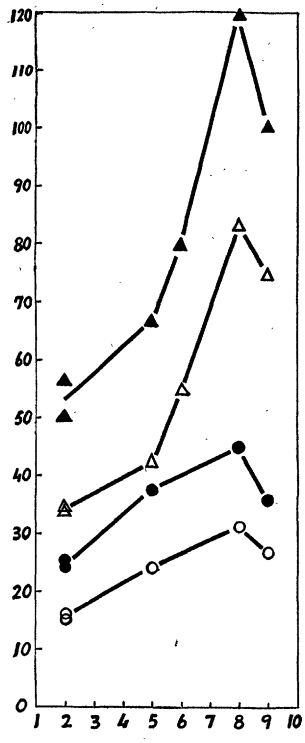

FIG. 1. Variation of the total niacin and niacinamide content in the pupal stage of *Bombyx mori*. Ordinate: content of total niacin and niacinamide (γ). Abscissa: age of pupae in days. ▲, Total niacin per individual; ●, total niacin per g; △, niacinamide per individual; ○, niacinamide per g.

worm pupae, although we cannot yet identify tryptophan as the precursor.

It is natural that *Tenebrio* and *Drosophila* have no mechanism of niacin biosynthesis, because they receive niacin in their food in the larval stage, whereas according to Kikkawa (*5*) 3-hydroxykynurenine is absent or very scanty in the larval stage of the silkworm, but becomes suddenly increased biosynthetically at the beginning of the prepupal stage and is maintained through almost the whole pupal stage except the last short period. We can therefore readily suppose that the niacin biosynthesis is carried on in the pupal stage.

References

1. *Nutrition Revs.*, **8**, 85 (1950).
2. *Ibid.*, **6**, 223 (1948).
3. FRAENKEL, G., and STERN, H. R. *Arch. Biochem.*, **30**, 438 (1951).
4. KATO, M., and SHIMIZU, H. *Science*, **114**, 2949 (1951).
5. KIKKAWA, H. *Sanshi Shikenjo Hokoku* (in Japanese), **11**, 311 (1943).

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An Oxidative Metabolite of Desoxycorticosterone

George M. Picha, Francis J. Saunders, and D. M. Green

Divisions of Chemical and Biological Research, G. D. Searle and Company, Chicago, Illinois

The general procedure of perfusing an isolated organ with a circulating medium enriched by the addition of a selected steroid precursor has found utility both as a means of determining the metabolic path of the precursor under conditions resembling those present in the intact animal (*1*) and as a method of biosynthesis (*2*). We have employed this procedure in a study of the metabolism of desoxycorticosterone (DOC) in a mammalian liver.

The liver is known to play an important role in the inactivation of DOC, as judged by the failure of this hormone to elicit a full physiological response when it is administered orally or introduced into a site drained by the portal circulation (*3, 4*). Studies based on urinary excretion products (*5–9*) in various species have shown that the administration of DOC (or its acetate) is followed by an increased elimination of pregnanediol, generally isolated and characterized as sodium pregnanediol glucuronide. The amount of administered DOC which can be accounted for as pregnanediol usually varies from 1% to 15%. In Addisonian patients a similar conversion of 11-dehydrocorticosterone to 11-keto-pregnanediol has been reported (*10*). Assays of the blood sera of monkeys have indicated a partial transformation of DOC acetate into progesterone (*11*). The administration of progesterone is also known to give rise to pregnanediol (*6*) and to pregnane-3α-ol-20-one (*12*).

More recently Schneider and Horstmann (*13*) have incubated DOC with rat liver tissue and have observed reduction of the conjugated unsaturated system in Ring A, shown by ultraviolet spectroscopy and by the isolation of allopregnane-3β, 21-diol-20-one. These investigators have also demonstrated that extensive attack takes place on the side chain, involving cleavage or reduction beyond the stage of the α-glycol, as they observe a loss of formaldehydogenic steroids during incubation. This result is explainable by the formation of pregnanediol or, as Schneider and Horstmann suggest, by the possibility of cleavage to a 19-carbon atom steroid. There is, however, some evidence (*14*) that administered DOC does not give rise to 17-ketosteroids, and no metabolite of DOC having other than 21 carbon atoms has previously been reported.

In our procedure rat livers were perfused via the superior vena cava with oxygenated, citrated beef blood containing about 500 mg of added DOC per liter. Adsorbable constituents were removed from the hemolyzed perfusate by activated carbon, and these materials were then removed from the carbon by extraction with chloroform and benzene in a Soxhlet apparatus.

The Soxhlet extract was subjected to extensive chromatography on silica columns, terminating in a final column on which the steroidal portion was partitioned, on 65 times its weight of silica, into 40 fractions by means of increasingly polar mixtures of ethyl acetate and benzene. Elution in the vicinity of 1:2 ethyl acetate-benzene resulted in crystalline fractions containing unaltered DOC and a 20-carbon atom transformation product, difficult to separate by direct chromatography because of its close resemblance to DOC in its behavior on the column.

Identification of this material as 3-keto-4-etiochol-

enic acid followed from the assignment of its 3 oxygen atoms to an α,β-unsaturated ketone and a carboxylic acid group. A sample recrystallized from ethyl acetate for analysis melted at 244°–246° (totally immersed Anschutz thermometer; literature mp, variously 236°–262°). Calculated for $C_{20}H_{28}O_3$: C, 75.91; H, 8.92. Found: C, 76.01, 75.90; H, 8.99, 8.90. $[\alpha]^{27}_D + 156°$ (chloroform). Ultraviolet absorption maximum at 241 mμ (log ε 4.21). No depression of melting point was observed when this product was mixed with an authentic sample.

