2.4. Konzentrationsbestimmung des 8-Hydroxychinolin-carbonsäure-2-methylesters und der Steroide im Nativsekret

Für die UV-spektrometrische Konzentrationsbestimmung war eine vorherige chromatographische Trennung des Rohsekrets unumgänglich, da sich die Absorptionsbanden der sechs Substanzen im UV stark überschneiden. Nach gel- und dünnsschichtchromatographischer Separierung der Einzelkomponenten ergaben sich folgende Konzentrationen:

	Menge [$\mu\text{g/Käfer}$]
8-Hydroxychinolincarbonsäure-2-methylester	350
4-Pregnen-20 β -ol-3-on	1
4-Androsten-17 β -ol-3-on	28
1.4-Androstadien-17 β -ol-3-on	16
Östron	2
17 β -Östradiol	19

Diese Angaben sind Mittelwerte, die aus der gemeinsamen Aufarbeitung der Prothorakalwehrrüsen von 40 Käfern gewonnen wurden.

Die Untersuchungen sind ermöglicht worden durch die dankenswerte Unterstützung der Deutschen Forschungsgemeinschaft, der Dr. Otto-Röhm-Gedächtnis-Stiftung und dem Fonds der Chemischen Industrie. Frl. Dr. KRAUSS danken wir für die Aufnahme und Deutung der Massenspektren.

Influence of X-irradiation on enzyme induction *: phosphoenolpyruvate carboxykinase in the liver of irradiated mice

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(Z. Naturforsch. **24 b**, 1534—1537 [1969]; eingegangen am 5. August 1969)

Normal or adrenalectomized mice were whole-body X-irradiated with 690—4000 R either after hydrocortisone administration or during a 24 h fasting period, and PEP carboxykinase activity measured after the appropriate time. The results show that the induction of PEP carboxykinase is not affected by the irradiation, neither in hormone treated nor in fasting normal or adrenalectomized mice as compared to the controls. These findings support the assumption that the protein synthesizing system in the living cell is very resistant to ionizing radiation.

Investigations on the influence of X-irradiation on the induction of enzymes¹ in mammals were performed mainly with tryptophan pyrrolase¹⁻⁴ and tyrosine transaminase^{2,5}. It was shown that the hormone dependent induction of these enzymes remains unaffected after irradiation. However, these and other enzymes are also induced by their respective substrates and substrate induction seems to be more sensitive to X-irradiation. The induction of tryptophan pyrrolase by tryptophan and of tyrosine transaminase by tyrosine^{1,2} and the induction of serine dehydrogenase and ornithine transaminase by

casein hydrolysates^{3,5} is affected by irradiation. Only the findings of MISHKIN and SHORE⁶ contradict these observations. These authors found that radiation interfered with hormonal induction of tryptophan pyrrolase but not with the induction caused by tryptophan.

It is well documented, that phosphoenolpyruvate (PEP) carboxykinase is one of the rate limiting enzymes in gluconeogenesis. The enzyme catalyzes the conversion of oxalacetate to PEP and the enzyme activity is increased by hormonal treatment or starvation in tissues with gluconeogenic capacity

* Enzyme induction is defined as an increase in the rate of enzyme synthesis and will result in an increased enzyme level.

¹ H. KRÖGER and B. GREUER, *Biochem. Z.* **341**, 190 [1965].

² O. GRENNGARD, G. T. BARKER, M. L. HOROWITZ, and W. E. KNOX, *Proc. nat. Acad. Sci. USA* **56**, 1303 [1966].

³ H. C. PITOT, C. PERAINO, and C. LAMMAR JR., *Radiat. Res.* **33**, 599 [1968].

⁴ J. F. THOMSON and F. J. KLIPPEL, *Proc. Soc. exp. Biol. Med.* **95**, 107 [1957].

⁵ H. C. PITOT, C. PERAINO, and C. LAMMAR JR., *Science [Washington]* **150**, 901 [1965].

⁶ E. P. MISHKIN and M. L. SHORE, *Radiat. Res.* **33**, 437 [1968].

(e.g. liver and kidney)⁷⁻¹⁰. The rise in the level of PEP carboxykinase after starvation occurs in normal as well as in adrenalectomized animals, indicating that adrenocortical hormones are not solely responsible for enhancing enzyme activity⁷. It is as yet unknown which factors beside the glucocorticoides effect the increase in PEP carboxykinase activity in the animals, but obviously a *de novo* synthesis of the enzyme occurs⁷.

