Table II. Everted small intestine of albino male rat (Wistar strain) weighing about 250 g. Jejunum portions starting 20 cm from pylorus and weighing about 0.5/1 g were chosen. In each everted sac 1 ml of the Krebs solution without glucose and containing $^{24}NaCl$ [a] (total c.p.m. 10^4–$2 \cdot 10^4$), was initially introduced and the sac was then incubated throughout 1 h 30 min in 50 ml of the Krebs solution or of a modified one (see Table) containing 13.9 mM/l of glucose. The radioactivity appearing in this fluid was determined at the end of the experiment. Temperature of incubation 28°C. Oxygenation with a mixture of O_2 95% and CO_2 5%. The single values per g fresh weight and per h are reported

Incubating mucosal fluid	Mean serosal Sodium conc. mE/l	Sodium total outflow $\mu E\ g^{-1}\ h^{-1}$
Krebs + glucose	134.5	141.3
13.9 mM/l	135.5	158.5
Na conc. 143.5 mE/l	135.5	152.0
	135.0	157.2
	m = 135.1	m = 152.2
Krebs + glucose	123.0	116.5
13.9 mM/l	118.0	126.4
Na conc. 54 mE/l	117.0	107.5
NaCl partially replaced by isosmotic urea	114.5	148.5
	m = 118.1	m = 124.7
Krebs + glucose	137.0	159.4
13.9 mM/l	139.0	138.5
Na conc. 143.5 mE/l	138.5	156.4
DNP 0.1 mM/l	134.5	137.0
	m = 137.2	m = 147.8

[a] Kindly supplied by Centro Studi Nucleari E. Fermi, Milano.

Presumably there exist diffusional fluxes (outflux and influx) which cross the intestinal wall in sites different from those utilized by the sodium pump. If such an assumption is correct, one may attempt to calculate the active sodium influx (ϕ_i^a) by the following formula:

$$\phi_i^a = \Delta\mathrm{Na} + \phi_o^d - \phi_i^d$$

where (ϕ_o^d) and (ϕ_i^d) are the diffusional out- and influxes, which are tentatively assumed to be only proportional to the serosal, respectively mucosal, sodium concentration, and (ΔNa) is the net sodium gain of the serosal medium. By introducing in the above equation the mean value of (ΔNa) determined in the urea experiments (Table I) and the corresponding values of (ϕ_o^d) and (ϕ_i^d) calculated on the basis of the experimental mean value of (ϕ_o^d) (reported in Table II: 124.7 $\mu E\ g^{-1}\ h^{-1}$), an active sodium influx of 110 $\mu E\ g^{-1}\ h^{-1}$ has been obtained:

$$\phi_i^a = 50.1 + \frac{110.5 \cdot 124.7}{118.1} - \frac{54 \cdot 124.7}{118.1} \cong 110\ \mu E\ g^{-1}\ h^{-1}$$

This flux (Table I, in brackets) is clearly higher than the net sodium transfer; with the lowering of the sodium pump the glucose transfer decreases more strikingly and the behaviour of the intestine poisoned with DNP seems no longer to disagree with that observed in urea experiments. In our opinion, this correlation between sodium and glucose transfers, besides signifying that the presence of a definite sodium concentration into the epithelial cells is necessary for the functioning of the sugar transfer, as supposed by CSÁKY[12], means that the sodium pump itself must function so that the glucose transfer may take place.

Riassunto. L'assorbimento di glucosio da parte del digiuno di ratto, rovesciato ed incubato *in vitro*, appare relativamente poco sensibile al trasporto attivo del sodio, mentre il trasporto di glucosio dalla mucosa alla sierosa è fortemente correlato e nello stesso senso, con il trasporto attivo di sodio.

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Istituto di Fisiologia Generale dell'Università di Milano (Italy), February 4, 1963.

[12] T. Z. CSÁKY, Fed. Proc. *22*, 3 (1963).

