It is likely that a substance derivable either from phenylalanine, tyrosine or *ortho*-amino-benzoic acid is the agent effective in reversing the effects obtained with chloramphenicol. Chloramphenicol possibly inhibits the endogenous production of such a material. Precise identification of this material will be of interest.

Zusammenfassung. Hühnerembryonen, die in vitro mit Chloramphenicol behandelt wurden, zeigten verschiedene Missbildungen, wie Mikrocephalie, offene Neuralrinne, gerade bleibenden Herzschlauch, verkürzte Körperachse. Nachbehandlung solcher Chloramphenicol-Keime mit äquimolaren Lösungen von Phenylalanin, Tyrosin oder *Ortho*-Aminobenzoesäure ergibt weitgehend oder völlig normale Entwicklung. Durch Behandlung mit Alanin, Phenylmilchsäure oder *Para*-Aminobenzoesäure wird dagegen die Chloramphenicolwirkung nicht aufgehoben. Auf Grund dieser Ergebnisse wird der mögliche Mechanismus der Chloramphenicolwirkung diskutiert.

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Whole-Body γ-Irradiation, Food Intake and Glucuronic Acid Excretion in Rats

In previous work it was found that the sensitivity to naphthalene was increased after whole-body irradiation in rats and that after 1000 R (whole body) of gamma rays the urinary glucuronic acid excretion was diminished[1]. Also it was found by HARTIALA and his group that in vitro local X-ray radiation decreased the ability of tissue from the intestinal tract[2] and liver[3] to conjugate *o*-amino-phenol with glucuronic acid. We found that same effect in the conjugation of anthranilic acid by duodenal tissue[4] in whole-body irradiation. All these results could have the same common denominator. However, a more detailed study of these problems showed that the low excretion of glucuronic acid by these animals is related to the dose of irradiation but mainly to the fact that animals are anorexic after irradiation[4].

The primitive hypothesis that some substances will be excreted as glucuronides after irradiation was found correct when the experiment was conducted with animals in starvation.

The condition of the experiments are reported elsewhere[1,4].

Total glucuronic acid in urine was generally determined by the method described by MEADS et al.[5]. Results are expressed as 'total glucuronic acid' in mg/24 h. The term 'total glucuronides' is used inferring that no free glucuronic acid is excreted by the kidney; free and conjugated glucuronic acid were determined by the method of FISHMAN et al.[6].

In preliminary studies, in which rats were irradiated with 1000 R, the excretion of total glucuronides was found to diminish and to reach the lowest values on the third day after irradiation.

In order to gain more information on this effect, experiments were done in which groups of 8 rats were irradiated with different doses: 1000, 400 and 100 R. Their daily food intake and daily excretion of total glucuronide were measured. The results (Figure 1) show the correlation of irradiation dose with food intake and with the amount of glucuronide excreted.

With 1000 R the food intake decreased severely for 4 days. With 400 R, there was a slight decrease during the first 3 days after irradiation. Therefore, the reduction of glucuronide excretion after irradiation must be correlated with the low food intake of the animals during this period.

An experiment was conducted on 8 rats to test the effect of starvation on the excretion of total glucuronides. Total glucuronide excretion was measured for 3 days before food was withheld. After 7 days food was again supplied. With a standard Purina diet, normal excretion of total glucuronides averages 40.5 mg/day determined

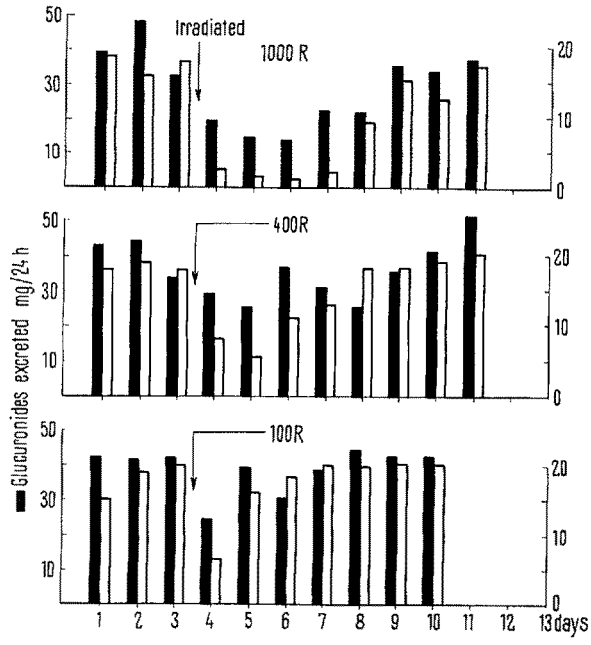

Fig. 1. Daily excretion of glucuronides and food intake □ before and after irradiation.

