

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

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.

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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).

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

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).

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

Steroid	Wavelength (nm) of main peaks after incubation of steroid for:		
	5 min	30 min	60 min
5 α -Pregnane-3, 6, 20-trione 5 β -Pregnane-3, 6, 20-trione	{ 500-550 (53)	{ 495 (75) Shoulder at 550 (60)	{ 490 (83)
5 β -Pregnane-3, 6-dione 5 α -Cholestane-3, 6-dione 3, 6-Dioxo-5 β -cholanolic acid	550 (41) 550 (39) 550 (43)	500 (45) { Broad peak from 500-560	495 (30) 490 (35) 490 (41)
3 β -Acetoxy-20 β -hydroxy-5 α -pregnan-6-one 3 α -Hydroxy-5 β -cholanolic acid-6-one 3 β -Hydroxy-5 α -cholestan-6-one	{ No reaction at any time		
5 α -Pregnan-3-one 5 α -Cholestan-3-one 5 β -Pregnan-3-one	550 (59) 550 (58) 550 (22), 360 (45) Shoulders at 415 (26), 440 (26)	{ Peak decreases with time of incubation 550 (23) 360 (54) Shoulders at 415 (34), 440 (34)	
3-Oxo-5 β -cholanolic acid	550 (33), 360 (73) Shoulders at 415 (40), 440 (33)	550 (31), 360 (73) Shoulders at 415 (46), 440 (38)	Shoulders at 360 (61), 415 (42), 440 (35)
5 α -Pregnane-3, 20-dione 5 β -Pregnane-3, 20-dione	550 (66) 550 (38), 360 (62) Shoulders at 415 (41), 440 (37)		490 (60) 490 (60) Shoulders at 360 (71), 415 (58), 440 (56)
3 β -Hydroxy-5 β -pregnan-20-one 3 α -Hydroxy-5 β -pregnan-20-one 3 β -Hydroxy-5 α -pregnane-6, 20-dione 3 α , 6 α -Dihydroxy-5 β -pregnan-20-one	{ Peak at 490 nm increasing with time of incubation		490 (51) 490 (54) 490 (51) 490 (48)

Figures in parenthesis denote molar extinction coefficient.

5 α - and 5 β -3-oxosteroids; when a 6-oxo group was present this difference between the isomers disappeared. Neither the 6-oxo nor the 6-hydroxyl group had any effect on the characteristic peak at 490 nm shown by a C₂₀-oxo group.

Pregnane-3, 6, 20-triones gave a higher extinction at 490 nm after 1 h than the corresponding 3, 20-diones. That this was not due entirely to the 20-oxo group was shown by the fact that 3, 6-diones also showed slight absorption at 490 nm after 1 h, presumably due to interaction of the 3- and 6-oxo groups as the 6-oxo group did not affect the reaction of a C₂₀-oxo group.

Zusammenfassung. Die Farbreaktion nach Zimmermann von Steroiden mit 3-, 6- oder 20-Oxogruppen wurde spektrophotometrisch untersucht. Während 6- und 20-Oxogruppen sich gegenseitig nicht störten, war eine Beeinflussung zwischen den 3- und 6-Oxogruppen zu beobachten.

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15th November 1966.*

Action of Reserpine and Imipramine on Intracellular Storage of 5-Hydroxytryptamine in Blood Platelets

In blood platelets of guinea pigs, reserpine and imipramine markedly decrease the uptake of 5-hydroxytryptamine (5HT) from the incubation medium, e.g. Tyrode solution^{1,2}. Reserpine also diminishes the osmophilic organelles which seem to be the intracellular storage sites of 5HT in platelets of rabbits³. It has not yet been demonstrated whether interference with the 5HT uptake by imipramine is accompanied by a decrease of the intracellular 5HT storage organelles in situ. Platelets of rabbits do not seem to be appropriate models for study-

ing this question since, according to preliminary experiments, the uptake of 5HT is only moderately diminished by imipramine. Platelets of guinea pigs, on the other hand, which are very sensitive to imipramine, contain only very few 5HT storage organelles³, so that their quantitative estimation is difficult.

¹ F. B. HUGHES and B. B. BRODIE, *J. Pharmac. exp. Ther.* 127, 96 (1959).

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