cells show morphologic injury. A large number of granulocytes show changes similar to those characteristic of the L.E. cells. These changes are reversible if the shock plasma is replaced by normal plasma. 2) Shock plasma produces a severe depression of the phagocytic index; the percent of bacteria ingested is some 40% less than in normal plasma, and the percent of cells containing bacteria is some 30% less than in normal plasma. Shock plasma also reduces the bacteriostatic power of the phagocytes. The granulocyte in this respect appears to be damaged more severely than the macrophage. The extent of the injury produced by the plasma from rabbits in advanced shock appears to be no greater than that produced by the plasma of rabbits after two hours of shock, *i.e.* while they are still responsive to transfusion. The injurious property of plasma of the reversibly shocked rabbit persists, with undiminished potency, for at least four hours after transfusion. 3) It is concluded that a leucotoxin develops in the blood of the rabbit in hemorrhagic shock, and that this leucotoxin severely impairs the antibacterial potential of the animal.

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Reduction of Androstane-3,17-dione by Liver Homogenates.* (22795)

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Androstane-3, 17-dione is formed *in vitro* from androstane-3α, 17β-diol by homogenates of the liver and kidney of the rat, guinea pig and rabbit(1), from 4-androstene-3, 17-dione by guinea pig liver and kidney homogenates (2) and from androsterone and epiandrosterone by guinea pig liver homogenates(3). Furthermore, androstane-3, 17-dione has been postulated as the precursor of urinary androsterone and epiandrosterone(4). It would appear that androstane-3, 17-dione occupies a key position in the intermediary metabolism of androgens. Therefore, the metabolism of this substance by liver homogenates has been investigated.

Methods and procedures. Purification of androstane-3, 17-dione.† The material was submitted to adsorption chromatography on alumina (washed Harshaw). Those fractions which showed only androstane-3, 17-dione on paper chromatography (0.5 mg/spot) were pooled, treated with Darco charcoal, and recrystallized from acetone and water until a constant melting point was obtained. The purity of the material used in most of the experiments was tested further by infra-red analysis.‡ *DPNH and FDP.*§ The DPNH

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† The steroids were generously provided by Ciba Pharmaceutical Products, Inc., and Syntex, S. A.

‡ Analyses were performed with a Perkin-Elmer, Model 21, Infra-red Spectrophotometer.

§ The following abbreviations are used: DPN, diphosphopyridinenucleotide (Pabst Laboratories); DPNH, reduced DPN; FDP, fructose diphosphate (Nutritional Biochemicals Company); NA, nicotinamide (General Biochemicals, Inc.).

was prepared from DPN by the method of Green and Dewan(5) with some alterations. Reductive conversions of 90% were obtained only when DPN:dithionite ratios of 2:1 were employed. The barium salt of FDP was converted to the sodium salt(6). Livers were obtained from adult male guinea pigs, 6 to 10 months old, which were killed by a blow at the base of the skull and bled. The procedure for incubation and isolation of steroid materials was as previously described(7) except that the separation of ketonic from non-ketonic materials with Girard's T reagent was omitted. The fractions obtained after chromatography on alumina were pooled in accordance with their elution properties and a 0.5 mg aliquot of the pooled fractions was submitted to qualitative analysis by paper chromatography(8). Fractions consisting of mixtures or containing non-steroidal impurities were submitted to paper chromatography for separation and purification. In the case of the principal metabolites, purification was carried out until satisfactory melting points and infra-red spectra were obtained. In addition, derivatives were prepared. The fractions were assayed by a modified Zimmerman reaction(9).

Results. Metabolites. Androsterone and epiandrosterone were the principal conversion products. Androstan-17β-ol-3-one and androstane-3α, 17β-diol were detected in much smaller amounts by paper chromatography in some of the experiments. The following is an example of the physical properties of the metabolites. The values obtained for the authentic samples are given in parentheses. Androsterone, m. 186-7° (185-6°); mixed, m. 186-8°; acetate, m. 165-7° (166-8°); oxime, m. 213-15° (214-17°). Epiandrosterone, m. 167-73° (174-5°); mixed, m. 165-72°; oxime, m. 183-4° (185-7°); mixed, m. 184-7°. The infra-red spectra were identical with those of the authentic compounds.

