Steroid	Wavelength (nm) of main peaks after incubation of steroid for: 5 min	30 min	60 min
5α-Pregnane-3,6,20-trione	500–550 (53)	495 (75)	490 (83)
5β-Pregnane-3,6,20-trione		Shoulder at 550 (60)	
5β-Pregnane-3,6-dione	550 (41)	500 (45)	495 (30)
5α-Cholestane-3,6-dione	550 (39)	Broad peak from 500–560	490 (35)
3,6-Dioxo-5β-cholanic acid	550 (43)		490 (41)
3β-Acetoxy-20β-hydroxy-5α-pregnan-6-one	No reaction at any time		
3α-Hydroxy-5β-cholanic acid-6-one			
3β-Hydroxy-5α-cholestan-6-one			
5α-Pregnan-3-one	550 (59)	Peak decreases with time of incubation	
5α-Cholestan-3-one	550 (58)		
5β-Pregnan-3-one	550 (22), 360 (45) Shoulders at 415 (26), 440 (26)	550 (23) 360 (54) Shoulders at 415 (34), 440 (34)	Shoulders at 360 (35), 415 (24), 440 (21)
3-Oxo-5β-cholanic 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)
5α-Pregnane-3,20-dione	550 (66)		490 (60)
5β-Pregnane-3,20-dione	550 (38), 360 (62) Shoulders at 415 (41), 440 (37)		490 (60) Shoulders at 360 (71), 415 (58), 440 (56)
3β-Hydroxy-5β-pregnan-20-one	Peak at 490 nm increasing with time of incubation		490 (51)
3α-Hydroxy-5β-pregnan-20-one			490 (54)
3β-Hydroxy-5α-pregnane-6,20-dione			490 (51)
3α,6α-Dihydroxy-5β-pregnan-20-one			490 (48)

Figures in parenthesis denote molar extinction coefficient.

5α- and 5β-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 C_{20}-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 C_{20}-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 osmiophilic organelles which seem to be the intracellular storage sites of 5HT in platelets of rabbits[3]. 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 studying 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 organelles[3], so that their quantitative estimation is difficult.

[1] F. B. Hughes and B. B. Brodie, J. Pharmac. exp. Ther. *127*, 96 (1959).
[2] A. Pletscher, W. P. Burkard, J. P. Tranzer and K. F. Gey, Life Sci. *6*, 273 (1967).
[3] J. P. Tranzer, M. Da Prada and A. Pletscher, Nature *212*, 1574 (1966).

Therefore, in the present work, cat platelets have been used, since their number of osmiophilic storage organelles is sufficient for quantitative estimation and because reserpine as well as imipramine cause a marked inhibition of their 5HT uptake.

Experimental. Cats weighing 2–3 kg, fasted for 16 h, were exsanguinated in nembutal anaesthesia through a cannula in the carotid artery. The platelets were isolated as previously described[4], centrifugated and resuspended in modified Tyrode solution, 1 ml suspension containing the amount of platelets present in 1–2 ml of the original plasma. The following experiments were carried out at 37 °C:

(a) Incubation of the platelets with ^{14}C-5HT (specific activity 40 mC/mM) alone and with ^{14}C-5HT in the presence of imipramine or reserpine. After 2 washings of the centrifugated platelets with ice-cold Tyrode, the ^{14}C-5HT of one part of the platelets was extracted as previously described[5] and measured in a liquid scintillation counter. Another part of the platelets was fixed with glutaraldehyde-osmium tetroxyde and embedded into Epon. The ultrathin sections were contrasted with uranyl-acetate and lead citrate and subjected to electron microscopy[2]. Thereby, the osmiophilic organelles were estimated semiquantitatively as indicated earlier[3].

(b) Spectrophotofluorimetric determinations of the endogenous 5HT in platelets[5] incubated without and with imipramine or reserpine.

Results. (1) In platelets incubated with imipramine and reserpine the uptake of ^{14}C-5HT from the medium is markedly inhibited by reserpine as well as by imipramine. The inhibition increases with rising concentrations of the drugs. Already $10^{-7}M$ reserpine and $10^{-6}M$ imipramine are effective. At concentrations of $1.4–55 \cdot 10^{-6}M$ ^{14}C-5HT in the medium, $1.6 \cdot 10^{-6}M$ (1 γ/cm^3) reserpine and $10^{-5}M$ imipramine cause a marked inhibition of the amine uptake which is about equal for both drugs (Figure 1).

(2) Incubation of normal platelets with reserpine ($1.6 \cdot 10^{-6}M$) markedly decreases the endogenous 5HT (e.g. to 47 $\pm$ 4 and 22 $\pm$ 3 % of controls after 6 and 12 h respectively). Imipramine ($10^{-5}M$) has much less effect since the drug lowers the amine content to about 90 % only (Figure 1). Incubation of platelets in Tyrode alone does not significantly decrease the endogenous 5HT within 12 h.

