(0·1 N NH_4OH) butanol) contained only small amounts of 17-KS (3–6% of the total 17-KS), and naphthoresorcine positive material (1·8 mg of glucuronic acid equivalents).

After evaporation of the solvent, fraction one of the above column (eluted with butanol and 2% aqueous butanol) was redissolved in 50 ml of 0·1 m phosphate buffer (pH 7·0) and 3 times extracted with ether. The paper chromatographic analysis of the ether extract showed one pronounced 17-KS zone with the running rate of epiandrosterone, and several smaller 17-KS zones which corresponded to 3α-hydroxyetiocholan-11,17-dione, 3α, 11β-dihydroxyetiocholan-17-one and 3α,11β-dihydroxyandrostan-17-one.

From these findings it may be concluded that the 17-KS in guinea pigs are excreted in the urine partly as sulfuric acid esters; only a small portion appears in the urine as glucuronic acid conjugates. Fraction one of the chromatographic column contained 17-KS as 'free' steroids. It consisted mainly of free epiandrosterone. We are yet unable to determine whether these free steroids were not conjugated primarily or whether a spontaneous hydrolysis of conjugates had taken place in the urine. 17-KS sulphates may easily be hydrolyzed either by spontaneous hydrolysis, or by bacterial influences. In order to cut down on these uncertain disturbing processes we collected the urine under butanol and, until preparation of the extracts began, kept the specimens in a frozen state at −20°C.

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Zusammenfassung

Im Harn von weiblichen Meerschweinchen wurde im Gegensatz zu männlichen Tieren Epiandrosteron als Haupt-17-Ketosteroid nachgewiesen.

Untersuchungen über die 17-Ketosteroidkonjugate im Harn von weiblichen Meerschweinchen ergaben, dass ein grosser Teil (30–40%) als freie Steroide nachweisbar ist. 40–50% liegen als Schwefelsäurekonjugate und 3–6% wahrscheinlich als Glucuronide vor. Bei den konjugierten 17-Ketosteroiden handelt es sich vornehmlich um 11-oxygenierte Steroide.

Corticosteroids and the Radiation Effect in Regenerating Liver of the Rat

The finding that Cortisone inhibited mitosis in regenerating rat liver[1] prompted the suggestion by Professor J. S. Mitchell that part of the inhibitory effect of ionising radiation on mitosis *in vivo* may be due to endogenous corticosteroids.

To test this possibility liver regeneration rates in adrenalectomized rats were compared with normal rats after whole body irradiation.

Method. Male Wistar rats of 180 g were adrenalectomized under ether anesthesia 24 h prior to irradiation. Glucose saline and food given *ad libitum*. Whole body irradiation was given by means of a 220 kV Maximar 15 m.a. tube filtered by 1 mm Al + 1/2 mm Cu at F. S. D. of 1 m. 200 r caused considerable reduction of mitosis rate in normal rat regenerating liver, higher doses adversely affected the adrenalectomized rats. Normal controls shielded by small lead squares over the pituitary and adrenals were irradiated under light Nembutal anesthesia to prevent stress of restraint. Some adrenalectomized rats were shielded in this way to provide strict comparison. The size of lead shields was kept to a minimum though this resulted in imperfect shielding in some rats.

Partial hepatectomy as described by Higgins and Anderson[2] was performed under very light ether anesthesia 1–3 h after irradiation and rats were sacrificed after 30 h by decapitation. Slices of liver from the larger remaining lobes were fixed in Susa, 7 μ paraffin sections cut and stained with haematoxylin and eosin. Phases of mitosis were recorded and counts made of 200 fields which averaged 4000 cells from each liver section. Results are expressed as mitoses per 1000 cells (mitotic index) which is the basis for comparison in these experiments.

Results. Normal Wistar rats show mitotic rates of 40/1000 cells in regenerating liver (Cater, Holmes, and Mee[3]). Unpublished work on this strain of adrenalectomized rats show rates of 60 to 100 mitoses per 1000 cells after partial hepatectomy.

So far 60 adrenalectomized and 60 normal rats have been used but owing to the particularly arduous conditions only 24 adrenalectomized rats have survived. These were in fair condition and showed a mean mitotic rate of 61/1000 cells (± 19 S.D.).

