The results of the lymphocyte α-D-glucosidase determinations are shown in the Table. Extracts from both normal and leukemic lymphocytes were able to liberate 6-bromo-2-naphthol, indicating the presence of α-D-glucosidase in these cells. As observed with the salivary enzyme, the hydrolytic potencies of leukemic lymphocyte extracts showed considerable variability, unrelated to disease duration, treatment administered, or amount of glycogen detected with the PAS reagent. Normal lymphocyte extracts demonstrated similar differences but their mean value was higher than that quantitated for leukemic enzyme activity.

Discussion. The overlapping of individual glucosidase activity values obtained with normal and leukemic lymphocyte extracts questions the significance of the mean differences detected by these assays. It appears that leukemic lymphocytes contain appreciable amounts of α-D-glucosidase making it unlikely that a deficiency of this enzyme is responsible for the excess histochemically demonstrable glycogen seen in these cells.

The consistent failure, up to the moment, to show that lymphocyte glycogen storage is related to abnormalities per se of 3 different enzymes involved in the synthesis or catabolism of glycogen suggests an alternative metabolic defect. It is conceivable, for example, that a block in either the EMBDEN-MEYERHOF or hexose monophosphate (HMP) shunt pathways might lead to an accumulation of glycolytic intermediates which could ultimately enter the glycogen synthesis cycle and become manifest as increased PAS-positive intracytoplasmic material. Experiments in this laboratory, measuring quantitative evolution of $C^{14}O_2$ from glucose-1-C^{14} as an endpoint, imply that leukemic lymphocytes metabolize proportionately less carbohydrate via the HMP pathway[12] than do their normal counterparts. These preliminary studies, which support, but do not prove, the stated hypothesis, are being extended to more precisely localize this metabolic abnormality[13,14].

Zusammenfassung. Der einfache Nachweis von α-D-Glukosidase in leukämischen Lymphozyten gelang spektrophotometrisch. Dies lässt darauf schliessen, dass ein Enzymmangel nicht Ursache für die Glykogenspeicherung dieser Zellen sein kann. Es scheint sich um eine Ansammlung von glykolytischen Zwischenprodukten und nicht um einen Defekt der Glykogenolyse zu handeln.

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[13] Acknowledgment. The authors gratefully acknowledge the technical assistance of Miss ANNEYSE GOLDBERG.

[14] Supported by U.S.P.H.S. Research Grant No. CA 07000 and U.S.P.H.S. Training Grant No. 5 T1 CA5159.

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The Activities of Phenylalanine Hydroxylase and Hypoxanthine Dehydrogenase in Chick Embryos and an Attempt to Induce these Enzymes

Genic defect of metabolic enzymes in man results in congenital metabolic disturbances. Phenylalanine hydroxylase and hypoxanthine dehydrogenase are 2 such enzymes, the lack of which causes phenylpyruvic oligophrenia and xanthinuria, respectively. The fetal mammalian liver contains very little of the former enzyme, which starts to develop several days after birth[1,2]. The latter enzyme, which is present in embryonic chick liver, is also negligible, although it increases abruptly immediately after hatching[3,4]. The activity of hydroxylase in young rats[5] and of dehydrogenase in rat and chick embryos[4,6] varies with the administration of substrate or non-substrate substances. Among many inducers of enzymes in rat liver[7], X-irradiation has been reported as an effective stimulator of tryptophan pyrrolase in which the hypophysis-adrenal function appears to be a controlling factor[8]. In view of this, observations have been made on the developmental patterns of these enzymes in chick embryos and on the inducibility of these by X-irradiation.

Developmentally staged chicks of ages between 14 days of incubation and 12 h after hatching were used. For the 2 dose groups of irradiated embryos, a single dose of either 600 or 800 r was administered into the embryo side of the eggs by an X-ray apparatus (250 kv, 30 mA, HVL 1.4 mm Cu, 295 r/min, distance 60 cm), and they were immediately returned into the incubator. These and sham-irradiated control embryos were sacrificed simultaneously after 2 h of treatment. The procedures for preparation and incubation for measuring the activities of hydroxylase and dehydrogenase, from the supernatant after 16,000 and 34,000 *g* centrifugation, respectively, of the pooled liver homogenate, were the modified methods[2,3] of corresponding original workers. Crude preparations were used to secure all the enzyme content of the cells which might be present in multiple forms. At least 5 determinations were made from each batch of eggs at

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every day of incubation. The protein content of the preparations was also determined[9].