Galactose Intoxication Pathologic Study in the Chick[1]

LECOQ et al.[2] in 1943 reported that pigeons on a diet containing 66% galactose developed symptoms similar to vitamin B_1 deficiency. DAM[3] observed that young chicks fed a 55% galactose diet developed convulsions and died after several days. In 1953 RUTTER et al.[4] found that chicks tolerated galactose up to 10% but greater amounts resulted in ataxia, tremors and eliptiform convulsions. A purified diet containing 10% galactose was fed to chicks by FOX and BRIGGS[5]. The authors reported no observable change from chicks fed the basal diet, and no curled toes or convulsions were found in the galactose-fed chicks. No pathologic lesions have been reported in

[1] Supported by grant B 2951 (C1) from the National Institute of Neurological Diseases and Blindness, National Institutes of Health, United States Public Health Service.

[2] R. LECOQ, P. CHAUCHARD, and H. MAZOUE, C. R. Acad. Sci. *216*, 211 (1943) (Ref. from RUTTER et al.[4]).

[3] H. DAM, Proc. Soc. exp. Biol. Med. *55*, 57 (1944).

[4] W. J. RUTTER, P. KRICHEVSKÝ, H. M. SCOTT, and R. H. HANSEN, Poultry Sci. *32*, 706 (1953).

[5] M. R. S. FOX and G. M. BRIGGS, Poultry Sci. *38*, 964 (1959).

chickens fed excessive amounts of galactose. HANDLER[6] did not observe any pathologic changes in the brain of rats fed a high concentration of galactose. Mice fed a diet containing 63% galactose developed nervous symptoms similar to those resulting from vitamin B_1 deficiency[7] but no lesions were observed in the brain.

We have studied the changes in chicks fed galactose in concentrations greater than 10% and have found histologic lesions in the brain. Our observations are reported at this time.

Methods and Material. Different strains of chickens, varying in age from one day to adult, were used. In the preliminary experiments a Torula yeast was fed, similar to that reported by CREECH et al.[8] except that galactose was used in place of cerelose and the calcium and phosphorus levels in the diet were 1.7 and 0.85% respectively. In the subsequent experiments, the basal ration (Table) was fed to the control group. Other groups were fed this diet with adjustments in the level of cerelose and galactose so that diets contained 10, 20, 30, 40, or 54.41% galactose as the source of carbohydrate. Food and water were available at all times. The young chicks were kept in electrically heated batteries. The older chicks were fed a commercial ration preceding the time they were given the galactose ration. Some of the chickens, after showing clinical manifestations of galactose intoxication, were killed and autopsied immediately. In other experiments the autopsy was performed within a few minutes after death. In all cases the tissues were fixed in a 4% buffered solution of formaldehyde. Paraffin sections were prepared and stained routinely with hematoxylin and eosin. Select sections were stained by the Luxol fast blue (LFB) techniques and some by the Periodic acid Schiff's (PAS) technique. A 5% concentration of diastase was used to treat some sections before the PAS staining.

Results. Clinical manifestations of galactose intoxication varied in chickens of different age and in birds receiving different amounts of galactose. One day old chicks fed a ration containing 20% or more of galactose usually developed a fine tremor and appeared weak within 24 to 48 h. Some had convulsions and usually all died within 48 to 120 h. Chickens one to four weeks of age, when fed this same concentration of galactose, showed an increased activity characterized by excessive movement, jumping, and flying about the cage. They ran into the food and water containers and frequently pecked at what would seem to be an imaginary object. Often these chicks had a peculiar stare and peeped loudly. These clinical manifestations frequently preceded a convulsion, which was characterized by falling to the floor of the cage and lying on the back with excessive flapping of the wings and legs (Figure 1). After a few seconds all movements ceased. The legs became spastic, the eyes closed, respiration ceased and food sometimes regurgitated from the mouth. Respirations slowly returned after a few seconds and within 1–2 min the chickens rose and slowly walked away looking perfectly normal. However, convulsions recurred over an interval of several days but they did decrease in frequency and severity. When given a higher concentration of galactose the clinical manifestations and the convulsions returned, but subsided or decreased after a few days. Birds appeared normal between the convulsions and, after having several convulsions and placed on the commercial ration, they did not show any visible residual neurologic symptoms. Adult chickens fed high concentrations of galactose did not show any clinical symptoms of toxicity.

