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Effect of Triparanol Pretreatment of Rats on *in vitro* Adrenal Steroid Production.* (27321)

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Recently, Melby, *et al.* have shown that pretreatment of rats with Triparanol‡ decreases adrenal cortical steroid production by adrenal slices *in vitro* to less than 50% of control values(1). They also demonstrated that the adrenal glands of Triparanol-treated rats can utilize acetate-1-C^{14} or mevalonate-2-C^{14} for synthesis of corticosteroids. The specific activities of the corticoids produced by the adrenals of Triparanol-treated rats were higher than those of the controls. Since Triparanol inhibits the synthesis of cholesterol from acetate and mevalonate by partially blocking the conversion of desmosterol to cholesterol(2), Melby *et al.*, suggested that the labeled steroids were being produced by biosynthetic pathways which did not involve cholesterol.

We have made a similar study in which adrenal slices from rats pretreated with Triparanol were incubated with progesterone-4-C^{14}. Our purpose was to investigate the possibility that Triparanol might act as an inhibitor at one or more of the steps subsequent to the formation of progesterone.

Materials and methods. Young adult female Sprague-Dawley rats, averaging 160 g in body weight, were divided into 10 groups of 4 animals each. Five of the groups served as controls. The remaining animals received subcutaneous injections of 12.5 mg of Triparanol (saline suspension) per 100 g of body weight 5 times a week. Two of the treated animals died during this interval. The animals were housed in air-conditioned quarters and fed a regular diet with tap water *ad libitum*. After 6 weeks the animals were weighed and sacrificed by decapitation. The heart, left kidney, thymus and adrenals were removed from each rat. The organs were trimmed free of extraneous tissue and weighed. The adrenals were halved and stored in iced saline until termination of the autopsy. The adrenals of the control animals were divided into 5 pools, those from the treated animals into 4 pools.

The separate pools of adrenal tissue were pre-incubated for ½ hour in 5.0 ml Krebs-Ringer-bicarbonate-glucose at 37°C with 95% O_2-5% CO_2 bubbled continuously through the medium. The preincubation fluid

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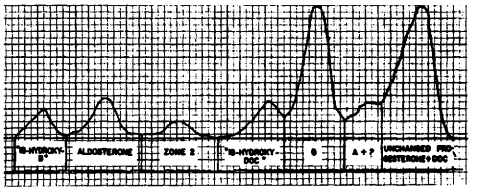
‡ Triparanol (MER/29); 1-ρ(β-diethylaminoethoxy)phenyl- 1 -(ρ-toyl)2-(ρ-chlorophenyl)ethanol, was graciously supplied by William S. Merrell Co., Cincinnati, Ohio.

was discarded. Five ml of fresh medium were then added to each pool along with 200 mU ACTH (Acton X§)/100 mg adrenal tissue. In addition, each incubation tube contained 10 μg progesterone-4-C^{14}. The incubations were then continued under the same conditions for one hour.

The medium from each incubation was decanted into a stoppered centrifuge tube. The tissues were washed with a small volume of saline and the washings combined with the medium. Radioactivity was quantitatively removed from the combined fluids by extracting 3 times with 2 ml of methylene dichloride. The adrenal tissues were discarded. Aliquots of the extract were taken for determination of total α-ketolic steroids using a corticosterone standard and the blue tetrazolium method of Nowaczynski(3). Additional small aliquots were taken for determination of total radioactivity. The remaining extract was chromatographed on paper to determine the individual radioactive and blue tetrazolium positive steroids(4). After development(4) the chromatograms were dried and the distribution of radioactivity quantitatively determined using an automatic recording windowless paper chromatogram scanner(5). An integrated trace of a characteristic recording is shown in Fig. 1. The total α-ketolic steroids in each of the chromatographic zones were determined by quantitative paper chromatography using methods previously described (4,6). Since authentic materials were not available in sufficient quantities for the preparation of standard curves, the quantities of 18-hydroxycorticosterone (18-HO-B), aldosterone and Zone 2 were estimated from standard curves based on cortisone; 18-hydroxydeoxycorticosterone (18-HO-DOC) quantities were similarly estimated using 6β-hydroxydeoxycorticosterone. A characteristic integrated recording of the blue tetrazolium positive steroids is shown in Fig. 2. The close correspondence observed between the zones detected by the blue tetrazolium and radioactive methods is evidence that the blue tetrazolium technic is measuring the same steroids



