

bei dem es sich den chromatographischen Untersuchungen zufolge höchstwahrscheinlich um Glukose handelt.

Der biologische Effekt des isolierten Extraktes wurde an Rattenmännchen verschiedenen Alters und an Mäusemännchen untersucht. Die Lösung wurde den Tieren s.c. eingespritzt, und zwar 0,3–0,5 ml/Tier und Tag während 5 Tagen. Es wurden insgesamt 27 Ratten und 10 Mäuse untersucht. In jeder Versuchsgruppe und Kontrollgruppe befanden sich Tiere aus demselben Wurf. Die Rattenmännchen waren 21–70 Tage alt. Die Wirkung des Extraktes wurde sofort nach dessen Gewinnung sowie nach 3 Monate dauerndem Aufbewahren bei Zimmertemperatur untersucht. Die Versuchsergebnisse sind in der Tabelle angeführt.

Die Angaben in der Tabelle zeigen, dass der Extrakt bei Rattenmännchen das Gewicht und die Grösse der Samenbläschen beeinflusst (Figur 1). Im frischen Zustand übt der Extrakt auch auf den Hoden seine Wirkung aus, und diese ist stärker, als wenn er längere Zeit aufbewahrt wurde. Bei jungen Tieren wird auch das Gewicht der Hoden erniedrigt, was bei älteren Tieren nicht der Fall ist. Der Hoden ist kleiner, sein Turgor ist herabgesetzt

(Figur 2). Histologisch kann man in Hodenschnitten eine starke Gonozytenzellenreduktion beobachten (Figuren 3 und 4). Bei Mäusemännchen wurde bei derselben Menge Extrakt kein Einfluss auf die Samenbläschen gefunden, und das Gewicht dieses Organs entsprach demjenigen der Kontrolltiere.

Summary. Extracts of lucerne with 0.1 N HCl prepared and fractionated by the method of ADLER¹⁶ give a filtrate which contains anantigonadal substance. This can be obtained from the extract after precipitation with lead acetate and evaporation. The residue after extraction with cold pyridin contains long needle-crystals insoluble in ethanol, methanol, ether and chloroform. A solution of these crystals injected into rats caused a decrease in weight of the seminal vesicles. In the mouse this substance is without effect.

J. CHURÝ

Biologisches Institut, Fakultät für Veterinärmedizin, Brno (ČSSR), 28. Oktober 1966.

Uridinediphosphoglucose: Glycogen α -4-Glucosyltransferase of Human Myometrium

It is now known that in animal tissues glycogen is synthesized from uridinediphosphoglucose. It was therefore surprising that BO and SMITH¹ could not demonstrate uridinediphosphoglucose-glycogen glucosyltransferase activity in the rat uterus, so that it had to be admitted that glycogen synthesis occurs in the uterus through the phosphorylase reaction. RUBULIS et al.², on the other hand, showed that uridinediphosphoglucose-glycogen glucosyltransferase is active in the rat uterus as well as in the human endometrium, while BARGONI et al.³ observed this reaction in the smooth muscle of the pigeon gizzard. Therefore I have measured uridinediphosphoglucose-glycogen glucosyltransferase, believed to be the rate-limiting enzyme for glycogen synthesis⁴, in myometrial tissue. Determinations of α -1,4-glucan:orthophosphate glucosyltransferase and glycogen were also made.

Table I. Uridinediphosphoglucose-glycogen glucosyltransferase in the human myometrium

Tissue	μ g uridinediphosphate formed/mg protein/15 min
Myometrium:	
Non-gravid	29
Gravid, at term	35 46 49 53
Prolonged pregnancy	25
Placenta praevia	18
Myoma	16

Human myometrial tissue obtained from the lower uterine segment during Caesarean section and from the anterior wall of uterine body during hysterectomy, was used for the experiments. The homogenate, prepared with 0.25 M sucrose and 0.001 M ethylenediaminetetraacetate, was centrifuged at 1200 rpm at 0°C for 10 min; 0.05 ml of the supernatant was used for the assay carried out according to LELOIR and GOLDBERG⁵. Glycogen phos-

Table II. Glycogen in the human myometrium

Tissue	mg glycogen/g fresh tissue
Myometrium:	
Post-menopausal	1.90 1.80
Non-gravid	3.09 3.10
Gravid, at term	3.50 3.50 4.20 3.70 4.10
Myoma	2.50

¹ M. J. BO and M. S. SMITH, Proc. Soc. exp. Biol. Med. 113, 814 (1963).

² A. RUBULIS, R. D. JACOBSON and E. C. HUGHES, Biochim. biophys. Acta 99, 584 (1965).

³ N. BARGONI, A. SISINI and C. MAJORANO, G. Biochim. 13, 54 (1964).

⁴ C. VILLAR-PALASI and J. LARNER, Archs Biochem. Biophys. 94, 436 (1961).