The time of death in a group of one-day-old White Leghorn chicks fed synthetic rations with 0, 10, 20, 40 and 54.41% galactose is shown in Figure 2. Chickens fed the control synthetic ration with no galactose gained in weight faster than those fed the commercial ration. Chickens fed the commercial ration and then changed to the synthetic ration did not eat it readily at first. There was no indication, however, of a change in eating habits of chickens fed the synthetic ration when increasing amounts of galactose were fed.

Pathologic studies were made on the chickens that had convulsions and either were killed or died as the result of galactose intoxication. No macroscopic changes were present. The only significant histologic lesion observed was in the brain. Neurons, primarily those in the area of the basal ganglia, medulla and occipital lobes, frequently

Basal diet

Ingredient	%
Cerelose	54.82
Soybean protein [a]	26.00
Vitamin E-free lard [b]	5.00
Wood pulp	3.00
Mineral mix [c]	9.83
Vitamin mix [d]	1.35

[a] C-1 Assay protein, Archer-Daniels Midland Co.

[b] Distillation Products Industries, Rochester, N.Y.

[c] Supplied the following per kg of diet: 16.03 g $CaCO_3$; 60.10 g $CaHPO_4 \cdot 2H_2O$; 5.0 g NaCl; 1.14 g $MnSO_4 \cdot 7H_2O$; 0.15 g $FeSO_4 \cdot 7H_2O$; 11.0 mg $CuSO_4 \cdot 5H_2O$; 0.137 g $ZnSO_4 \cdot H_2O$; 7.0 mg KI; 1.0 mg $CoCl_2$; 10.0 g KCl; 5.76 mg $SO_4 \cdot 7H_2O$; 0.00076 g $NaMoO_4 \cdot 2H_2O$; 0.1 ppm $Na_2SeO_3 \cdot 5H_2O$.

[d] Supplied the following per kg of diet: 2200 IU vitamin A; 44.0 mg *d*-α-tocopheryl acetate; 4800 IU vitamin D_3; 10.0 mg riboflavin; 40.0 mg D-calcium pantothenate; 10.0 mg pyridoxine HCl; 120 mg Niacin; 50.0 mg thiamine; 6.0 mg folic acid; 20.0 mg *para*-aminobenzoic acid; 1.0 g inositol; 0.2 mg biotin; 72.6 mg procaine penicillin; 0.5 mg vitamin K; 26.4 mcg vitamin B_{12}; 7.5 g methionine; 4.0 g glycine; 2.0 g choline chloride.

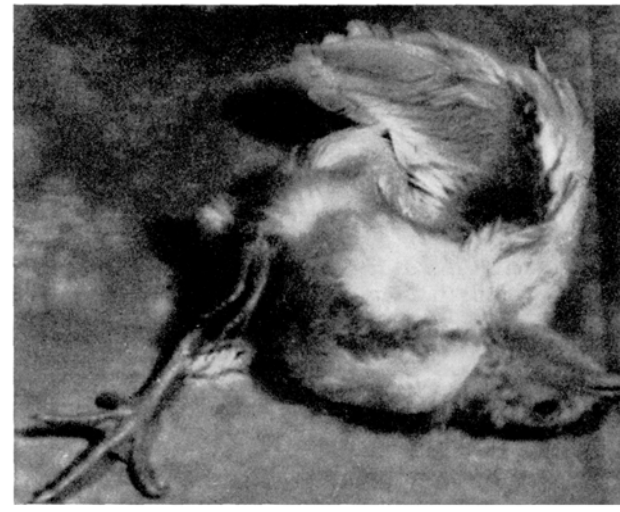

Fig. 1. C 593. Convulsion in chicken, 22 days of age. Fed a diet containing 40% galactose for 48 h. Chick recovered from this convulsion and was killed 1 h later. Lesions were present in the brain.

[6] PH. HANDLER, J. Nutrition *33*, 221 (1947).

[7] B. CH. CL. GUHA, Biochem. J. *25*, 1385 (1931).

