The formation of a new compound on irradiating (3655 Å) an aqueous solution of psoralen and a pyrimidine nucleoside was also confirmed by paper chromatography. After irradiation, in the chromatogram (examined at 3655 Å UV-light), within the spot of psoralen and other very slight spots due to the photolysis products of furocoumarin[5], there is an evident new spot with violet fluorescence.

The new spots obtained with various nucleosides have all the same violet fluorescence but different Rf values, as appears in Table III.

The spots of the nucleosides can be observed at 2537 Å UV-light and their Rf values are slightly smaller than those of the new compounds.

Preliminary experiments show that the formation of the new compounds takes place better by irradiating the mixture of psoralen and nucleosides in the solid state.

Irradiating an intimate solid mixture of thymidine and psoralen (2:1 mol) for 1 h with a Philips HPW 125 lamp (3655 Å) at a distance of 25 cm, preparing the chromatogram of the product obtained and eluting with methyl-alcohol the new spot with violet fluorescence and Rf 0.64 (see Table III), we have obtained a solution, which demonstrates a fluorescence spectrum (Figure 3) with a λ_{max} at 395 mμ, very similar to those (λ_{max} 400 mμ) shown by the irradiated solutions of psoralen and DNA or other pyrimidine nucleosides and nucleotides.

These are the first results of research work now under progress. We think we have obtained sufficient indications that a photoreaction occurs when a solution of DNA is irradiated in the presence of a *skin-active* furocoumarin.

At present, our goal is the isolation of the new-formed substances, the study of their biological significance, and the extension of these experiments to RNA.

Table III. Fluorescence and Rf values of new compounds formed in the photoreactions between psoralen and pyrimidine nucleosides (experimental conditions as in Table II)

Pyrimidine nucleosides	New chromatographic spots	
	Fluorescence (at 3 655 Å)	Rf butanol-acetic acid-water 4:1:5
Thymidine	violet	0.64
Cytidine	violet	0.32
Deoxy-cytidine	violet	0.52
Uridine	violet	0.43

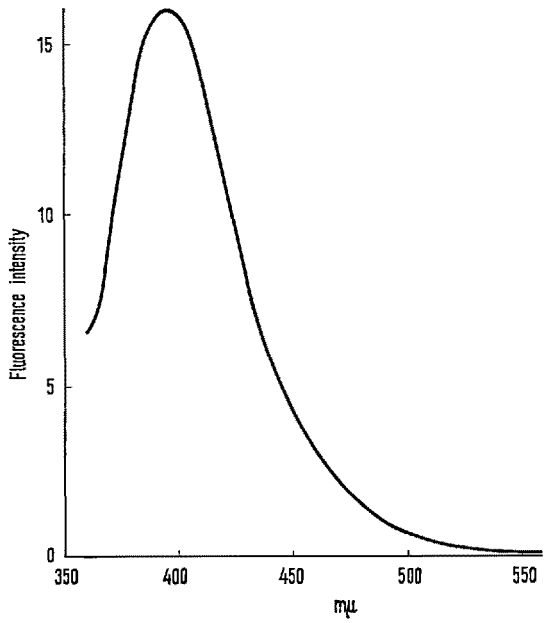

Fig. 3. Fluorescence spectrum of the new chromatographic spot obtained in the photoreaction between psoralen and thymidine. Activating wavelength: 330 mμ.

Riassunto. Modificazioni negli spettri di fluorescenza si hanno irradiando (3655 Å) soluzioni di DNA o di nucleosidi e nucleotidi pirimidinici in presenza di furocumarine fotosensibilizzatrici. La formazione di nuovi composti nelle fotoreazioni tra psoralene e nucleosidi pirimidinici è stata confermata per cromatografia su carta.

L. MUSAJO, G. RODIGHIERO, and F. DALL'ACQUA

Istituto di Chimica Farmaceutica dell'Università di Padova, Sezione III del Centro Nazionale di Chimica del Farmaco e dei Prodotti biologicamente attivi del Consiglio Nazionale delle Ricerche (Italy),
August 10, 1964.

[5] L. MUSAJO, G. RODIGHIERO, F. DALL'ACQUA, and G. CAPORALE, Gazz. chim. ital., in press.

The Steroid Hormone Synthesis in the Brown Adipose Tissue of Mice

The histological and biochemical differences between brown and white adipose tissue are so great that there are reasons for regarding them as different tissues. Although brown adipose tissue (BAT) has recently become the object of renewed interest, its physiological role has not yet been clarified. In extracts of BAT of hibernating and non-hibernating animals, the presence of steroid hormones has been stated[1-6]. The question whether the steroids revealed in BAT are not only stored up but also

[1] P. H. KRUTZSCH and W. W. WELLS, Proc. Soc. exp. Biol. Med. *105*, 578 (1960).
[2] W. PTAK, Endokrynologia Polska *13*, 111 (1962).
[3] W. PTAK, Folia Biol. *10*, 125 (1962).
[4] W. PTAK, Folia Biol. *11*, 347 (1963).
[5] A. R. RATSIMAMANGA, T. RAHANDRAHA, M. NIGEON-DUREUIL, and M. RABINOWICZ, J. Physiol. *50*, 479 (1958).
[6] L. ZIZINE, C. R. Acad. Sci. *242*, 681 (1956).

synthesized there, still remains unanswered. The aim of the present work is to demonstrate the possibility of steroid hormone synthesis in BAT and to state the conditions in which steroid output takes place.