⁵ L. F. LELOIR and S. H. GOLDBERG, J. biol. Chem. 235, 919 (1960).

phorylase was assayed by the method of DANFORTH et al.⁶ in a homogenate prepared with 0.15 M KCl. Glycogen was extracted from the tissue by the procedure of GOOD, KRAMER and SOMOGYI⁷ and measured according to

Table III. Glycogen-phosphorylase in the human myometrium

Tissue	$\mu\text{g Pi formed/mg protein/20 min}$
Myometrium:	
Non-gravid	3.8 4.1
Gravid, at term	6.4 8.5 11.0 8.6 11.2
Myoma	3.4

Table IV. Effect of glucose-6-phosphate and adenosine-3,5-cyclic phosphate on uridinediphosphoglucose-glycogen glucosyltransferase of human myometrium

Addition	$\mu\text{g uridinediphosphate formed/mg protein/15 min}$
None	11
Glucose-6-phosphate	34
Adenosine-3,5-cyclic phosphate	36

Zimmermann Reaction of 3-, 6- and 20-Oxosteroids

JAMES and FOTHERBY¹ found that either 5 α - or 5 β -pregnane-3,6,20-trione gave on paper a characteristic Zimmermann reaction; a blue-grey colour appeared initially, which after about 30 min had faded to a brownish-grey colour, and within 24 h the colour had almost completely disappeared. The colour obtained with 17-oxosteroids was stable under these conditions. It was shown with a limited number of steroids that the 6-oxo group did not react with the Zimmermann reagent²⁻⁵. However, the influence of the 6-oxo group on the chromogenicity of steroids in the Zimmermann reaction has received little attention. Recent work in this laboratory made available a number of steroids containing a 6-oxo group, and their behaviour in the Zimmermann reaction was studied.

0.25 ml of Zimmermann reagent (2:1 v/v mixture of 1% *m*-dinitrobenzene in ethanol and 40% benzyltrimethylammonium hydroxide) was added to triplicate 50 or 100 μg samples of the steroid. After incubation for 5, 30 and 60 min, 3 ml ethanol was added to each tube and the absorption spectrum of the solution read from

MONTGOMERY⁸. Protein was assayed by the method of LOWRY et al.⁹.

The results are summarized in Table I. As is shown, uridinediphosphoglucose-glycogen glucosyltransferase is clearly present in the human myometrium, although not in great amounts. Glycogen is also found in the myometrium in small quantities – decidedly less than in striated muscle and about as much as in the myocardium (Table II). Moreover, glycogen-phosphorylase activity being also fairly low (Table III) does not point to the fast degradation of the polysaccharide after its synthesis.

Like uridinediphosphoglucose-glycogen glucosyltransferase of other tissues, the enzyme of myometrium is activated by glucose-6-phosphate and by adenosine-3,5-cyclic phosphate (Table IV).

Riassunto. L'uridindifosfoglicoso glicogeno glucosil-transferasi si trova sicuramente nel miometrio umano. Ne sono attivatori glucoso-6-fosfato e adenosin-3,5-mono-fosfato ciclico.

A. LANZA

Institute of Biochemistry of the University of Torino and St. Anna Obstetric and Gynecologic Hospital (I Div.), Torino (Italy), 31st October 1966.

⁶ W. H. DANFORTH, E. HELMREICH and C. F. CORI, *Proc. natn. Acad. Sci. U.S.A.* **48**, 1191 (1962).

⁷ C. GOOD, H. KRAMER and M. SOMOGYI, *J. biol. Chem.* **100**, 485 (1933).

⁸ R. MONTGOMERY, *Archs Biochem. Biophys.* **67**, 378 (1957).

⁹ F. C. LOWRY, N. J. ROSEBROUGH, A. L. FARR and R. J. RANDALL, *J. biol. Chem.* **193**, 265 (1951).

320 to 620 nm against a reagent blank using a Beckman DB recording spectrophotometer. The time taken to scan the wavelength range was 7 min. The wavelength of the main peaks of the absorption spectra and the molar extinction coefficients for the steroids examined are shown in the Table.

Of the steroids with an isolated 3-oxo group the 5 α -isomers had a much higher molar extinction coefficient at 550 nm after 5 min than the 5 β -isomers. However, the 5 β -isomers showed a more complex spectrum than the 5 α ones; at 5 and 30 min a 360 nm peak was present with small shoulders at 415 and 440 nm. This 360 nm peak given by the 5 β -3-oxosteroids was suggested by BROADBENT and KLYNE⁵ to be useful in the differentiation of

¹ F. JAMES and K. FOTHERBY, *Biochem. J.* **95**, 459 (1965).

² K. KAZIRO and T. SHIMADA, *Hoppe-Seyler's Z. physiol. Chem.* **249**, 220 (1937).

³ N. H. CALLOW, R. K. CALLOW and C. W. EMMENS, *Biochem. J.* **32**, 1312 (1938).

⁴ A. E. HOLTORFF and F. C. KOCH, *J. biol. Chem.* **135**, 377 (1940).

⁵ I. E. BROADBENT and W. KLYNE, *Biochem. J.* **56**, 30 (1954).