sentence in the second paragraph under the heading "Iron transport" should read "The Fe^{+++}-B_1-globulin complex has a characteristic salmon-pink color with a maximum light absorption at 460 millimicrons. . . ."

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I am more and more convinced that our happiness or unhappiness depends far more on the way we meet the events of life than on the nature of those events themselves.—KARL VON HUMBOLDT.