PEP carboxykinase was chosen to study the effect of X-rays on the induction of this enzyme for two reasons: 1. Changes in the induction rate of this gluconeogenic key enzyme after irradiation must have serious consequences for gluconeogenesis. 2. No data are available on the induction of mammalian carbohydrate metabolizing enzymes in irradiated animals. Therefore, the influence of X-irradiation on the hydrocortisone-dependent or starvation-dependent induction of PEP carboxykinase was estimated in both, normal and adrenalectomized mice.

Material and Methods

Male white mice, 25–30 g, were fed on pellets (altromin). The liver was removed under light ether anesthesia and the organ was homogenized for 3 min with a Potter-Elvehjem homogenizer in a fivefold volume of 0.25 M sucrose solution. Then the homogenate was diluted 1:1 with the same solution and centrifuged for 30 min. at 105 000 *g*.

PEP-carboxykinase was measured in the 105 000 *g* supernatant (Norlie and Lardy, 11) in the following way: to the incubation mixture, containing TRIS-buffer pH 8.0 100 μ moles, $MnCl_2$ 1 μ mole, $MgSO_4$ 20 μ moles, KCl 10 μ mol, KF 10 μ moles, ITP- Na_2 7 μ moles, oxalacetate 3 μ moles, was added 0.05 ml of the 105 000 *g* supernatant (0.1–0.6 mg protein) and incubated for 15 min at 37°. The reaction was stopped by the addition of 0.1 ml trichloroacetic acid (3 M) and centrifuged. The supernatant solution was placed into graduated tubes and 1 ml of a solution containing 2,4-dinitrophenylhydrazine (DNP) (0.3% in 2 M HCl) was added. After 20 min the yellow solution was extracted twice with ethylacetate (saturated with water) and the ethylacetate layer, containing excess DNP and the phenylhydrazone of oxalacetate, was discarded. The remaining aqueous solution was neutralized with 3.5 M NaOH and adjusted with water to a definite volume.

Subsequent optical assay of an aliquot of the PEP solution was carried out as described by CZOK and ECKERT¹² with ADP, pyruvate kinase and lactate dehydrogenase. The specific activity of PEP carboxykinase is given in μ moles PEP formed per mg of protein per hour at 37 °C.

Protein was determined with the biuret method.

Hydrocortisone acetate was given intraperitoneally, 1 mg per mouse in a microcrystalline suspension in 0.9% NaCl. For enzyme measurements all animals were killed 6 h after hormone administration.

X-irradiation was performed at 150 kV, 20 mA with a dose rate of 148 R/min. Adrenalectomized mice received 0.9% NaCl solution instead of drinking water and were used for experiments 6–8 days after the adrenalectomy.

Results

Table 1 summarizes the results of hydrocortisone administration and starvation on PEP carboxykinase activity in normal and adrenalectomized mice. The enzyme activity is increased three- to fourfold after the hormone was given in both normal and adrenalectomized mice.

	PEP formation [μ moles/mg protein/h/37°]	
normal	3.3 $\pm$ 0.3 *	(24) **
normal, 6 h after 1 mg hydrocortisone	10.1 $\pm$ 0.7	(22)
normal, 24 h fasted	12.1 $\pm$ 0.7	(24)
adrenalectomized	3.9 $\pm$ 0.3	(23)
adrenalectomized, 6 h after 1 mg hydrocortisone	11.3 $\pm$ 0.6	(24)
adrenalectomized, 24 h fasted	13.2 $\pm$ 0.8	(16)

Table 1. PEP-carboxykinase activity in the liver of normal and adrenalectomized mice. Experimental details see method section. * Standard deviation of the mean. ** Number of animals.

In two sets of experiments the influence of X-irradiation on the hormone-dependent induction of PEP carboxykinase and on the starvation-dependent induction of the enzyme was investigated. Although the enzyme activities are the same in normal and adrenalectomized mice (Table 1), adrenalectomized animals were chosen in addition to normal mice in order to avoid any hydrocortisone excretion by the

⁷ H. A. LARDY, D. O. FOSTER, E. SHRAGO, and P. D. RAY, in: *Advances in enzyme regulation*, Vol. 2 (ed. G. WEBER), Pergamon Press, Oxford 1964, p. 39.

⁸ G. WEBER, R. L. SINGHAL, and S. K. SRIVASTAVA, *ibid.* Vol. 3 (1965), p. 43.

⁹ M. C. SCRUTTON and M. F. UTTER, *Ann. Rev. Biochem.* 37, 249 [1968].

¹⁰ W. SEUBERT and W. HUTH, *Biochem. Z.* 343, 176 [1965].

¹¹ R. C. NORDLIE and H. A. LARDY, *J. biol. Chemistry* 238, 2259 [1963].

¹² R. CZOK and L. ECKERT, in: *Methoden der enzymatischen Analyse* (ed. H. U. BERGMAYER), Verlag Chemie, Weinheim 1962, p. 229.

adrenals which could occur as a consequence of the stress caused by the irradiation.

