This inhibition was progressive, being just obvious after 4 hr. and very marked after 24 hr., and it ran parallel with the cell damage observed histologically.

2. Anaerobic glycolysis was also inhibited in the rabbit spleen, but only after a much longer time (48 hr.). At this late stage, histological damage was present.

3. No inhibition of anaerobic glycolysis or respiration was observed in brain slices from rats 48 hr. after poisoning.

4. We were not able to find any inhibitory effect on the anaerobic glycolysis of normal marrow cells by serum obtained from rabbits poisoned with the usual dose of *H*.

5. Neither haemolyzed red cells from a poisoned rabbit nor fat obtained from the marrow of such animals, had any effect on the anaerobic glycolysis of normal marrow cells.

6. Addition of *H* (0·0026M) to marrow cells *in vitro* caused immediate inhibition amounting to 50%. No effect was observed with the same amount of *H* after reaction with serum.

7. The acid production from *H* in serum and in whole blood took about 45 min. for completion at 38°; 85% was produced in 30 min.

8. Excluding part of the bone marrow of rats from the circulation for 1 hr. after injection of a lethal dose of *H* (2 mg./kg.) did not protect the marrow from the cell damage caused by *H*, as determined histologically.

This work was carried out for the Ministry of Supply in 1942–3, as part of the work of an Extramural Research Team under the direction of Dr M. Dixon, and was contained in a report to the Ministry dated 1944. We are indebted to the Chief Scientist Ministry of Supply for permission to publish this paper. We would also express our gratitude to Dr M. Dixon for his constant advice and interest.

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Precipitation of Steroid Ketones with Digitonin

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It is frequently held that a steroid is precipitable by digitonin by virtue of a free 3-hydroxyl group of the β-configuration (formula (I)). There are in fact at least four known exceptions to this rule, namely, *allo*-pregnan-3(β)-ol-20-one acetate, *allo*pregnane-3:20-dione (Butenandt & Mamoli, 1935), 'α'-oestradiol and its 3-benzoyl derivative (Wintersteiner, 1937). In the course of some work on the metabolism of cholest-4-en-3-one, it was discovered that this substance also is precipitable by digitonin. The following is a brief account of this reaction applied to some steroid ketones.

METHODS

The ketones, together with four parts by weight of digitonin, were dissolved with heating, in 90% ethanol-water. After at least 24 hr. at 18–20°, the digitonides were collected, washed with cold 90% ethanol-water, and ether, dried at *c.* 70° and decomposed by a single pyridine-ether treatment in the usual way. The ketones, recovered by evaporation of the washed ether, were crystallized from dilute ethanol. Table 1 shows the weights of digitonide and of crystalline recovered ketone. The amounts used were 50 mg. of ketone and 200 mg. of digitonin in all cases except that of progesterone, when 25 mg. of ketone and 100 mg. of digitonin were used. Recovered ketones were identified by mixed melting-point determinations.

The digitonin used in the above experiments did not itself crystallize out in 24 hr. at 18–20°, although it sometimes did so on longer standing. Unlike the precipitates of digitonides (except, perhaps, that from progesterone) the digitonin crystals themselves were readily soluble in cold water.

Table 1. *Yields of digitonides and of recovered ketones from digitonin precipitation in 90% ethanol. Amounts of starting materials given in text*

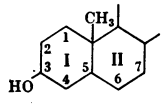
Steroid	Vol. of 90% ethanol used to dissolve the mixture of steroid and digitonin (ml.)	Weight of digitonide (mg.)	Weight of recovered ketone (mg.)
Cholestanone	16	170	40
Coprostanone	5	0	0
Cholest-1-en-3-one	5	0	0
Cholest-4-en-3-one	5	190	40
Cholest-5-en-3-one	10	180	35
β-Sitost-4-en-3-one	5	180	40
Progesterone	2	60	10

RESULTS

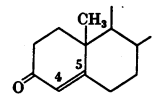
These are shown in Table 1.

DISCUSSION

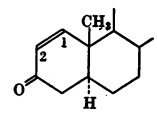
Since cholest-4-en-3-one (II) yields a digitonide whilst cholest-1-en-3-one (III) does not, it might seem that the reaction depended upon enolization from C_2 to give a 3(β)-hydroxyl group (IV); if this were so, it is difficult to see why coprostanone (V)



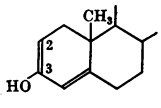
I



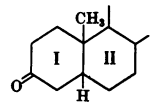
II



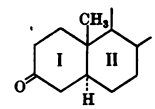
III



IV



V



VI

does not react. The absence of the reaction with coprostanone (V) in contrast to cholestanone (VI) suggests that a *cis*-configuration of rings I and II is less favourable to digitonide formation than a *trans*- or flat (Δ^4- or Δ^5-) configuration. Nevertheless, the lack of precipitation with cholest-1-en-3-one (*trans*) remains unexplained.

From a consideration of the literature it is clear that digitonin precipitation does not depend only on a 3(β)-hydroxyl group, and may fail when such a group is present (cf. Butenandt & Fleischer, 1937; Reichstein & Gätzi, 1938; Spring & Swain, 1941; Wintersteiner & Ruigh, 1942). The reaction is evidently complex, but no steroid without an oxygen atom at C_3 has so far been found to precipitate with digitonin. Digitonin precipitation can be given approximately quantitative expression (Noller, 1939; Haslewood, 1948) and the figures obtained may assist in planning experiments and in interpretation of their results, especially where studies of steroid metabolism are concerned.

SUMMARY

Digitonides have been prepared from certain steroids with a keto group at C_3: the ketones have been recovered from these derivatives.

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