Homogenate. The amounts of metabolites formed were never very large, ranging from 0.4 to 1.2% in the case of androsterone, and from 0.8 to 2.5% in the case of epiandrosterone (Table I). No significant variation in the amounts of metabolites formed was observed when the pH was varied from 5.2 to 8.0, or when the length of the incubation period was varied to 2.5 hours. These results indicated that either only a small amount of apoenzyme for the reduction of the 3 and none for the 17 ketone group was present or that the amount of endogenous co-factor was not adequate to provide a substantial reduction. It is known that under similar conditions oxidation of the 17-hydroxyl of testosterone is greatly accelerated by the addition of DPN(10) with an optimum at about 4 mg/g of guinea pig liver (unpublished). The endogenous DPN of guinea pig liver, however, is only about 0.5 mg/g and the DPNH even lower, 0.1-0.2 mg/g(11,12). Therefore, it seemed reasonable to assume that the homogenate (apoenzyme) needed exogenous DPNH to become fully effective.

TABLE I. Reduction of Androstane-3,17-dione by Guinea Pig Liver Homogenates.*

	Exp. No.	Metabolites† And., %	Epi., %	Recovered androstanedione, %
Boiled controls	3	0	0	86.2±1.3
Experimental	4	.9±.2	1.8±.4	84.2±2.4

* Adult non-fasted male guinea pigs used. Steroid (100 mg), 12 g liver and 50 ml Krebs-Ringer bicarbonate buffer, pH 7.3 (one exp. at 7.5) mixed in Waring blendor for 2 min. Mixture transferred quantitatively by washing with 50 ml buffer to Fernbach flask. Incubation at 27° for 2.5 hr; 95% O_2–5% CO_2 was the gaseous phase. Values are means and their stand. errors.

† And. = Androsterone; Epi. = Epiandrosterone.

Effect of co-factors. Since DPNH can be formed effectively by liver homogenates from FDP and DPN(6), this procedure was utilized (Table II). DPN alone did not increase the yield of metabolites. The addition of FDP with DPN, however, produced a substantial increase in the production of epiandrosterone but not androsterone. A further increase in the formation of epiandrosterone and also an increase in androsterone was secured by increasing the pH from 5.1 to 6.7. The same results were observed when an atmosphere of nitrogen was used. The omission of nicotinamide, however, prevented the increased production of both metabolites presumably due to a destruction of the nucleo-

TABLE II. Influence of pH, Fructose Diphosphate (FDP), Diphosphopyridine Nucleotide (DPN) and Gaseous Atmosphere in Reduction of Androstane-3,17-dione by Guinea Pig Liver Homogenates.

Exp. No.	FDP, mg*	pH	Metabolites§ And., %	Epi., %	Aa., %	Total recovery, %‡
			Air atmosphere			
1		5.2	1.0	1.8		89.7
1	170	5.2	2.2	5.8		86.6
2	330	5.1	1.1	4.8		91.9
1	170†	6.5	2.1	1.7		96.6
2	250	6.7	6.0	9.0	.4	83.6
			N. atmosphere			
2	250	6.7	5.8	8.9	.3	95.2

* Liver (12 g) from adult non-fasted male guinea pigs and 50 ml 0.2 M Na_2HPO_4 buffer mixed in Waring blendor for 20 sec. Mixture transferred with 50 ml of buffer to Fernbach flask containing 50 mg steroid, 50 mg DPN and 400 mg NA. Incubation at 37° for 1.5 hr. Air was the gaseous phase.

† NA omitted.

‡ Metabolites plus recovered androstanedione. Control in which liver was boiled gave a recovery of 86.1%.

§ And. = Androsterone; Epi. = Epiandrosterone; Aa. = Androstanolone.

tides by the endogenous nucleotidase(13,14).

Effect of pH. Since pH seemed to be a factor in the yield of metabolites, studies were carried out to determine the optimum pH of the reductase when DPNH was used. The maximum formation of epiandrosterone occurred at about pH 6.7 (Fig. 1), and the yield was approximately twice that observed when FDP and DPN were used (Table II) but the amount of androsterone not only was less than that obtained at pH 6.7 with FDP and DPN but also was not affected by the change in pH. Small but significant amounts of androstan-17β-ol-3-one were formed.

Amount of DPNH. The addition of more than 19 mg of DPNH to the nicotinamide fortified liver homogenate at pH 6.6-6.7 produced only a slight further increase in the formation of epiandrosterone and had no further effect on the yield of androsterone (Fig. 2).

