Male sham operated to female sham operated 1.45 to 1.07

Discussion. It is apparent from these results that between the ages of one and 3 months, the female rat develops a marked increase in sensitivity to the paralytic effects of curare. Our results are also in close agreement with those obtained by Gallagher and Koch even though their end point, the determination of the LD50, was different from our own. From our data it is not possible to determine whether the increased sensitivity in the females is due to an increased rate of peritoneal absorption of dTC, an increased sensitivity to dTC at the neuromuscular junction, or a decreased rate of removal of the drug from the end plate areas. These questions can only be answered by more experiments. For example, it would be useful to determine *in vitro* whether there is any difference in the sensitivity of male and female mammalian neuromuscular preparations to d-tubocurarine.

Since gonadectomy fails to abolish the sex difference in curare sensitivity it appears that this difference is not mainly determined by the sex hormones but by some other sex linked genetic mechanism.

Our results suggest that it is necessary to consider the age and sex of animals used in any comparison of d-tubocurarine with other drugs capable of producing muscle relaxation. Since a search of the literature has not revealed a reference dealing with a difference in sensitivity to d-tubocurarine of human females *vs* human males, a clinical evaluation of this point is warranted.

Summary. One- and three-month-old male and female rats were injected intraperitoneally with d-tubocurarine in various doses. These animals were then placed on their sides and their ability to regain the upright position determined. No difference in sensitivity to d-tubocurarine was found to exist between one-month-old females, one-month-old males, and 3-month-old males. The 3-month-old females, however, were significantly more sensitive than any of the other groups. Gonadectomy performed at age one week failed to abolish the sex difference in curare sensitivity in animals tested at 3 months of age.

We would like to express our appreciation to Dr. A. Varma for statistical analysis of the data, and to Drs. H. Houston Merritt, and L. P. Rowland for review of the manuscript. We would also like to thank Mrs. Arleene Ozerkis, Mr. Robert Rodvien, Mr. Robert Lattes, and Mrs. Barbara Wolf for technical help. We are grateful to E. R. Squibb for supplies of Intocostrin.

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Received June 17, 1964. P.S.E.B.M., 1964, v116.

Inhibition of Adrenal Cholesterogenesis *in vitro* by AY-9944, 22,25-Diazacholestanol and by Triparanol. (29481)

M. L. GIVNER AND D. DVORNIK (Introduced by C. Chappel)
(With technical assistance of Sheila Gosselin)
Department of Biochemistry, Ayerst Research Laboratories, Montreal, Canada

The over-all course of hepatic cholesterogenesis is known and, with the exception of some steps involved in conversion of lanosterol to cholesterol(1,2) the normal sequence of reactions constituting the biogenetic pathway is well established(3). It is generally believed that the extrahepatic biosynthesis of cholesterol follows the same pathway. Similarity of the hepatic and adrenal patterns of cholesterol biosynthesis depends on the presence of similar enzyme systems in both organs. A method of revealing the existence

TABLE I. Effect of Glucose on Incorporation of 2-C^{14}-Acetate by 3-Hours-Old Bovine Adrenal Slices.

	Total radioactivity, as cpm*		
	Nonsaponifiable	5,6-Dibromocholestanol	Total F.A.
Control	2,693	1,001	307
Glucose added (200 mg/ml)	38,047	17,344	7,203

* Avg values of quadruplicate incubation. Each flask contained 10 μc of 2-C^{14}-acetate.

of fundamentally similar metabolic pathways could be based on the use of appropriate inhibitors. We have therefore studied adrenal cholesterol biosynthesis *in vitro* by examining the incorporation of 2-C^{14}-acetate into cholesterol by bovine adrenals in the presence of agents known to interfere with hepatic cholesterol synthesis. We have selected AY-9944(4),22,25-diazacholestanol (SC-11952) (5) and triparanol (Mer-29)(6), three potent inhibitors. In view of the limited incorporation of 2-C^{14}-acetate into nonsaponifiable lipids by beef adrenal homogenates, adrenal slices were used.