[1] J. CHIRIBOGA, Nature *198*, 803 (1963).
[2] K. J. HARTIALA, W. V. NANTO and U. K. RINNE, Acta physiol. scand. *43*, 77 (1958).
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[4] J. CHIRIBOGA, Puerto Rico Nuclear Center Report No. 80, San Juan, Puerto Rico (1965).
[5] J. A. R. MEADS, J. N. SMITH and R. T. WILLIAMS, Biochem. J. *68*, 61 (1958).
[6] W. H. FISHMAN and S. GREEN, J. biol. Chem. *215*, 527 (1955).

by MEAD's method. After food withdrawal, the excretion decreases, reaching values < 10 mg/day. This level of excretion could be accounted for by endogenous sources (Figure 2).

The results indicated that $^4/_5$ of the total glucuronides excreted by our normal rats are of exogenous origin. These are healthy rats with a 50% rate of spontaneous tumorogenesis with aging.

The decrease in food intake of irradiated rats produces a corresponding decrease in the normal total glucuronide excretion that could mask the endogenous changes due to irradiation. In an experiment done to test this possibility, 8 rats were starved for 3 days and then irradiated with 1000 R. The excretion of total glucuronides was measured each day.

The results (Table) clearly show the increased excretion during the first 24 h after irradiation compared with that of the controls (8.82 ± 0.39 mg for controls and 14.56 ± 0.86 for irradiated animals). A t test comparing the 2 groups indicates the high significance of these values. This data agreed with the normal ability of irradiated rats to excrete toxin substances conjugated with glucuronic acid[7,8]. The results (Figure 3) show that the increased excretion includes both free and the conjugated acid forms.

The free acid found may be due to the action of β-glucuronidase present in the urine. Many substances, principally hormones or degradation products, could be responsible for the increased glucuronide excretion in starved rats after irradiation. These substances could explain at least in part the higher resistance of intact animals to irradiation compared with adrenalectonized ones found the first time by EDELMAN[9].

Epinephrin is excreted as a glucuronide and is released after irradiation[10]. This hormone has a protective effect as a result of its antioxidant activity.

The substances excreted as glucuronides could have effect in many other metabolic events. It is already reported that immediately after whole-body irradiation nuclear protein synthesis[11] is stimulated and also, it was suggested, its correlation with adrenal substances.

Elucidation of these points could contribute to the understanding of the physiological reaction of higher animals to radiation. Identification of these substances excreted as glucuronides is the next step in this line of study[12].

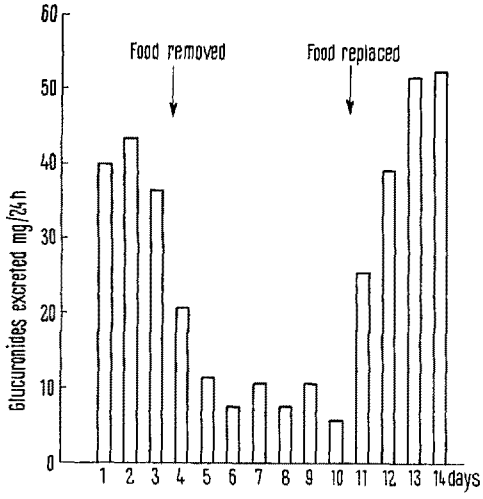

Fig. 2. Daily excretion of glucuronides in rats before and after starvation.

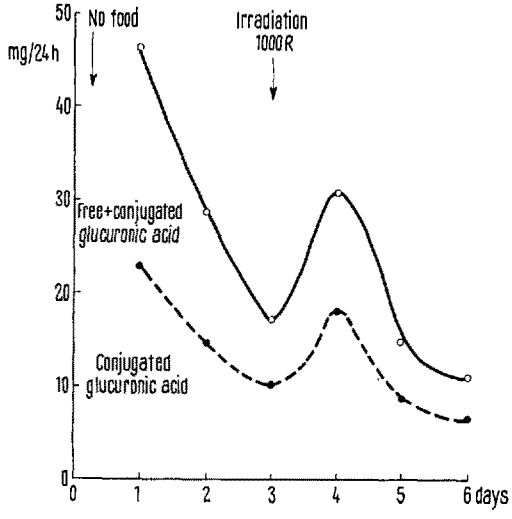

Fig. 3. Daily excretion of free and conjugated glucuronic acid by rats under starvation before and after irradiation.

Total urinary glucuronide excretion of starved rats (mg/24 h) after whole-body irradiation

Day	Controls	Irradiated	Significance of Δ between control and irradiated group
1	19.59 ± 0.55[b]	18.59 ± 0.84	not significant
2	12.58 ± 0.59	14.22 ± 0.66	not significant
3	11.22 ± 0.83	9.94 ± 0.65	not significant
1000 R administered			
4	8.82 ± 0.39	14.56 ± 0.86	$P < 0.005$
5	8.00 ± 0.51	8.88 ± 0.80	not significant
6	6.48 ± 0.28	7.85 ± 0.81	not significant

[a] Starved at day 0. [b] Standard error of the mean.

Zusammenfassung. Ganzkörperbestrahlung bei Ratten führt zur Verminderung der Futteraufnahme und zur Reduktion der ausgeschiedenen Glukoronide. Die Ausscheidung der Glukoronide erhöht sich nach dreitägigem Hunger.

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