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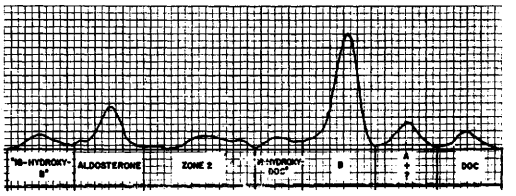

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FIG. 1. Integrated scan of a chromatogram showing radioactive steroids produced by Triparanol treated rat adrenal slices incubated with progesterone-4-C^{14}. Progesterone and DOC are not separated by this chromatographic system.

FIG. 2. A characteristic integrated densitometric recording of a chromatogram showing the blue tetrazolium positive steroids produced by incubating rat adrenal slices *in vitro*.

as those produced by adrenal glands in the presence of added radioactive corticosteroid precursors.

The presence of aldosterone, corticosterone and the 18-hydroxylated derivatives of corticosterone and deoxycorticosterone in rat adrenal incubates has been established by Peron(7). Identification of the steroids shown in the various zones (Fig. 1 and 2) was tentative and based on the following evidence. The steroid in the zone designated 18-HO-B had chemical properties and chromatographic mobilities similar or identical to the compound characterized as 18-hydroxycorticosterone (X_1) by Peron(7). In addition, this zone became labeled with tritium when rat adrenals were incubated with authentic 18-HO-DOC-H^3 for 5 hours under the conditions described above.‖ Further characterization has not been attempted since authentic 18-HO-B is not yet available. Identification of the aldosterone zone was based on evidence previously described(6). The steroid(s) of Zone 2 have not been identified. The zone immediately following had the same chroma-

§ We are indebted to Mr. K. Antoft of Nordic Biochemicals for Acton X ACTH.

‖ The 18-HO-DOC-H^3 was a gift of Dr. F. Peron, Worcester Foundation for Experimental Biology, Shrewsbury, Mass.

TABLE I. Effect of Triparanol Treatment on Final Body and Organ Weights of Female Sprague-Dawley Rats.

Group	No. rats	Body wt, g	Thymus wt, mg	Heart wt, mg	Kidney wt, mg	Total adrenal wt, mg
Controls	20	211 ± 3*	330 ± 11	752 ± 15	790 ± 19	73 ± 1
MER/29	18	218 ± 6	379 ± 17	818 ± 16	863 ± 20	79 ± .3
P (t test) <		—	.025	.005	.01	.001

* Stand. dev. of mean.

TABLE II. Distribution of Total Corticosteroid Radioactivity by Chromatographic Zones (%).

Group and No. determinations	18-HO-B	Aldosterone	Zone 2	18-HO-DOC	B	A + ¶	Progesterone + DOC
Control (5)	1.9 ±.3	4.0 ± .4	1.3 ±.3	4.0 ±.4	21.6 ± 1.2	3.3 ±.2	63.3 ± 2.1
MER/29 (4)	3.0 ±.8	5.9 ±1.1	2.2 ±.4	6.7 ±.9	28.9 ± 1.8	5.2 ±.8	47.3 ± 3.4
P <	.05	—	.025	.001	.025	.005	.001

tographic mobility as either 6β-hydroxy- or 18-hydroxydeoxycorticosterone. This material was eluted, converted to the diacetate and rechromatographed in hexane : toluene: methanol:H_2O; 667:337:800:200v/v. The steroid migrated with a mobility identical to that of the authentic acetate of 18-HO-DOC. The 6β derivative migrated much more rapidly in this system. The major peak (Zone B) was given by a steroid having chromatographic and chemical properties identical to those of corticosterone using the same procedures used for the identification of aldosterone(6). Zone A is a mixture according to evidence obtained using the same chromatographic methods(6). The zone contains approximately equal amounts of dehydrocorticosterone and of an unidentified steroid whose acetate is less polar than the 21-acetate of dehydrocorticosterone. The steroid in the final zone had chemical and chromatographic properties identical to deoxycorticosterone using criteria previously described(6).