The investigations were carried out on normal and adrenalectomized and castrated (AC) white male mice, Porton strain, weighing 20 g, two weeks after operation. The interscapular BAT was cut into pieces and placed in a Krebs-Ringer calcium-free phosphate medium (pH 7.4) containing 11 m*M* glucose, 0.7 m*M* ATP, 0.65 m*M* DPN, 25 m*M* nicotinamide and 20 m*M* sodium fumarate. The incubation was carried out at 37°C within 3 h of continuous gassing with pure oxygen. 800 mg of BAT were incubated in 10 ml of Krebs-Ringer solution. The experiments were taken up in 3 groups, each of them with BAT taken from normal and AC mice. In the first group the incubation was performed without any additional components, in the second group 2 IU of the commercial preparation of ACTH (Polfa) were added, in the third group pituitary glands of AC mice were added (2 per incubation vessel). In the control experiments pituitary glands of AC mice were incubated as above. The medium and tissue from flasks (10 in each group of experiments) were extracted three times with two volumes of chloroform. Extracts were further purified on silica gel column through which subsequently petroleum ether, benzene, chloroform, acetone and methanol were passed. The final extracts were again dried down. Paper chromatography was carried out on washed Whatman No. 1 paper at 24°C with the Bush B_5 (chloroform, acetone and methanol eluents) and cyclohexane-benzene/formamide solvent systems. Standard steroids were applied to lanes running parallel to the unknown samples. Steroids were detected on developed chromatograms by comparing their mobilities with those of the standards and by the following tests: reaction with blue tetrazolium, fluorescence with NaOH, $SbCl_3$, and H_3PO_4, UV-absorption of methanole eluates over the range 200–300 mμ in a Hilger spectrophotometer, and sulphuric acid spectrum.

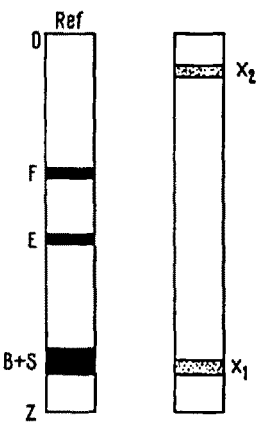

Diagram of chromatograms of extracts of brown adipose tissue incubation media (adrenalectomized and castrated mice) and of reference standards (Ref.). Solvent system: Bush B_5 at 24°C. Stippled spots represent the unknown substance, black areas correspond to the standard steroids run simultaneously. 0 – starting line, Z – solvent front. Reference standards: F – cortisol, E – cortisone, B – corticosterone, S – 17α-hydroxy-11-desoxycorticosterone. Unknown steroids: X_1 – Rf 0.90, blue tetrazolium positive, NaOH fluorescent yellow, $SbCl_3$ fluorescent orange, H_3PO_4 fluorescent orange. Sulphuric acid spectrum-peaks at 280 and 535 mμ (17α-hydroxy-11-desoxycorticosterone). X_2 – Rf 0.09, blue tetrazolium positive, NaOH fluorescent blue, $SbCl_3$ fluorescent orange, H_3PO_4 fluorescent negative (unknown, 17α,19-dihydroxycortexone?).

Chromatograms representing the first group, as well as incubated pituitary glands, revealed no steroid spots. In the second group steroid compounds were seen in only trace quantities. Distinct steroid spots were found only in the third group of chromatograms representing AC mice, opposite to the 17α-hydroxy-11-desoxycorticosterone standard. On the basis of colour reactions and sulphuric acid spectrum (peaks at 280 and 535 mμ) the unknown steroid compound was identified as 17α-hydroxy-11-desoxycorticosterone (Compound S). We failed to identify a high polar steroid which in trace quantities was seen to appear on the chromatograms (17α,19-dihydroxycortexone?) (see Figure). Quantitative determinations of the size of the steroid synthesis in BAT were carried out at 254 mμ in a spectrophotometer, and results were calculated as μg of Compound S/100 mg of BAT/1 h. This output ranges from 1.7 to 2.5 μg, and is thus approximatively 10–30 times smaller than the average steroid synthesis in adrenals.

There is a close relationship between the established results and those which were published in our former reports. In 1963 we demonstrated the presence of Compound S in extracts of BAT in mice and suggested the possibility of a steroid synthesis there[6]. As shown by our observations, BAT can take up the steroid hormone production if a steroid demand at the periphery exists (adrenalectomy and castration). This is further substantiated by the fact that equal quantities of BAT of normal mice fail to yield detectable amounts of steroids. The secreted steroid compound has, however, only a weak glycotropic action and exerts very slight influence on Na retention. As early as 1958, ZIZINE[7] ascertained that BAT in rats was able to synthesize desoxycorticosterone from progesterone (unfortunately without giving methodic details). In our experiments we cannot confirm any steroid production in BAT of normal mice. Only simultaneous adrenalectomy and castration of mice, and the addition to the incubating medium of the pituitary glands of AC mice, caused the stimulation of BAT. Which of the hypophyseal hormones is responsible for this stimulation remains unexplained, since the preparations of ACTH used by us were ineffective. As in any in vitro system, one cannot assume that the secretory product observed corresponds to those normally secreted by the BAT.

Zusammenfassung. Braunes Fettgewebe adrenalektomierter und kastrierter weisser Mäuse synthetisiert in vitro 17α-hydroxy-11-desoxycorticosteron in einer Menge 1,7–2,5 μg/100 mg des Fettgewebes/h, wenn dem Inkubationsmedium Mäusehypophyse zugesetzt wird. Zugabe von ACTH bewirkt keine Stimulierung des braunen Fettgewebes.

W. PTAK

Department of Medical Microbiology, Medical Academy, Kraków (Poland), August 10, 1964.

[7] L. ZIZINE, C. R. Acad. Sci. *246*, 1091 (1958).