As it is shown in Table 1, PEP carboxykinase activity is increased three- to fourfold within 6 h after the injection of 1 mg of hydrocortisone per mouse. In the first experiment mice were irradiated during this induction period with different X-ray doses and at different time intervals after the hydrocortisone injection. One can assume that the influence of irradiation on the enzyme induction might be most effective soon after the hormone administration when the enzyme synthesis starts. Therefore, X-irradiation was carried out during 2 hours after hormone injection. Only with 690 R also other time intervals were chosen.

Fig. 1. Influence of 690 R on the hydrocortisone dependent induction of PEP carboxykinase in the liver of adrenalectomized mice. Mice received 1 mg of hydrocortisone acetate per mouse i.p. At the time indicated after hydrocortisone injection mice were irradiated. Animals were killed 6 h after hydrocortisone injection and PEP carboxykinase activity was measured as described in methods section. 6 mice were used in each experiment. Standard deviations of the mean. The control values on the ordinate are the same as in Table 1.

Fig. 2. Influence of 1000 R and 2000 R on the hydrocortisone dependent induction of PEP carboxykinase in the liver of adrenalectomized mice. Details see legend to Fig. 1.

Fig. 3. Influence of 3000 R on the hydrocortisone dependent induction of PEP carboxykinase in the liver of adrenalectomized and normal mice. Details see legend to Fig. 1.

Fig. 4. Influence of 4000 R on the hydrocortisone dependent induction of PEP carboxykinase in the liver of adrenalectomized and normal mice. Details see legend to Fig. 1.

In Figs. 1–4 is shown the influence of 690, 1000, 2000, 3000 and 4000 R, respectively, on the induction of PEP carboxykinase in adrenalectomized and normal mice after hydrocortisone administration. Under all conditions employed here PEP carboxykinase activity was changed somewhat by the irradiation, but these changes were not significant.

After a 24 h starvation period PEP carboxykinase is also increased three- to fourfold as compared with fed animals (see Table 1). In the second set of experiments the influence of X-irradiation on this induction caused by starvation was examined. No

Fig. 5. Influence of X-irradiation on the starvation dependent induction of PEP carboxykinase in the liver of normal (n) and adrenalectomized (a) mice. No hormone was given in this experiment. Starvation begins at zero time. At the time indicated after beginning of fasting mice were irradiated with the respective radiation doses. PEP carboxykinase activity was determined after a total fasting period of 24 h. The control values on the ordinate are the same as in Table 1.

hormone was given in these experiments. As indicated in Fig. 5, normal and adrenalectomized mice were irradiated with 690, 2000 and 4000 R, respectively, during the 24 h starvation period. The irradiation was carried out at 0.5, 2, 4, and 8 h after the beginning of the fasting period. PEP carboxykinase activity was measured after 24 hours of fasting.

As it was observed in hormone treated mice (Figs. 1–4), enzyme activity remained almost unchanged as compared to the unirradiated fasted controls. No difference was obtained between normal or adrenalectomized mice, but normal mice were used only in the 2000 R experiment.

Discussion

In the present study the induction rates of PEP carboxykinase in irradiated mice were compared with those of unirradiated controls. It could be shown that the induction of PEP carboxykinase is not affected by the irradiation, neither in hydrocortisone treated nor in fasting normal or adrenal-

ectomized mice. Our findings on PEP carboxykinase induction confirm the observations of KRÖGER¹, GREENGARD² and PITOT³, showing that the hormone dependent induction of tryptophan pyrrolase and tyrosine transaminase remains unaffected after irradiation. Our results support further the assumption that the protein synthesizing machinery in the living cell is very resistant to ionizing radiation. Rather a stimulation often is observed^{13–15}. Although it is as yet uncertain which factor(s) effect the induction of PEP carboxykinase in adrenalectomized fasting mice, it does not seem to be an endogenous substrate induction. Otherwise a diminished induction rate should be found after irradiation in the adrenalectomized fasting mice. A diminished induction was observed so far with all substrate induced enzymes^{1–5}. Probably other hormones besides the glucocorticoids might play an important role in the induction of PEP carboxykinase.

This investigation was supported by the Bundesministerium für wissenschaftliche Forschung.

¹³ A. M. REUTER, A. SASSEN, and F. KENNES, *Radiat. Res.* **30**, 445 [1967].

¹⁴ J. CORLESS and J. GRAY, *Radiat. Res.* **31**, 775 [1967].

¹⁵ D. W. JOHN and L. L. MILLER, *J. biol. Chemistry* **243**, 268 [1968].