Materials and methods. Bovine adrenal glands were collected within minutes following death of the animal, immersed in physiological saline solution and kept on ice during transport to the laboratory. The glands were freed of extraneous tissue, split in half, carefully demedullated and the cortex sliced with a Stadie-Riggs microtome. The slices (500 mg) were incubated in 5 ml of Krebs-Ringer bicarbonate buffer(7) with 2-C^{14}-acetate (Merck, Sharp & Dohme of Canada Ltd.) at 37°C in an atmosphere of 95% O_2 and 5% CO_2. The medium contained 200 mg/100 ml glucose(8) (Table I). The slices were preincubated for 30 minutes, the buffer renewed and the agents to be tested were added: 22,25-diazacholestanol* and AY-9944 in a final concentration of 1×10^{-5}M in water; with triparanol,† added in a 2% gelatin suspension, the final concentration was 1×10^{-4}M. After 3 hours the incubation was terminated by 20 N KOH (1 ml). Water (6 ml), ethanol (4 ml) and carrier cholesterol (200 mg) were added, the suspension heated for 1 hour at 75-80°C and the nonsaponifiable lipids extracted with petroleum ether (b.p. 30-40°C). After bromination(9), 5-6-dibromocholestan-3β-ol was isolated and crystallized to constant radioactivity (4 times from methanol-ethyl acetate).

Two sets of experiments were conducted. In the first set, incubation was started 3 hours after the death of the animals. After the saponification total fatty acids (FA) were extracted with light petroleum ether from the acidified aqueous solution; the extract was washed with aqueous potassium acetate, the solvent was removed, the residue dissolved in acetone and titrated. In the second set, the adrenal slices were 6 hours old when incubation was started. In both sets of experiments the inhibitors to be tested were added at final concentrations which completely blocked the incorporation of 2-C^{14}-mevalonate into cholesterol by rat liver homogenates, *i.e.* triparanol and 22,25-diazacholestanol at 1×10^{-4} and 1×10^{-5}M respectively(10) and AY-9944 at 1×10^{-5}M(4). Samples were counted in a D-47 gas flow counter mounted with a Mylar Micromil window (Nuclear Chicago Corp.).

Results. The enhancing effect of glucose on the incorporation of 2-C^{14}-acetate into cholesterol and total fatty acids by (unusually active) bovine adrenal slices is presented in Table I. In the first set of experiments (Table II), AY-9944 depressed the incorporation of 2-C^{14}-acetate into beef adrenal cholesterol and total fatty acids by 89 and 91% respectively. In the second set (Table III), incorporation of 2-C^{14}-acetate into cholesterol by bovine adrenal slices was suppressed by triparanol (—32%) and by AY-9944 (—65%) whereas 22,25-diazacholestanol caused complete inhibition.

Discussion. Biosynthesis of adrenal choles-

* Kindly provided by Dr. V. A. Drill, G. D. Searle & Co.

† Kindly provided by Dr. C. A. Bunde, Wm. S. Merrell Co.

TABLE II. Effect of AY-9944 on Incorporation of 2-C^{14}-Acetate by 3-Hours-Old Bovine Adrenal Slices.

	Total radioactivity, as cpm*	
	5,6-Dibromo-cholestanol	Total F.A.
Control	2,082	144
AY-9944, 1×10^{-5}M†	231 (–89%)	11 (–91%)

* Avg values of quadruplicate incubations. Each flask contained 10 μc of 2-C^{14}-acetate.
† Final concentration.

terol, first revealed in bovine adrenal cortex, was later found to occur in a number of other species including man(11). However, it should be emphasized that the contribution of local biosynthesis to the adrenal cholesterol pool varies from species to species. Thus, it is rather small in the rat and dog (12) and considerably higher in the guinea pig(13).

The present study shows that 22,25-diazacholesterol, AY-9944 and triparanol suppressed the incorporation of 2-C^{14}-acetate into cholesterol by bovine adrenal slices. AY-9944 and 22,25-diazacholesterol differ in their primary site of inhibition: AY-9944 inhibits the 7-dehydrocholesterol Δ^7-reductase, *i.e.* it blocks the conversion of 7-dehydrocholesterol to cholesterol(2,14), whereas 22,25-diazacholestanol(10), like triparanol(15) inhibits the 24-dehydrosterol Δ^{24}-reductase, *i.e.* it prevents saturation of the peripheral Δ^{24}-bond in post-lanosterol precursors of cholesterol. The finding that both types of inhibitors suppressed adrenal synthesis of cholesterol indicates the presence in bovine adrenals of the enzymes involved (a) in transformation of 7-dehydrocholesterol to cholesterol and (b) in reduction of the peripheral Δ^{24}-bond of lanosterol and its metabolites. Hence, the findings suggest similarity in enzymatic reactions constituting the biosynthetic pattern of hepatic and adrenal cholesterol formation.

TABLE III. Effect of Triparanol (MER-29) 22,25-Diazacholestanol (SC-11952) and of AY-9944 on Incorporation of 2-C^{14}-Acetate into Cholesterol by 6-Hours-Old Bovine Adrenal Slices.