[8] B. G. CREECH, G. L. FELDMAN, T. M. FERGUSON, B. L. REID, and J. R. COUCH, J. Nutr. *62*, 83 (1957).

were degenerated. Often all the nerve cells in a specific nucleus were pyknotic while those in an adjacent nucleus of the same chicken were normal. In the LFB-Nissl stain the normal nerve cell has a large clear nucleus and a few light purple-staining granules within the cytoplasm (Figure 3). Numerous vacuoles were present in some of the basal ganglia (Figures 4 and 5). There was a correponding decrease in the number of nerve cells in those areas where vacuoles were present. Apparently some of these spaces resulted from the dropping out of degenerated cells (Figures 6 and 7). At the periphery of some of the degenerating cells were clear spaces, suggestive of edema (Figure 8). The nucleus was absent and the cytoplasm appeared coagulated in some of the degenerated nerve cells. No inflammatory reaction accompanied these lesions in the brain. The degenerative changes usually were present in the chickens that died following a convulsion but not in all of those that were killed. The degree of brain damage varied widely in the different chickens.

Discussion. Galactose is a normal constituent of cerebrosides and in association with lipids has been termed

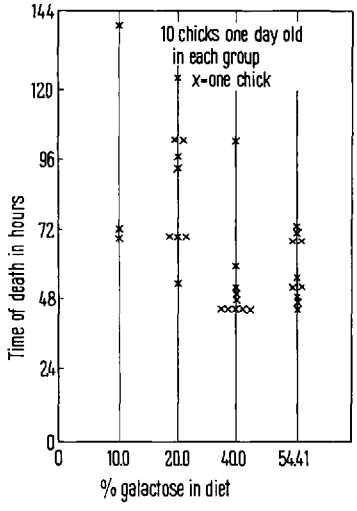

Fig. 2. Time of death in chickens one day of age fed a diet with 10, 20, 40, and 54.41% galactose.

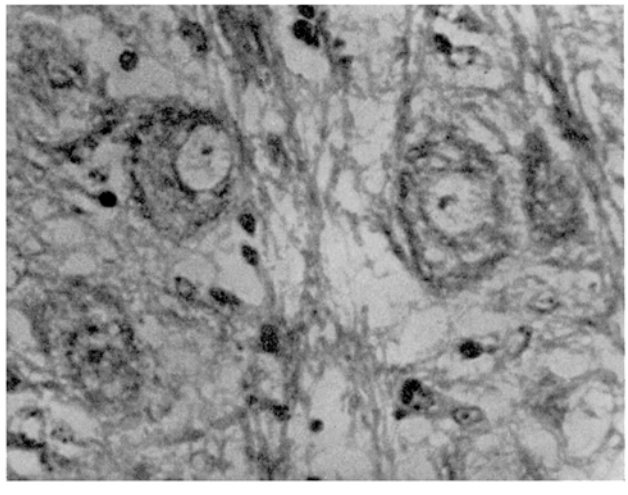

Fig. 3. C 512-4A. Normal nerve cells in a chick. LFB-Nissl stain ×461.

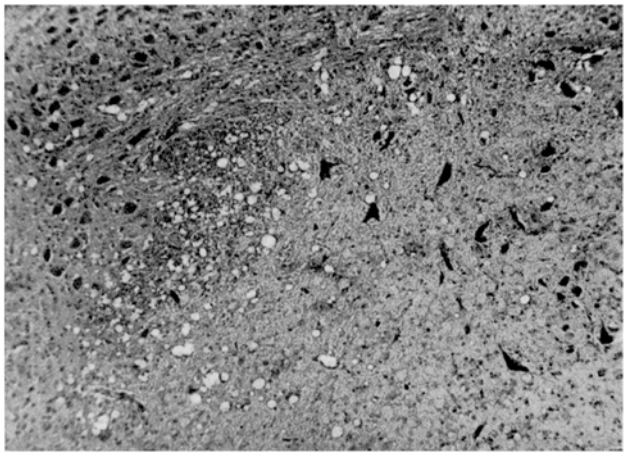

Fig. 4. C 475. A local group of vacuoles in area of basal ganglia in 5 day old chick fed Torula yeast diet with galactose. H&E stain, ×50.