In control liver the hydroxylase activity was negligible throughout the experimental period; all the average values of the specific activity (mμmoles of tyrosine formed/h/mg protein) at each incubation day were between 8 and 20, without notable change immediately after hatching. Similarly, the dehydrogenase level in the pre-hatching period was very low, averages of the specific activity (mμmoles of NADH formed/min/mg protein) being between 25 and 41. This enzyme, however, increased immediately after hatching to 161, 4 times that of the previous day level. In the case of each enzyme, neither dose group of the irradiated liver showed any meaningful, statistically significant change in activity from that of the control. The result was also inconclusive in the case of hydroxylase with a single injection of 2.5 μg cortisone in saline into the air chamber of the eggs immediately after or before the irradiation, at several stages of incubation. A dose of 400 r was not successful in enticing a change in the dehydrogenase activity.

The developmental pattern of the hydroxylase in the chick embryo seems to be similar to that of mammals, including the human[1,3], although a more positive view has been expressed[10]. The sudden increase of the dehydrogenase confirms previous work[4] and is comparable to that of other mammalian enzymes[11,12]. The low conversion rate of phenylalanine to tyrosine in the new-born rat was attributed to the low content of the co-factors[13], and injection of molybdenum enhanced the dehydrogenase activity in the chick embryo[4]. Although both enzymes are co-enzyme-dependent, the pyridine nucleotides were not an effective stimulator[14]. Despite much emphasis on the endocrine functions in the adult, several lines of experimental evidence indicate that factors such as the initial level and the rate of formation of the enzyme could play a more significant role in embryonic enzyme induction: (1) contrary to the condition in the adult, substrate injection did not activate the fetal liver enzyme[11], and this appears to be true in the chick embryo[4]; (2) the sudden increase of enzyme in the chick embryo[4] or in mammalian liver[15] was completely blocked by inhibiting protein synthesis; (3) many enzymes are influenced by age, sex, and nutrition, in addition to hormones[2,12,16]. Therefore, the lack of enzyme activation in this experiment could be attributed to these general conditions.

Résumé. Bien que l'activité de l'hydroxylase phenylalanine et de la déhydrogénase hypoxanthine dans l'embryon de poulet ne soit pas évidente, la déhydrogénase hypoxanthine augmente d'une façon rapide justement après l'éclosion. L'induction des enzymes par l'irradiation par les Rayons X n'est pas effective non plus.

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Onset of Post-Irradiation Depression of Chlortetracycline-Induced Resistance of Faecal Coliforms in Young Conventional Pigs

The coliform component of faecal microflora of conventional piglets, which were maintained for 1–2 weeks after weaning on a diet supplemented with chlortetracycline (CTC) (daily dose $\simeq$ 10 mg/kg body weight) includes a high percentage – practically 100% – of strains resistant to tetracyclines. We demonstrated a considerable temporary depression of this resistance after total-body sublethal and half-lethal doses of X-irradiation, when the piglets – after being 'prepared' by low CTC doses for a short period – were fed a diet without a supplementary dose of CTC during the whole experiment[1].

The mechanism of the effect of these doses of ionizing radiation (550 and 600 r), which are insufficient for any direct influence on bacteria[2], is not known. We assume that the macroorganism damaged by the radiation or, more accurately, its intestinal tract which has a relatively high degree of radiosensitivity and which represents the life environment for intestinal microorganisms, is a mediator of this effect. There is presumably some 'triggering' function of a relatively low dose of radiation for some mechanisms of infectious genetics which might be involved in inducing this phenomenon.

In order to confirm our hypothesis, an attempt was made to reveal the onset moment of the resistance depression. Thus, it would be possible to estimate the number of generations between the 'hit' of irradiation and the consequences on the characteristic of the bacteria – their resistance to tetracyclines.

We used 3 conventional healthy piglets (age 7 weeks; weight 9.5, 9.9 and 11.0 kg respectively), which were irradiated by the dose of 600 r, $\simeq LD_{50/30}$. (For the conditions of care, feeding and irradiation see the previous contribution). The piglets, which had been put on a diet without CTC immediately after they had been delivered to the institute, were sampled 8 times and their faecal coliforms tested on the resistance during the pre-irradiation period of 18 days. After irradiation the samples of faeces were taken 10 times at 3 h intervals (from 3–30 h)

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