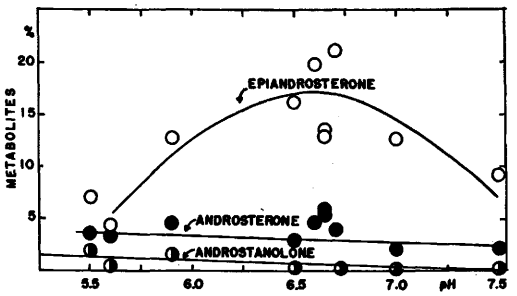

FIG. 1. Effect of pH on reduction of 50 mg of androstane-3,17-dione by 12 g guinea pig liver homogenate in presence of from 37 to 40 mg DPNH and 400 mg NA. Incubation for 1.5 hr at 37° in air atmosphere.

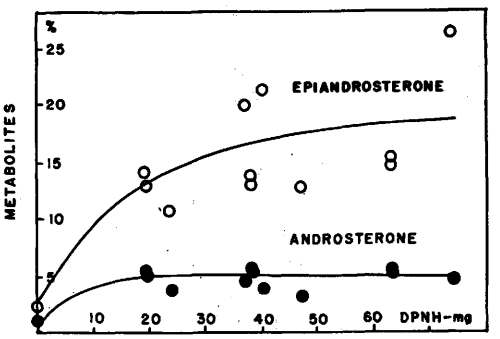

FIG. 2. Effect of quantity of DPNH on reduction of androstane-3,17-dione by guinea pig liver homogenate at pH 6.6-6.7 in presence of 400 mg NA. Incubation was 1.5 hr at 37° in air atmosphere. Total recoveries were from 91-98%. A boiled control yielded no metabolites and 86.1% recovery of androstanedione.

Distribution of androstane-3, 17-dione reductive activity. The liver homogenate was centrifuged at 1500 x g for 5 minutes at 3-8°.‖ The pellet fraction was resuspended in buffer for use. Approximately 75% of the ability to reduce androstane-3, 17-dione remained in the supernate (Table III). The re-

‖ Centrifugations were performed on an International Refrigerated Centrifuge, Model PR-2 provided by the American Cancer Society.

TABLE III. Distribution of Androstanedione Reductive Activity in Adult Male Guinea Pig Liver.

Fraction	Metabolites recovered*			Recovered Ae.,* %	Total recovery, %
	And., %	Epi., %	Aa., %		
Homogenate	4.6 ± .0	23.0 ± 3.2	trace	57.0 ± .0	84.6 ± 3.2
Supernatant	3.6 ± .8	15.5 ± .1	"	70.5 ± 3.1	89.6 ± 4.0
Sediment	2.7 ± .3	4.7 ± 2.5		88.4 ± .0	95.8 ± 2.8

* And. = Androsterone; Epi. = Epiandrosterone; Aa. = Androstanolone; Ae. = Androstanedione.

Tissue (12 g) and 50 ml 0.2 Na_2HPO_4 buffer (pH 6.6) mixed for 20 sec. in Waring blendor. Centrifugation 5 min. at 3-8° and 1500 × g; supernatants were decanted and sediments resuspended in 50 ml buffer. DPNH added in 50 ml solution. Each flask contained 50 mg steroid and 400 mg NA. Incubation was for 1.5 hr at 37°. Air was the gaseous phase. Results are avg of 2 series of experiments in which 37 and 74 mg of DPNH were used.

mainder of the activity was present in the resuspended pellet. There appeared to be a greater sedimentation of the androsterone-forming activity than of the epiandrosterone-forming activity.

Discussion. The reduction of androstanedione to androsterone and epiandrosterone apparently is accomplished by two different enzyme systems. A similar separation has been demonstrated in preparations of Pseudomonas(15).

Summary. Guinea pig liver homogenates reduce androstane-3, 17-dione to androsterone, epiandrosterone, androstan-17β-ol-3-one and androstane-3α, 17β-diol. The latter two compounds were observed only in trace amounts when addition of co-factors to the incubation mixture produced a marked increase in the amount of metabolites formed. Anaerobic conditions had no effect on the conversion produced by DPN, FDP and Na. The system which produced epiandrosterone exhibited an optimum pH of about 6.7. The formation of the other metabolites, however, was not affected by changes in pH. Approximately three-fourths of the ability to reduce androstane-3, 17-dione was in the supernatant fraction of homogenates centrifuged at 1500 x g.

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