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Galactose-Poisoning in Chicks.*

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During a study concerning the significance of carbohydrate sources in the production of symptoms of vitamin E deficiency young chicks were given a diet containing 54.6% of galactose. A few days after being transferred to such a diet the animals became severely ill, sat shivering or shaking and were seized by tetanic or by clonic spasms of leg and wing muscles, often accompanied by screaming. Although the initial attacks were usually followed by temporary recovery, several additional attacks during the next few days resulted in death. The symptoms were relatively infrequent when the animals were deprived of food for some time, but occurred frequently when feeding was resumed. The body weight declined during the galactose feeding. The brain of the affected animals showed no macroscopic changes. This disorder is unrelated to an insufficiency of vitamin E in the diet since it develops much earlier than the symptoms of this deficiency and can be prevented by replacing the galactose with any other carbohydrate. The following preliminary investigation of this galactose-poisoning in chicks, which the author for practical reasons is unable to carry further at the present time, is reported in the hopes that it may be of some interest to investigators in this and related fields.

Procedure. The diet was the following: alcohol-extracted casein 15 g, ether-extracted yeast 10 g, McCollum's salt mixture No. 185 7.2 g, $CuSO_4 \cdot 5H_2O$ 0.01 g, $MnSO_4 \cdot 4H_2O$ 0.02 g, galactose 54.6 g, gelatine 8 g, gum arabic 5 g, choline chloride 0.1 g, l-cystine 0.1 g, diacetate of 2-methyl-1,4-naphthohydroquinone 0.1 mg, cod liver oil 5 g. Another group of chicks received the same diet with the exception that sucrose was used instead of galactose.

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Glycogen in liver and muscles was determined by the method of Pflüger[2] modified in the following way: 1 g of the minced tissue was heated with 1 cc 60% KOH in a graduated centrifuge tube on a boiling water-bath 2 to 3 hours (covered to prevent drying), cooled and diluted with water to 4 cc. After centrifugation 3 cc of the supernatant liquid was taken out and added to 3 cc of 96% alcohol. After 15 minutes the precipitated glycogen was centrifuged down and washed twice with a mixture of 1 vol. 15% KOH and 2 vols. 96% alcohol. The precipitate was slowly dissolved in water and neutralized with conc. HCl. Water was added to make 10 cc. After heating on a boiling water-bath 3 hours the contents of the tube were cooled to room temperature and the volume readjusted to 10 cc. This solution which represents 0.75 g of tissue was used for the glucose determination after Benedict. The amount of solution taken out for the sugar determination varied from 0.5 to 2 cc according to the amount of glycogen in the organ. The sample was neutralized before the addition of the copper reagent.

Calcium was determined in the blood plasma of two birds after Clark and Collip's method.[3]

The results of the chick experiment are presented in Table I. In order to compare the galactose-disease in chicks with the corresponding disease in rats described by other authors,[4] 5 newly weaned rats were given the same galactose diet as the chicks. The results are presented in Table II.

Discussion. The brief experiments indicate

1 Somogyi, M., *J. Biol. Chem.*, 1928, **78**, 117.

2 Pflüger, E., *Pflügers Arch.*, 1903, **96**, 98.

3 Clark, E. P., and Collip, J. B., *J. Biol. Chem.*, 1925, **63**, 461.

4 Mitchell, H. S., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 971.

TABLE I.
Blood and Tissue Analyses of Chicks Fed Diets Containing 54.6% Galactose and 54.6% Sucrose, Respectively (Non-fasting Values).

Chick No.	Age in days: Feeding began	Symptoms first observed	At death	Killed for tissue analysis	Total blood sugar, mg%	Blood galactose, mg%	Liver glycogen, mg%	Muscle glycogen, mg%	Plasma Ca, mg%
Galactose diet:									
2341	7	10	10						
2342	7	10		11				**109**	
2343	7	8		9	502	280	43		
2344	7	8		9	624	400	6		
2345	7	10		11				350	
2346	7	9		11	550	328	9		
2347	7	10		11				468	
2348	7	12	12						
2349	7	9	10						
2350	7	8	11						
2405	28	32		32					10.6
Sucrose diet:									
2353	7			**9**	252	0	420		
2355	7			11				420	
2356	7			11				312	
2357	7			11	200	0	401		
2359	7			9	208	0	464		
2360	7			11				447	
2402	28			32					10.6

TABLE II.
Blood Sugar and Liver Glycogen in Rats Reared from Weaning on Diet Containing 54.6% Galactose (Non-fasting Values).