Results. No significant differences in final body weight were observed between treated and control animals. The thymus, heart, kidney and adrenal glands were significantly enlarged in the Triparanol treated animals (Table I).

Radioactivity equivalent to 40 ± 3% of the initial progesterone-C^{14} was recovered in the media from both the treated and control groups. The distribution of radioactivity by chromatographic zones is shown in Table II. The treated groups incorporated more radioactivity into all zones except the zones containing unchanged progesterone and DOC.

Total steroid production¶ by adrenal slices was markedly decreased in the Triparanol treated groups, 5.4 ± .2 μg/100 mg tissue/hour compared to 8.4 ± .7 μg for the controls. The difference is significant at the level of P<.001. The distribution of steroid among the various chromatographic zones is shown in Table III. Steroids in the 18-HO-B zone of the Triparanol groups were present in proportionately higher amounts than in the corresponding groups of the controls. Also, in contrast to the controls, no DOC could be detected in the Triparanol groups.

Discussion. It has been previously reported that Triparanol inhibits normal growth in rats(8,9). However, in this study the treated rats were able to maintain their body weights as well as the controls. This difference may be due to the fact that the drug was administered subcutaneously rather than orally. Our finding of adrenal hypertrophy in rats chronically treated with Triparanol is in agreement with the observations of others(8,9). The adrenal enlargement is probably due to increased ACTH release in response to the decreased *in vivo* adrenal steroid production. The thymus gland hypertrophy observed in the treated group is

¶ In this study the phrase "total steroid production" refers to corticosteroids released by the adrenals into the incubation medium.

TABLE III. Distribution of *Total* Blue Tetrazolium Positive Steroids by Chromatographic Zones (%).

Group and No. determinations	18-HO-B	Aldosterone	Zone 2	18-HO-DOC	B	A + ?	DOC
Control (5)	10.7 ±2.3	23.9 ±2.9	18.3 ± .5	.9 ±.3	40.2 ± 2.1	2.9 ± .9	3.2 ± .7
MER/29 (4)	18.0 ±4.0	27.1 ±3.4	14.6 ±4.8	1.2 ±.3	35.8 ± 4.7	3.2 ±1.3	.0
P <	.01	—	—	—	—	—	(?) –

also believed to be a consequence of decreased adrenal steroidogenesis. The increased kidney and heart weight observed in the Triparanol treated rats suggests that chronic administration of high doses of this drug may have caused changes in blood pressure or cardiovascular-renal damage. This possibility is currently being investigated.

The total conversion of progesterone-C^{14} to corticosteroids was greater in the Triparanol group than in the controls (Table II). The individual chromatographic zones in the Triparanol groups also contained more radioactivity than the equivalent control zones (excluding the last zone which contained mainly unchanged progesterone). This increase was statistically significant in all but the aldosterone zone. The increased utilization of exogenous progesterone by the adrenals of the treated animals is probably related to low concentrations of endogenous corticosteroid precusors. This interpretation is supported by the concurrent observation of diminished rates of total adrenal steroid production *in vitro* in the Triparanol treated groups. These results also suggest the possibility that the inhibitory effects of Triparanol on adrenal function *in vivo* could be partially overcome by simultaneous treatment with adrenal steroid precusors such as progesterone or pregnenolone. Further, Triparanol apparently does not specifically inhibit any of the enzymatic steps in corticosteroid synthesis subsequent to formation of progesterone.