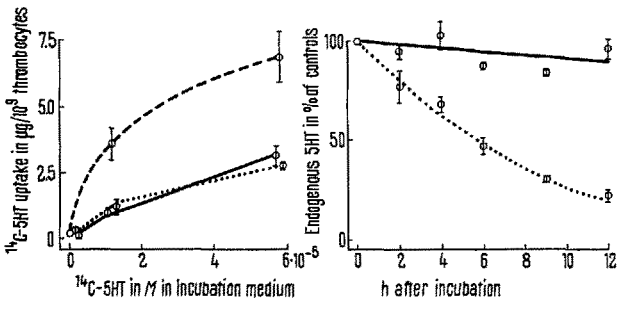

Fig. 1. Effect of imipramine and reserpine on the uptake of ^{14}C-5-hydroxytryptamine (left) and on endogenous 5-hydroxytryptamine (5HT) (right) of cat platelets. In the experiments with ^{14}C-5HT (left) the platelets were incubated for 1 h. The endogenous 5HT was related to that of platelets incubated in Tyrode alone (controls). Absolute values for endogenous 5HT of normal platelets: 12.5 $\pm$ 3.2 μg/10^8 thrombocytes. Each point represents an average of 2–5 experiments $\pm$ S.E. ------- controls, ——— imipramine ($10^{-5}M$), ... reserpine ($1.6 \cdot 10^{-6}M$).

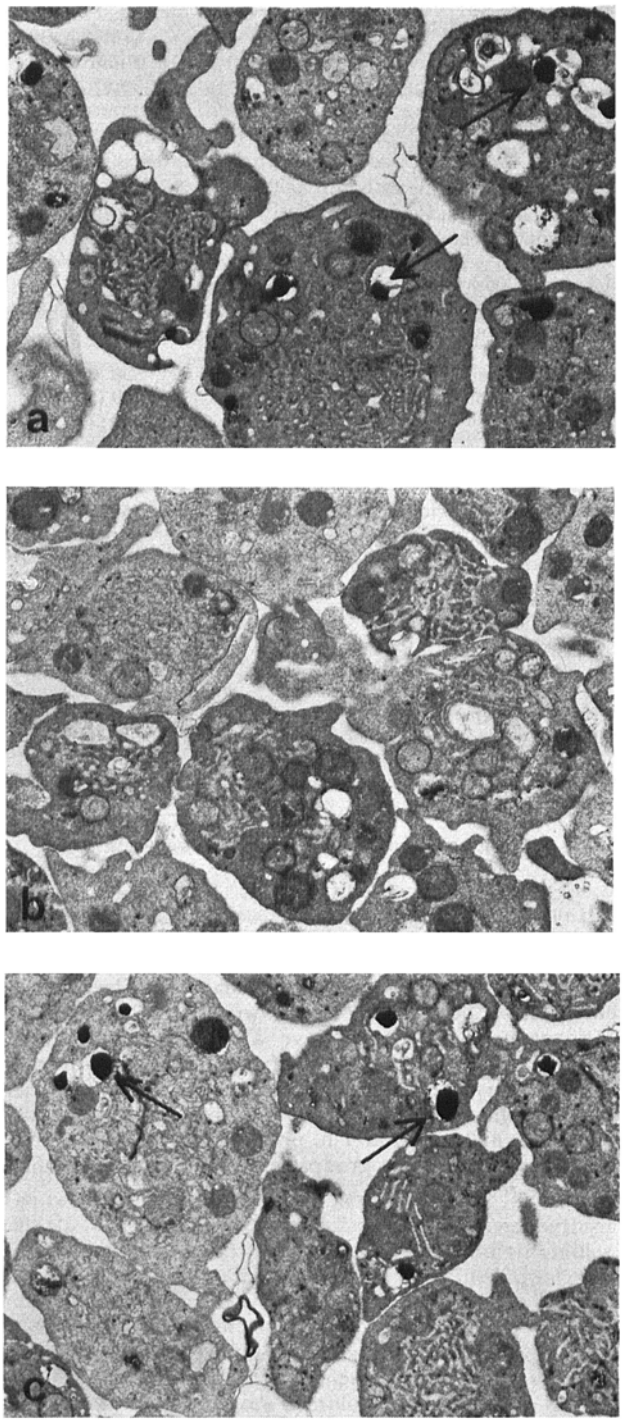

Fig. 2. Electron micrographs of cat platelets incubated in vitro for 12 h at 37 °C (a) in Tyrode alone, (b) in Tyrode containing reserpine $1.6 \cdot 10^{-6}M$, (c) in Tyrode containing imipramine $10^{-5}M$. Considering the long incubation times, the ultrastructure is reasonably well preserved. 5HT organelles (dense osmiophilic bodies —→) are present in (a) and (c) but not in (b). × 14,000.

[4] G. Bartholini, A. Pletscher and K. F. Gey, Experientia *17*, 541 (1961).

[5] D. F. Bogdanski, A. Pletscher, B. B. Brodie and S. Udenfriend, J. Pharmac. exp. Ther. *117*, 82 (1956).

(3) The change of the intracellular storage organelles of the platelets is similar to that of the total endogenous 5HT. Thus, after incubation of 6 and 12 h respectively, reserpine diminishes the number of the organelles to 32 ± 4 and $18 \pm 2\%$, whereas imipramine causes no significant diminution (105 ± 4 and $102 \pm 2\%$ compared with controls) (Figure 2).