	Total radioactivity, as cpm*	
	Non-saponifiable	5,6-Dibromo-cholestanol
Control	5,686	1,037
MER-29, 1×10^{-4}M†	5,265	708 (–32%)
SC-11952, 1×10^{-5}M	3,066	0 (–100%)
AY-9944, 1×10^{-5}M	4,187	362 (–65%)

* Avg values of duplicate incubations. Each flask contained 20 μc of 2-C^{14}-acetate.
† Final concentration.

In view of this similarity, an inhibitor of cholesterol biosynthesis can affect the functions of the adrenals *in vivo* in at least two ways: (1) indirectly, by inhibiting hepatic cholesterogenesis and depressing serum cholesterol, (2) directly, by inhibiting adrenal cholesterol formation *in situ*. A major role of adrenal cholesterol is its convertibility to steroid hormones. Hence, inhibition of adrenal cholesterogenesis may indirectly affect the formation of adrenocortical hormones(13,15) in species whose adrenal cholesterol is mainly synthesized *in situ*.

Summary. AY-9944, 22,25-diazacholestanol and triparanol, known to inhibit hepatic cholesterogenesis, significantly suppressed the incorporation of 2-C^{14}-acetate into cholesterol by bovine adrenal slices. Thus, by suggesting the presence in beef adrenals of 7-dehydrocholesterol Δ^7-reductase and of 24-dehydrosterol Δ^{24}-reductase, the finding indicates a similar pattern of hepatic and adrenal cholesterol synthesis.

We wish to thank Mr. A. Smith, Canada Packers for the generous supply of adrenal glands and to Dr. J. P. Giroud, Montreal Children's Hospital for helpful suggestions.

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Received May 13, 1964. P.S.E.B.M., v117, 1964.

Neutralizing Antibodies Against Simian Viruses SV_5 and SV_{20} in Human Sera.* (29482)

C. G. AULISIO, D. C. WONG AND J. A. MORRIS

Division of Biologics Standards and National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Md.

Since 1955 there have been recovered from tissues and excreta of chimpanzees and monkeys approximately 100 viruses(1,2,3): the rhesus monkey has been the source of about 50 of these(2). With the exception of herpesvirus simiae (B virus)(4), SV_{40}(5,6), and the chimpanzee coryza agent (RS virus)(7,8), little effort has been made to determine whether other viruses of lower primate origin are infectious for man. This paper reports and interprets findings in an examination of sera obtained from people in 2 different ecologic settings (New Guinea and the United States) for presence of neutralizing antibodies against 2 simian viruses: parainfluenza virus SV_5 and adenovirus SV_{20}.

Materials and methods. Viruses: Viruses employed were SV_5 virus, strain Norris, and SV_{20} virus, strain Chin (A57). Both viruses were recovered as contaminants in primary cultures of rhesus monkey kidney cells, the former in the laboratories of the Division of Biologics Standards, and the latter in the laboratories of Dr. Tom Chin (U.S.P.H.S., Kansas City). Seed viruses were prepared by inoculating rhesus monkey kidney cells and fluids infected with SV_5 or SV_{20} into cultures of primary vervet monkey kidney cells for SV_5 and into cultures of BS-C-1 cells for SV_{20}. Maintenance medium used to support the viability of host cells during viral growth consisted of serum-free 199 medium. After 4 to 7 days incubation infected cells and fluids were harvested, centrifuged at low speeds to remove cellular debris and the resultant supernatant fluids representing virus seeds were stored in convenient amounts at —60°C.

Sera: Blood samples were drawn from 267 persons from 1 to 60 years of age. New Guinea sera were obtained in March, 1963, by Dr. D. C. Gajdusek from residents of Sabron Village, West New Guinea. The New Guinea donors had never received vaccine of any kind and it is reported that they had never had contact with a primate other than man. The U. S. sera were obtained in the summer of 1960 from military recruits and dependents prior to and, in some instances after administration of adenovirus vaccine. These sera were given to us by Dr. E. L. Buescher. For certain tests use was made of sera obtained in 1956-57 from patients with clinically diagnosed infectious hepatitis in United Nations military hospitals in Japan, Korea, and Okinawa. All sera were stored frozen at —20°C.

Virus neutralization tests. To 0.3 ml volumes of serial 2-fold dilutions of serum (in-

* This study was made possible through the generous cooperation of the following persons: Drs. T. O. Y. Chin, E. L. Buescher, R. M. Chanock and D. C. Gajdusek.