Of the normal rats 54 survived and owing to the difficulties of shielding can be divided into two groups:

(1)—36 rats showing radiation inhibition had low mitosis rates averaging 14/1000 cells (± 4 S.D.).

(2)—18 rats showing high mitosis rates comparable with and having similar histological appearances to adrenalectomized rats. Much of the early shielding has been inadequate and either pituitary or adrenals or both were affected by radiation causing reduced corticosteroid output.

Histological Appearances. Irradiated normal rats show a sharp crenated outline to a pyknotic nucleus and vacuolation of cytoplasm. These changes, with the paucity of mitoses, are similar to those noted in earlier work on unirradiated cortisone treated rats.

Irradiated adrenalectomized rat liver shows degenerating, fragmented, and aberrant nuclear forms with frequent bridges between daughter cells in telophase. Earlier phases show very large 'blown out' forms with occasional shrunk highly pyknotic metaphases. The co-existence of radiation damage and disintegrating cells which still show many mitosing forms, is most striking.

Discussion. 200 r causes considerable reduction of mitoses in normal rat regenerating liver and moderate to severe nuclear damage in both normal and adrenalectomized rats. In spite of this damage a high mitotic rate is seen in liver from adrenalectomized rats, mitoses often proceeding in nuclei obviously breaking down.

The fact that mitosis rate can be separated from nuclear degeneration indicates that the usually accepted radiation effect comprises at least two factors.

(1)—A hormonal inhibition mediated by corticosteroids as part of the general stress response.

(2)—Breakdown of nuclei due to a block in nucleic acid synthesis with accumulation of deoxyribonucleotides as

[1] J. T. Hemingway and D. B. Cater, Nature *181*, 1065 (1958).

[2] G. M. Higgins and R. M. Anderson, Arch. Path. *12*, 186 (1931).

[3] D. B. Cater, B. E. Holmes, and L. K. Mee, Acta radiol. *46*, 655 (1956).

shown by ORD and STOCKEN[4]. One of the largest accumulations is of deoxycytidine which has been shown by PARIZEK *et al.*[5] to be excreted in urine in direct proportion to ionizing radiation.

The hormonal inhibition of mitosis by corticosteroids as a result of radiation suggests that cortisone is an adjuvant to radiation in achieving this end and exogenous cortisone used in treating the radiation syndrome should not detract from the therapeutic radiation effect.

Animals with intact adrenals tolerate radiation better as shown by many workers, but this study demonstrates considerably less nuclear degeneration in normal rats than in those adrenalectomised.

My thanks are due to Professor J. S. MITCHELL for advice and encouragement, to Dr. K. A. MUNDAY, University of Southampton for facilities and kindness during part of this work and to Miss N. BURR for histological assistance.

J. T. HEMINGWAY

Department of Radiotherapeutics, University of Cambridge, December 1, 1958.

Résumé

La vitesse de régénération du foie chez le rat surrénalectomisé, irradié, est comparée à celle du rat normal radioprotégé, afin d'établir si la sécrétion des corticostéroides est en partie responsable de l'inhibition de la mitose provoquée par la radiation.

Dans le présent travail, il est démontré que le rat surrénalectomisé possède une vitesse de mitose quatre fois supérieure à celle du rat normal, et ceci malgré d'importants dégâts causés au foie par la radiation.

[4] M. G. ORD and L. A. STOCKEN, Biochem. biophys. Acta *29*, 201 (1958).

[5] J. PARIZEK, M. ARIENT, Z. DIENSTBIER, and J. SKODA, Nature *182*, 721 (1958).

A Contribution on the Mode of Action of D 860

From many reports it is known that hypoglycaemic sulphonylurea compounds are ineffective in depancreatised dogs, rabbits, rats, toads and man, in rabbits and rats with severe alloxan diabetes, and in some severe forms of human diabetes. These facts support the hypothesis which explains the mechanism of the action of hypoglycaemic sulphonylurea compounds in terms of stimulating the beta cells of the islets of LANGERHANS to increased insulin secretion[1], or by enhancing the action of endogenous insulin[2,3]. Diabetic animals, however, do differ from normal not only in the absence of beta-cells but also in a high glucose concentration in the extracellular fluid. In the present paper we are therefore concerned with the problem of the effect of D 860 on intact rats with hyperglycaemia induced by the exogenous administration of glucose.