The distribution of the total blue tetrazolium positive steroids (Table III) in the control and treated groups demonstrates that proportionately more 18-HO-B is produced by the adrenals of Triparanol treated rats and concomitantly, little or no DOC. The data on DOC may be interpreted as evidence for the view that rate of conversion of desmosterol to DOC becomes a rate limiting factor in steroid biosynthesis in the treated groups, but not in the controls. The relatively higher proportion of 18-HO-B produced by the adrenals of the Triparanol groups may have biological significance since this compound is probably an immediate precursor of aldosterone(10). The calculated absolute quantities of 18-HO-B and aldosterone are approximately equal in both groups in spite of substantial inhibition of total corticoid biosynthesis in the Triparanol treated groups. In view of this it appears that there are mechanisms which preferentially shunt available substrate into pathways leading to aldosterone synthesis whenever endogenous adrenal cholesterol levels are significantly depleted.

Summary. Adrenal slices from rats pretreated with Triparanol produce less total blue tetrazolium positive steroids *in vitro* than do control slices. At the same time, however, adrenals from Triparanol treated animals incorporate significantly more added progesterone-C^{14} into cortical hormones. These data indicate that the conversion of desmosterol to progesterone becomes a rate limiting factor in corticoid biosynthesis in the adrenal glands of Triparanol treated rats and that Triparanol does not specifically inhibit corticoid synthesis from progesterone. Measurement of the relative quantities of the different cortical steroids indicates that biosynthetic pathways leading to aldosterone production are favored when endogenous cholesterol levels are significantly depleted in the adrenal gland.

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Orally Administered Cation Exchange Resin-Sorbital Regimen for Treatment of Ammonia Intoxication in Dogs.* (27322)

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Ammonia intoxication, a complication of advanced liver disease, is believed responsible for part of the clinical syndrome of hepatic coma. When massive hemorrhage occurs from gastroesophageal varices, intestinal microorganisms break down blood proteins to ammonia. Portal-systemic venous collateral channels permit portal blood with high ammonia content to bypass the liver and enter the systemic circulation, thereby avoiding conversion to urea. Elevated levels of blood ammonia in the systemic circulation act on the central nervous system, and are thought to contribute to confusion, irritability and stupor.

Ammonia intoxication may be produced experimentally in dogs by oral administration of blood or protein substances to Eck-fistula animals. Certain cation exchange resins are known to have an affinity for ammonium ions. Oral administration of such resins following administration of the challenging dose of blood would place the resin at the site of ammonia formation within the intestines. This should permit the resin to act with maximal efficiency, and prevent intestinal absorption of ammonia.

This report presents our laboratory experience with a sodium cycle sulphonic polystyrene resin,‡ which exchanges sodium ions for ammonium and potassium ions. It is available in particles of small size (5-10 μ diameter), thereby providing large surface area for interaction and exchange.

These experiments tested the ability of this cation exchange resin to prevent development of ammonia intoxication in Eck fistula dogs given a challenging dose of blood by gastric tube.

Methods. Eight healthy, mongrel dogs weighing 9 to 15 kg were selected and end-to-side portacaval shunts were constructed under intravenous pentobarbital sodium anesthesia (30 mg/kg). The dogs were allowed 5 to 7 days to recover from the operation before the specific experiments were performed.

The first experiment consisted of obtaining baseline ammonia response curves to a standard dose of blood. Each dog was fasted for 18 hours, and a 20 cc sample of venous blood was taken to measure ammonia, blood urea nitrogen, total serum protein, sodium, potassium and chloride. Stored human blood (40 ml/kg) was given by gastric tube in 3 equally divided doses over a period of one hour. Blood samples were taken at hourly intervals for the following 8 hours for analysis of ammonia-nitrogen concentration. Electrolytes, serum proteins and blood urea nitrogen were again measured at the end of the experiment.

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‡ Kayexalate, product of Winthrop Laboratories, New York.