quée de 5-HT dans la paroi de l'estomac et dans l'iléon. Dans la rate de cet animal, on n'y observe pas d'augmentation de 5-HT.

L'administration intraluminale de la substance *P* (60–80 U) dans l'iléon isolé du cobaye, augmente aussi sensiblement la quantité de 5-HT.

On the Excretion of 17-Ketosteroids in Guinea Pigs

Several investigations have revealed that the adrenocortical activity in guinea pigs is relatively high. They therefore excrete a number of 17-ketosteroids (17-KS) in the urine. PERON *et al.*[1] isolated the following 17-KS from *male* guinea pig urine:

3α-hydroxyetiocholan-17-one, 3α-hydroxy-$\Delta^{9(11)}$-etiocholen-17-one, 3α-hydroxyetiocholan-11,17-dione, 3α-,11β-dihydroxyetiocholan-17-one,3α-hydroxy-$\Delta^{9(11)}$-androsten-17-one, 3α-hydroxyandrostan-11,17-dione, 3α,11β-dihydroxyandrostan-17-one.

They were all 3α-compounds, of which the final identification was made by infrared analysis.

In our experiments, which will be reported in detail elsewhere, we isolated a *3β-hydroxy-17-ketosteroid* from the urine of normal untreated *female* guinea pigs. This has not previously been reported, under similar experimental conditions. Moreover, we confirmed the findings of PERON *et al.*[1].

The urine of 10 female guinea pigs was collected daily for 6 days. After β-glucuronidase hydrolysis and subsequent cold acid hydrolysis at pH 0·5 with continuous ether extraction (for 48 h), the 17-KS containing extracts were chromatographed on alumina by gradient elution technique[2]. We obtained 7–8 Zimmermann-positive fractions. As is shown in Table I, fraction II was the greatest. It amounted to 44·9% of the total Zimmermann chromogens eluted and contained a 17-KS, which showed the same running rate as crystalline *epiandrosterone* on the paper chromatogram (chromatographic system used: propylene glycol/ligroin). In the mixed chromatogram of both the isolated substance and pure epiandrosterone, no separation occurred.

The sulphuric acid spectrum of the unknown revealed maximal optical density at 310 mμ. No increased absorption was apparent at 405 mμ, which is characteristic for dehydroepiandrosterone. The paper chromatographic properties of epiandrosterone and dehydroepiandrosterone are not much different. Both steroids have almost the same running rate in the system propylene glycol/ligroin.

The 3β-configuration of the isolated steroid was demonstrated by digitonin precipitation of the purified fraction II. Less than 10% of it appeared in the α-fraction, while the bulk of Zimmermann positive material was measured in the β-fraction. The infrared spectrum of the β-fraction was identical with that of crystalline epiandrosterone. The described substance could be isolated repeatedly in different series of experiments.

The question as to the nature of the 17-KS conjugates appearing in the urine of guinea pigs seemed to be of particular interest. In order to investigate the mode of conjugation of 17-KS in guinea pigs, we followed the procedure of CREPY *et al.*[3] given for the extraction and separation of steroid conjugates in human urine.

A 6-day-urine specimen collected from 10 female guinea pigs was adjusted to pH 11, followed by the addition of sodium chloride until a saturation of approximately 10% was achieved. The urine was then extracted twice with the same volume of n-butanol. The extract so obtained was chromatographed on alumina as reported by CREPY *et al.*[3]. In this manner it was separated into 3 Zimmermann-positive fractions, the second of which (eluted with 10% aqueous butanol) corresponded to 17-KS sulphates, the third of which (eluted with 15% aqueous (0·1 N NH_4OH) butanol) to 17-KS glucuronides (Table II). Subjected to high voltage paper electrophoresis the 10 % aqueous-butanol fraction revealed the mobility of Rb = 0·86, which is typical for 17-KS sulphates[4]. After cold acid hydrolysis (at pH 0·5) and simultaneous continuous ether extraction (for 48 h) the paper chromatographic evaluation of the above fraction disclosed 3α-hydroxyetiocholan-11,17-dione and higher polar 17-KS (probably 3α,11β-dihydroxy etiocholan-17-one and 3α,11β-dihydroxyandrostan-17-one). The glucuronide fraction (eluted with 15% aqueous

Table II

10 female guinea pigs-urine collection for 6 days. Extraction of conjugates with butanol at pH 11; chromatography of the extract on alumina (according to CREPY[3]).

Eluate	17-Ketosteroids (% of total amount eluted)	Glucuronic Acid
Butanol; 2% aqueous butanol	30–40%	–
6% aqueous butanol	–	–
10% aqueous butanol	40–50%	–
15% aqueous (0·1 N NH_4OH) butanol . . .	3–6%	1·8 mg

[1] F. G. PERON and R. I. DORFMAN, J. biol. Chem. *223*, 877 (1956).
[2] T. K. LAKSHMANAN and S. LIEBERMAN, Arch. Biochem. Biophys. *53*, 258 (1954). – W. STAIB and W. SCHILD, Klin. Wschr. *36*, 166 (1958).
[3] O. CREPY, M. F. JAYLE, and F. MESLIN, Acta endocrinol. *24*, 233 (1957).
[4] H. PELZER and W. STAIB, Clin. chim. Acta *2*, 407 (1957).

Table I

10 female guinea pigs-urine collection for 6 days. Gradient elution chromatography on alumina after β-glucuronidase hydrolysis and subsequent cold acid hydrolysis (at pH 0·5) with continuous ether extraction (for 48 h).

Total 17-Ketosteroids	Relative distribution of the 17-KS; recorded in % of the total 17-KS-excretion						
15·9 mg	Fraction I 10·3	Fraction II 44·9	Fraction III 5·3	Fraction IV 9·1	Fraction V 10·9	Fraction VI 14·3	Fraction VII 5·2

(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

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[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).