Discussion. According to the present results, reserpine ($1.6 \cdot 10^{-6} M$), in contrast to imipramine ($10^{-5} M$, markedly decreases the endogenous 5HT of the platelets although both drugs inhibit the uptake of exogenous (radioactive) 5HT to a similar extent. These findings confirm that reserpine and imipramine diminish the uptake of ^{14}C-5HT in platelets by 2 different mechanisms. Reserpine probably interferes with the storage of 5HT in the intracellular organelles leading to a decrease of the endogenous 5HT as well as of the number of the organelles storing 5HT. This has also been shown in earlier experiments in rabbits and guinea pigs to which the drug was administered in vivo[2,3].

Imipramine does not markedly impair the amine storage in the 5HT organelles according to the present experiments. The drug seems, however, to inhibit the 5HT transport through the platelet membrane. Thus, in guinea pig platelets incubated with ^{14}C-5HT, imipramine diminished the formation of ^{14}C-5HT metabolites without interfering with the mitochondrial monoamine oxidase (MAO)[2]. This indicates that the penetration of the amine into the cell is inhibited. In preliminary experiments a similar action of imipramine has been demonstrated in cat platelets.

Since imipramine markedly inhibits the uptake of ^{14}C-5HT by platelets, it probably also interferes with the re-uptake of spontaneously released 5HT by platelets under physiological conditions (e.g. in plasma or Tyrode). According to the present experiments, however, the drug does not markedly affect the stored endogenous amine in the cells. It may therefore be assumed that under physiological conditions spontaneous release of stored 5HT into the extracellular space and re-uptake of the amine into the storage organelles do not occur to a major extent. Interference with the intracellular storage mechanism, e.g. by reserpine, is therefore more effective in depleting the endogenous platelet 5HT than inhibition of the amine transport through the cell membrane as exerted by imipramine.

Zusammenfassung. In isolierten Blutplättchen von Katzen vermindert Imipramin im Gegensatz zu Reserpin das endogene 5-Hydroxytryptamin (5HT) und die 5HT-Speicherorganellen nicht wesentlich, obwohl es wie Reserpin die Aufnahme von exogenem 5HT herabsetzt. Hemmung des Membrantransportes von 5HT durch Imipramin scheint also das intrazellulär gespeicherte 5HT nur relativ wenig zu beeinflussen.

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Medizinische Forschungsabteilung der F. Hoffmann-La Roche & Co. AG, Basel (Switzerland), 27th December 1966.

Comparative Effects of Carbon Tetrachloride and Colchicine on Xanthine Dehydrogenase

Our laboratory has been concerned with changes in serum xanthine oxidase (XO) following CCl_4 administration[1]. It has also been demonstrated that colchicine is able to produce increased levels of rat serum XO[2] and reduced levels of the enzyme in the liver under identical experimental conditions[3].

Experiments on the simultaneous changes in serum and liver enzyme activity after CCl_4 or colchicine administration were undertaken in order to study the disappearance of the enzyme from liver and the possible correlation of these changes with a disturbance in the lipid metabolism under these experimental conditions.

Materials and methods. Adult male Wistar rats ranging in weight from 100–150 g were used throughout. The animals were injected i.p. with a single dose of either CCl_4 (0.1 ml/100 g body weight) or colchicine (0.1 mg/100 g body weight). The colchicine solution contained 20 mg of the alkaloid/100 ml of 6.8% ethanol solution (v/v) and the results presented in this paper for colchicine treated rats have already been corrected for the values obtained for animals injected with a single dose of a 6.8% alcohol solution.

The animals were starved for 20 h, unless otherwise stated. Blood samples were collected by heart puncture under light ether anaesthesia, at the proper time intervals (usually 20 h). In some cases, the liver was removed, weighed, and homogenized in a Warring blender in $0.015 M$ pyrophosphate buffer, pH 8.6, so that the fina homogenate contained 200 mg of tissue/ml of homogenate. Dry weight was determined in all cases.

The xanthine dehydrogenase (XD) activity was measured in 0.5 or 1 ml of clear blood serum incubated with 0.1 ml of a $0.05 M$ hypoxanthine solution as substrate and 0.3 ml of a 0.1% triphenyltetrazolium chloride solution as hydrogen acceptor in an evacuated Thunberg tube.

XO activity was determined manometrically in a Warburg respirometer at 37 °C as described previously by Villela and Mitidieri[4]. XO as well XD activity determinations were carried out in pyrophosphate buffer, pH 8.6.

Iodine value was determined according to Yasuda[5] and total lipids were estimated colorimetrically following the method of Bragdon[6].

Results and discussion. Serum XO and XD activities of normal control rats, of rats treated with CCl_4, and of rats

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[3] O. R. Affonso, E. Mitidieri and G. G. Villela, Nature *192*, 666 (1961).
[4] G. G. Villela and E. Mitidieri, Nature *175*, 208 (1955).
[5] M. Yasuda, J. biol. Chem. *94*, 401 (1931).
[6] J. Bragdon, J. biol. Chem. *190*, 513 (1951).