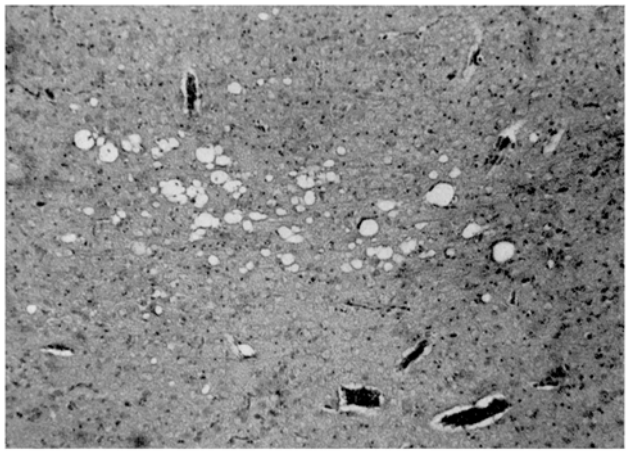

Fig. 5. C 475. A local group of vacuoles in area of basal ganglia in same chick as shown in Figure 4. H&E stain, ×57.

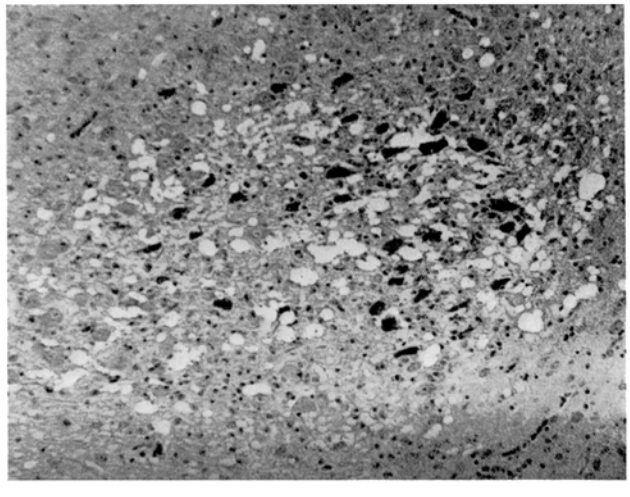

Fig. 6. C 475. A local group of vacuoles and pyknotic nerve cells in area of basal ganglia of same chick as shown in Figure 4. H&E stain, ×84.

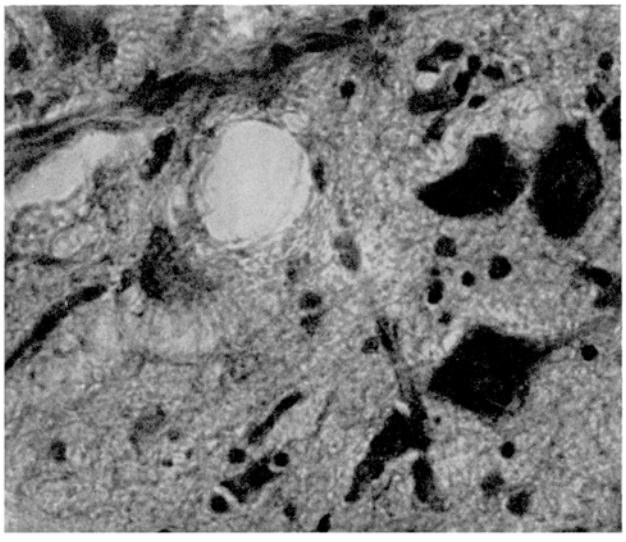

Fig. 7. C 479. A space apparently left by a degenerated neuron. This chick was killed when 12 days of age after being on the Torula yeast diet with galactose for 52 h. LFB-Nissl stain, ×435.