Rat No.	Days exper. before cataract appeared	Blood sugar at the 34th day of exper.: Total mg%	Blood sugar at the 34th day of exper.: Galactose, mg%	Liver-glycogen at the 35th day of exper., mg%
71	18	254	60	1720
72	16	273	123	1470
73	12	230	53	2000
74	12	206	59	1680
75	16	370	73	1700

that the spasms observed in galactose-fed chicks are not related to low blood-sugar or to a deficiency of muscle-glycogen. Although the exceedingly low liver-glycogen and high blood-galactose appears to be an essential feature, there is as yet insufficient evidence to indicate how this is related to the symptomatology observed. It is possible that the high blood-galactose in some way or other causes a dysfunction of the central nervous system. Changes in the total oxalate-precipitable calcium in the plasma seem not to occur.

In rats the galactose-feeding did not result in low liver-glycogen and the blood-galactose was not as high as in the chicks. This indicates that rats are able to utilize galactose better than chicks. The rats did not show clinical symptoms resembling those of the chicks, but cataract developed as previously described by Mitchell[4] and the growth was subnormal, the average weight gain in 5 weeks being about 20 g.

The failure of cataract to appear in the chicks may be due to the acute and much more severe character of the disorder in this species resulting in early death before sufficient time had elapsed to induce the eye lesions. In another series of experiments 10% of galactose was added to the sucrose diet without giving rise to clinical symptoms or to disturbance of growth in chicks. A sys-

tematic investigation with varying amounts of galactose in the diet might throw some more light on this question.

Summary. Chicks fed a diet containing a high percentage of galactose develop violent spasms and die within few days. There is a high content of galactose in the blood during the spasms but about normal blood glucose. Muscle glycogen is about normal but liver glycogen nearly zero. The mechanism of the disorder is not yet clear.

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Use of a Germicidal Quaternary Ammonium Salt in Nutritional Studies.

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Until sterile rats can be raised routinely, studies involving synthesis of vitamins by bacteria existing in this animal will have to depend largely on the use of suitable agents to eliminate at least some of the microörganisms. Certain sulfa drugs have proven to be of much value in such studies, but it is recognized that their usefulness is limited because they attack only certain types of bacteria while others survive and indeed tend to fill in the "vacancy" created by removal of the sulfonamide-susceptible types.[1,2] It seems logical therefore that each new germicide whose properties suggest that it might be useful in nutritional studies should be given a trial. This report summarizes preliminary observations on the effect of feeding one of the quaternary ammonium salt germicides to rats on various diets.

Experimental. The compound Phemerol* was chosen because of its potency, purity, low toxicity, and availability.[3,4] In all the studies, 21-day-old, male, albino Sprague-Dawlev rats were used. The basal ration consisted of sucrose 76, Labco casein 18, salts IV[5] 4, corn oil 2; vitamins (mg per 100 g ration): choline 100, nicotinic acid 0.250, calcium pantothenate 2.0, thiamine 0.200, pyridoxine 0.250, riboflavin 0.300, 2-methyl-1,4-naphthaquinone 0.100, biotin 0.010 (S.M.A. conc.) Two drops of haliver oil containing 1 mg α-tocopherol were fed weekly to each rat. When used, para-aminobenzoic acid and inositol were added at a level of 25 mg per 100 g of ration and vitamin C at 100 mg %. Three rats were used per group and growth results given in Table I are averages over a 5-week period.

It was found that when crystalline Phemerol was incorporated in the diet at a level as low as 0.1%, rats refused to eat the ration and soon died from inanition. A ration containing

1 Light, R., Cracas, L., Oliott, C., and Frey, C., *J. Nutr.*, 1942, **24**, 427.

2 Gant, O., Ransone, B., McCoy, E., and Elvehjem, C., PROC. SOC. EXP. BIOL. AND MED., 1943, **52**, 276.

* The Phemerol used in these studies was supplied by Parke, Davis and Co., Detroit, Mich.

3 Rawlins, A., Sweet, L., and Joslyn, D., *J. Am. Pharm. Assn.*, 1943, **22**, 11.

4 Joslyn, D., You, K., and Rawlins, A., *J. Am. Pharm. Assn.*, 1943, **22**, 49.

5 Hegsted, D., Mills, R., Elvehjem, C. A., and Hart, E., *J. Biol. Chem.*, 1941, **138**, 459.