That 3-keto-4-etiocholenic acid was a true transformation product of DOC and not merely a normal constituent of the liver or the blood was concluded from the quantity of this product that was isolated. Thus, from the perfusion of 10.2 g of DOC a total of 3.1 g of crude, crystalline steroids was isolated. By direct chromatography and by extraction of the DOC-rich eluate residues with potassium bicarbonate solution, 340 mg of reasonably pure (minimum mp, 236°) etio acid was obtained. Therefore the conversion was 3.3%, the yield was 4.6%, and the etio acid represented 11% of the recovered crystalline steroids.

Evidence is lacking to demonstrate whether the relatively low recovery of crystalline steroids is due to more extensive degradation of DOC or to inadequacies in the isolation procedure, although indications are that both these factors are important. Total steroid recoveries from nonperfused blood samples treated by the same technique are known to average about 60%. The fate of a glucuronide in our procedure is not known, although it is quite possible that it would not be adsorbed from the perfusate and would be lost. Various amorphous fractions from chromatography are undergoing further investigation.

Turfitt (*15*) has shown that soil bacteria of the genus *Proactinomyces* oxidize cholesterol (via cholestenone) to 3-keto-4-etiocholenic acid. However, the formation of this etio acid by liver perfusion of DOC is considered remarkable, since the commonly recognized transformation to pregnanediol involves reduction at both the unsaturated ketone grouping and the side chain, whereas the formation of the etio acid is an oxidative change. There is, of course, the possibility that the perfused liver in our experiments had available a larger supply of oxygen than the liver in an intact animal.

Precedence for the degradative bio-oxidation of a steroid side chain by mammals can be found in the oxidation of cholesterol to the bile acid stage (*16*) and in the presence of radioactive carbon dioxide in expired air after intraperitoneal injection of radioprogesterone labeled at C_{21} (*17*).

References

1. SCHILLER, J., and PINCUS, G. *Science,* **98**, 410 (1943).
2. HECHTER, O., *et al. J. Am. Chem. Soc.,* **71**, 3261 (1949).
3. EVERSOLE, W. J., and GAUNT, R. *Endocrinology,* **32**, 51 (1943).
4. GREEN, D. M. *Ibid.,* **43**, 325 (1948).
5. CUYLER, W. K., ASHLEY, C., and HAMBLEN, E. C. *Ibid.,* **27**, 177 (1940).
6. WESTPHAL, U. *Z. physiol. Chem.,* **273**, 13 (1942).
7. FISH, W. R., HORWITT, B. N., and DORFMAN, R. I. *Science,* **97**, 227 (1943).
8. HOFFMAN, M. M., KAZMIN, V. E., and BROWNE, J. S. L. *J. Biol. Chem.,* **147**, 259 (1943).
9. HORWITT, B. N., *et al. Ibid.,* **155**, 213 (1944).
10. MASON, H. L. *Ibid.,* **172**, 783 (1948).
11. ZARROW, M. X., HISAW, F. L., and BRYANS, F. *Endocrinology,* **46**, 403 (1950).
12. DORFMAN, R. I., ROSS, E., and SHIPLEY, R. A. *Ibid.,* **42**, 77 (1948).
13. SCHNEIDER, J. J., and HORSTMANN, P. M. *J. Biol. Chem.,* **191**, 327 (1951).
14. CUYLER, W. K., *et al. J. Clin. Endocrinol.,* **2**, 373 (1942).
15. TURFITT, G. E. *Biochem. J.,* **42**, 376 (1948).
16. BLOCH, K., BERG, B. N., and RITTENBERG, D. *J. Biol. Chem.,* **149**, 511 (1943).
17. RIEGEL, B., HARTOP, W. L., JR., and KITTINGER, G. W. *Endocrinology,* **47**, 311 (1950).

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The Aerial Transport of Mammalian Ova for Transplantation

W. G. R. Marden and M. C. Chang[1]

School of Agriculture, University of Cambridge, England,* and *Worcester Foundation for Experimental Biology, Boston University, Massachusetts

Since Heape (*1*) succeeded in transferring fertilized ova from one rabbit to another, Pincus (*2*) has employed the technique to determine the possibility of fertilization *in vitro* and parthogenesis of rabbit ova. Chang (*3*) has been able to store *in vitro* at 10° C fertilized rabbit ova in the early stages and in the late blastocyst stage. The possible practical application of such transplantation techniques in agriculture and medicine has aroused interest in recent years. This note reports the first successful aerial shipment of fertilized rabbit ova for transplantation from one country to another.

Two Californian rabbits were superovulated and inseminated with the semen of a Californian buck at the Worcester Foundation for Experimental Biology. About 24 hr after insemination they were sacrificed and the Fallopian tubes were flushed with whole rabbit serum. Altogether, 74 fertilized ova at the 2-cell stage were recovered and placed in 4 small flasks (1 ml capacity) containing whole serum, with a drop of penicillin solution added. The flasks were packed in a small thermos flask containing 2 small ice balloons. The thermos was shipped from Boston by air to London and then by train to Cambridge.

The time interval from recovery of the ova to the time of transplantation was 27 hr, and the temperature in the thermos was 12° C at the time of packing and 19° C at the time of unpacking.

Three black does kept in the School of Agriculture at Cambridge University were intravenously injected with Prolan and two of them were successfully bred by a colored vasectomized buck to induce ovulation about 26 hr before transplantation. Midline incision was made on the recipients, and 5 segmented ova were placed into the ampulla of each Fallopian tube. Ex-

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