

to consider age as a more important factor than body-weight.

Experiments were carried out on piglets suckling regularly. As we discovered, even though intake and digestion of foods in piglets are important factors in metabolism, they cannot significantly influence the reported findings².

In conclusion it can be stated that development of chemical thermoregulation in the postnatal period of piglets occurs in three phases. Up to sixth-day thermogenesis, even if it exists, is unsatisfactory; from the ninth day it starts asserting itself, and around the twentieth day it is nearly perfect.

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¹ Tomme, N. F., "Obměn veščetství i energii u selskochozajstvennych životnych", Moskva (1949).

² Holub, A., Ježková, D., and Forman, Z. (in the press).

Glucuronic Acid Formation in Epiphyseal Cartilage Homogenate

IN a previous communication¹ the formation of hexosamine in epiphyseal cartilage homogenate of young rabbits was reported.

Cartilage was shown to synthesize the amino-sugar from glucose-6-phosphate and glutamine. The enzymatic fixation of sulphuric and acetyl radicals by cartilage has been reported by other workers^{2,3}; we then envisaged the presence of all the enzymes concerned in chondroitin sulphate biosynthesis, inasmuch as chondroitin sulphate is largely present in cartilage and possibly connected with the fixation of calcium ions during the mineralization process.

Strominger *et al.*⁴ have shown that the formation of glucuronic acid is accomplished through oxidation of uridine diphosphoglucose to uridine diphosphoglucuronic acid. This dehydrogenase was shown to be dependent on DPN⁺ and was isolated from calf liver by Strominger *et al.*⁴.

We were therefore interested to see whether this enzyme was present in epiphyseal cartilage.

Epiphyseal cartilage of growing rabbits was used; the tissue was excised from proximal and distal ends of long bones at 0° C. and homogenized with cold water in a Potter-Elvehjem homogenizer. Two 10-ml. centrifuge tubes were used, each containing: 0.5 ml. cartilage homogenate (10 per cent), 1.5 ml. 0.014 M phosphate buffer pH 7.4, 20 μM magnesium chloride, 1 μM DPN⁺ and 1 μM of uridine diphosphoglucose (Sigma Chem. Co.). One tube immediately put in boiling water for 10 min. was taken as a blank; the other tube was incubated at 38° C. for three hours and the reaction was blocked as in the blank tube. In both tubes chondroitin sulphate was precipitated by the addition of 1 ml. of acetic acid and 2.5 ml. of ethanol (95%), and glucuronic acid was measured on the supernatant with the method of Dische⁵.

Furthermore, we have studied the glucuronic acid formation omitting in the reaction mixture magnesium chloride or DPN⁺ or uridine diphosphoglucose, as well as replacing the last-named with uridine triphosphate and glucose-1-phosphate.

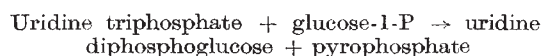
Table 1. GLUCURONIC ACID FORMATION IN EPIPHYSEAL CARTILAGE HOMOGENATES

Values obtained in 3 hr. at 38° C. and pH 7.4 for 100 mgm. of cartilage

No. exper.	DPN ⁺ μM	MgCl ₂ μM	UDPG μM	UTP μM	Glucose-1-P μM	Glucuronic acid μgm.
11	1	20	1	—	—	136
2	1	—	1	—	—	110
4	—	20	1	—	—	52
6	1	20	—	—	—	12
2	1	20	—	2	3	100

In Table 1 average values of some experiments are collected.

From Table 1 it appears that uridine diphosphoglucose is oxidized to uridine phosphoglucuronic acid by cartilage homogenate: the dehydrogenase which is responsible for this oxidation is dependent on DPN⁺, as reported by Strominger *et al.*⁴, since omission of this nucleotide in the reaction mixture yields a reduced formation of glucuronic acid. Uridine diphosphoglucose may be substituted by uridine triphosphate and glucose-1-phosphate: this observation indicates that uridine diphosphoglucose can be synthesized from these compounds according to the reaction⁶:



The capacity of cartilage to synthesize uridine diphosphoglucose is further support of the hypothesis that all the enzymes concerned in chondroitin sulphate biosynthesis are present in epiphyseal cartilage.

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² Boström, H., and Månsson, B., *J. Biol. Chem.*, **196**, 483 (1952); *Ark. Kemi*, **6**, 23 (1953).

³ Boström, H., and Månsson, B., *Acta Chem. Scand.*, **6**, 1559 (1952).

⁴ Strominger, J. L., Kalckar, H. M., Axelrod, J., and Maxwell, E. S., *J. Amer. Chem. Soc.*, **76**, 6411 (1954).

⁵ Dische, Z., *J. Biol. Chem.*, **167**, 189 (1947).

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Lipotropic Action of Ethyl Trichloracetate

DURING a study of the biological effects of halogen derivatives of aliphatic acids, the effects of ethyl monochloracetate and ethyl trichloracetate on rat liver have been compared. Ethyl monochloracetate has a toxic effect on liver, whereas equimolecular doses of ethyl trichloracetate cause no apparent change. However, when ethyl trichloracetate is given subcutaneously to choline-deficient rats, the high level of liver lipid is significantly reduced.

Young rats were placed on a basic choline-deficient diet containing 8 per cent casein, 12 per cent gelatin, and 12 per cent fat, the diet being essentially similar to that used by Ridout, Lucas, Patterson and Best¹. After 21 days, a group of animals was given six subcutaneous injections of 0.1 ml. of ethyl trichloracetate (approx. 720 μmoles) per 100 gm. body-weight over three days. Control animals and injected animals were killed after 24 days on the basic diet. Mean lipid content of livers from the control group was 18.6 per cent wet weight of liver and 9.6 per