Method. Wistar rats were used, weighing 200–300 g and fed on a standard diet, containing 25% protein, 20% fat and 55% carbohydrate (LARSEN's diet). Hyperglycaemia was induced by subcutaneous administration of glucose, 180 mg/100 g of body weight, spaced over 20 min intervals, in three fractional doses. In order to avoid any uncontrollable loss of glucose in the urine, the ureters were ligated bilaterally under ether anaesthesia 1 h before the experiment was begun. 2 h after the last dose of glucose, physiological saline was given intravenously to the first group (5 animals); to the second group (5 animals) 0·2 u. insulin (Novo)/kg of body weight was given; to the third group (6 animals) we administered 150 mg/kg D 860 (Artosin, Boehringer) as sodium salt intravenously. To the fourth group (6 animals), insulin 0·2 u. plus D 860 150 mg/kg of body weight was given intravenously. In two groups of intact rats (7 animals) with a normal blood sugar level, we tested the hypoglycaemic action of insulin and D 860. Blood sugar was estimated by a modification of the Somogyi-Nelson method (FRANK and KIRBERGER, 1950).

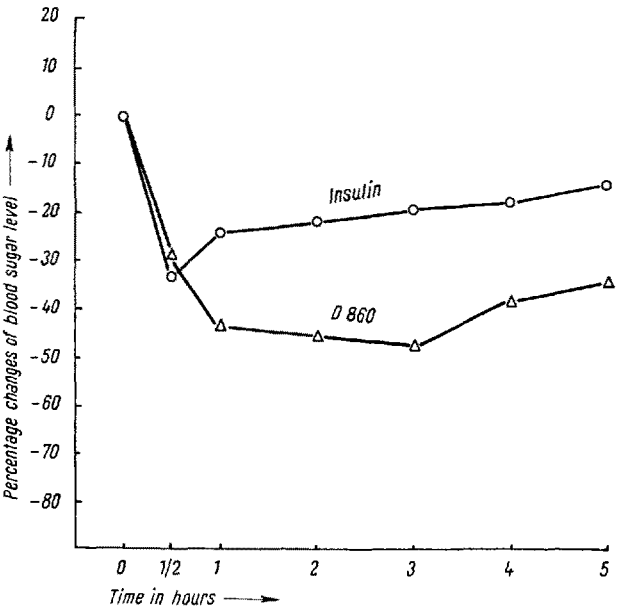

Fig. 1. – Percentage changes in blood sugar levels in rats with a normal initial value (104·7 ± 5·26* and 103·4 ± 4·86 mg% after a single intravenous injection of 0·2 u. insulin, and an intravenous injection of 150 mg D 860/per kg body weight). * S.E. of mean.

Results. Changes of the blood sugar level after a single dose of 0·2 u. insulin/kg body weight or 150 mg D 860 are shown in Figure 1. The maximum decrease in the blood sugar level after insulin occurs after half an hour, and subsequently it returns slowly to the initial level. After the administration of D 860 the blood sugar level decreases by 45%; the fall in blood sugar still being marked and statistically significant ($P < 0{\cdot}001$) 5 h after injection. Figure 2 shows the percentage changes in blood sugar levels after a glucose load. In rats with a normal blood sugar, D 860 caused a drop in blood sugar of 45%. In animals with an initial hyperglycaemia, the same dose leads to a slowing down of the rate of return to normal values. 5 h after the administration of D 860, the blood sugar level is 108% higher than in the group after injection of physiological saline. A single dose of 0·2 u. insulin/kg body weight in animals with an initial hyperglycaemia leads to a more rapid return of the blood sugar to normal. The changes of the blood sugar level after the administration of insulin and D 860 is practically the same as after insulin alone. The blood sugar value after 5 h in the D 860 group was 357 ± 61·1 mg%; in the control group 164 ± 36·8 mg%; in the insulin group 127 ± 21·6 mg%;

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