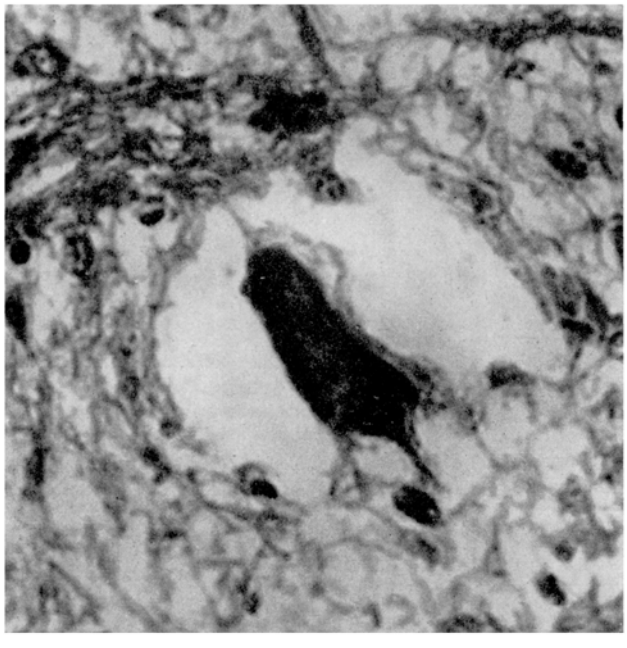

Fig. 8. C 512-4A. This nerve cell is pyknotic and has an adjacent pericellular clear space that probably is edema. This day old chick was fed the synthetic diet containing 40% galactose; 48 h later it was weak, had a tremor, convulsions and died. LFB-Nissl stain ×616.

galactosphingoside[9]. Apparently little is known of the mechanism which produces tremor, convulsions and death in young chicks fed an excessive amount of galactose. The morphologic changes in the brain, resulting from galactose intoxication, are characterized by acute degenerative changes in the neurons. Accompanying this degenerative change is pericellular edema. The nerve cells injured by excessive amount of galactose usually are within the basal ganglia. It is suggested that an excessive amount of galactose in young chicks in some manner injures the neurons, probably by acting through some local enzyme system. The early effect of galactose apparently is that of cell stimulation, as manifested by tremor and convulsions. This effect is reversible without any residual damage. Death, however, may occur in chickens fed the higher concentrations of galactose and they may have severe damage to the nerve cells in the basal ganglia.

The occurrence of convulsions often preceded by aurae is the characteristic clinical manifestation of galactose intoxication in the chicken. Convulsions may occur in man and animal in a wide variety of circumstances, among which may be mentioned hypoglycemia and hypoxia. Blood glucose levels in these chickens fed excessive amounts of galactose are within the range of normal. There is nothing clinically to support anoxia in the birds.

The mechanism of damage to the neurons in the basal ganglia of young chicks fed galactose in concentrations above 10% is unknown. However, the ease by which convulsions and degeneration of the nerve cells can be produced offers an excellent opportunity for the neurochemist, anatomist and pathologist to study specific nerve cell injury and the occurrence of edema[10].

Résumé. Des poussins nourris avec une ration contenant plus de 10% de galactose développent des tremblements, des convulsions et meurent. Une dégénération des neurons et de l'oedéma péri-cellulaire se présente dans la zone des ganglions basals, dans la zone médullaire et dans les lobes optiques.

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[9] W. E. STONE, chap. 18 in *Neurochemistry. The Chemical Dynamics of Brain and Nerve* (Ed. by K. A. C. ELLIOT, IRVINE H. PAGE, and J. H. QUASTEL; Charles C. Thomas, Springfield, Ill. 1955), p. 485.

[10] A 16 mm Kodachrome movie illustrating the clinical and histologic changes in galactose intoxication is available for those who are interested.

Histochemical Localization of Acid Phosphatase and Cathepsin-like Activities in Regressing Tails of Xenopus Larvae at Metamorphosis[1]

The atrophy of the larval tail, which occurs during anuran metamorphosis, represents a striking example of tissue regression. At the biochemical level this process coincides with a marked increase in the activity of cathepsins[2,3], acid phosphatase[4] and other 'lysosomal' enzymes[5]. Since larval tails include various structural elements, comprising very different types of tissue, the problem arises as

[1] This work was supported by the 'Swiss National Foundation for Promotion of Scientific Research' (Project 1613).

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