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Effect of Anabolic Steroids on Liver Function Tests in Rabbits (28558)

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An increasing number of reports of abnormal liver function tests following administration of certain drugs have appeared in the recent literature(1,2). Noteworthy among the compounds that produce these changes are the anabolic steroids(3,4,5). The two compounds most extensively studied have been methyltestosterone and norethandrolone (17γ-ethyl-19-nortestosterone). These compounds not infrequently cause an increased bromsulfalein (BSP) retention and less frequently increased serum transaminase activity. Published studies have shown that most of these abnormalities disappear following withdrawal or even with continued administration of the drug(5,6). Increases in BSP retention and serum transaminase activity have not been reported in animal studies following administration of drugs which have been shown to cause these changes in man.

Although Popper and Schaffner(7) detected changes in the microvilli of some bile canaliculi in human and rat liver by electron microscopy following norethandrolone administration, Gass and Umberger(8) reported daily administration of 10-100 mg of methyltestosterone and norethandrolone for periods up to 50 days had no effect on BSP retention in dogs. Recently Clodi *et al.*(9) and Kolb *et al.*(10) published reports regarding the effect of anabolic steroid administration on elimination of certain organic dyes in the bile of rats. Clodi and associates showed an initial decrease (0-45 minute interval) followed by an increase (45-90 minute interval) in BSP elimination in rat bile after 7-day administration of 6 mg/kg/day of methyltestosterone. Kolb and associates measured the effect of 8 different anabolic steroids on bile elimination of I-131 labeled rose bengal in bile-fistula rats. Seven of the 8 steroids including methyltestosterone and norethandrolone, administered in doses of 5 mg/kg/day, caused a retardation in bile elimination of varying significance on first and/or 7th and/or 14th test day, but none on 21st test day.

This report concerns experiments undertaken to determine the effect of methyltestosterone and norethandrolone administration on liver function in rabbits as measured by serum glutamic-oxolacetic transaminase activity (SGOT) and disappearance of BSP from the blood stream.

Material and methods. Male and female young white New Zealand rabbits weighing 2.5-3.5 kg were given Purina rabbit chow and water *ad libitum*. The compounds were prepared for injection in polyethylene glycol 200 (PEG), 25 mg/ml and administered IM daily—10 mg/kg of body weight for 6 days. Control animals were injected with an equivalent amount of PEG in the same manner. Percent BSP retention was measured prior to compound administration and on the 6th test day by a modified procedure especially designed for rabbits. Twenty mg of dye per kg of body weight was injected into marginal ear vein. A blood sample for assay was obtained by cardiac puncture exactly 20 minutes later using a #23 gauge one-inch needle and a 2 cc syringe wet with heparin to prevent clotting. To minimize the effect of lipemia associated with anabolic steroid administration, the BSP was assayed according to the procedure of Henry *et al.*(11). Blood for SGOT assay was drawn from marginal ear vein prior to injection of dye in each experiment and was measured by the method of Karmen *et al.*(12). It was neces-

TABLE I. Effect of 6-day Treatment with Steroids on BSP Retention and SGOT Activity in Rabbits.

Drug (10 mg/kg)	% BSP retention				SGOT			
	No. rabbits	Mean response	Standard deviation of mean response	Range of individual responses	No. rabbits	Mean response	Standard deviation of mean response	Range of individual responses
Methyltestosterone	7	2.8	.8	6.0	3	42	51	161
Norethandrolone	8	1.8	.4	2.8	8	42	5	39
Vehicle only (PEG)	8	−0.2	.1	.5	8	−8	5	41

(Response on an individual animal is computed as the value on treatment minus the value on control.)

sary to modify the method by increasing initial incubation time to correct for increased intrinsic oxidizing factors present in the sera.

The amount of BSP injected and time of blood sampling were determined from disappearance curves using varying dose levels of dye. Fig. 1 shows some typical results obtained following injection of 10 and 20 mg/kg doses in rabbits. A 5 mg/kg dose disappeared at such a rate as to make is unsatisfactory for consideration in our multiple animal testing procedure. Either the 10 or 20 mg/kg dose could be used; however, for our purpose the 20 mg dose and the 20 minute sampling time were chosen. This allows sufficient time for simultaneous testing of groups of 6-8 animals and produces an ample dye concentration for accurate assay. This procedure also allows one to detect abnormalities in the primary, as well as the secondary disappearance phase of the dye while minimizing errors in sampling time. Repeated tests on normal rabbits revealed that this procedure resulted in plasma BSP levels of less than 0.7 mg% or 1.5% retention—based on an average plasma volume of 42 ml/kg body weight.

Results and discussion. The effect of the compounds tested on the ability of the liver to remove BSP from the blood stream and on SGOT activity are summarized in Table I. After 6 days' administration of 10 mg/kg/day, the group of animals treated with methyltestosterone showed a significant increase in % BSP retention ($P<.01$). The group treated with norethandrolone also showed a significantly increased % BSP retention ($P<.01$); although the increases were not as marked. Similar changes were noted in a small group of 4 rabbits after only 3 days of daily IM administration of 2.5 and 5.0 mg/kg of methyltestosterone. Another trial study with methyltestosterone administered orally to a small group of rabbits showed similar changes, but only after 14-21 days of treatment. In man, Marquardt and associates(3) observed no significant differences in the increased BSP retention produced when comparing oral and IM routes of administration of norethandrolone.

The SGOT data are incomplete due to previously mentioned initial difficulty in assay procedure; however, from these limited data it appears that the anabolic agents cause an occasional moderate increase in SGOT activity. A slight increase in SGOT activity was also observed after 3 days in each of 4 rabbits treated daily with 2.5 and 5.0 mg/kg of methyltestosterone. There was no evidence that the cardiac punctures affected the SGOT. No changes were observed in control animals and the heart appeared normal on autopsy. Likewise, the vehicle (PEG) ap-

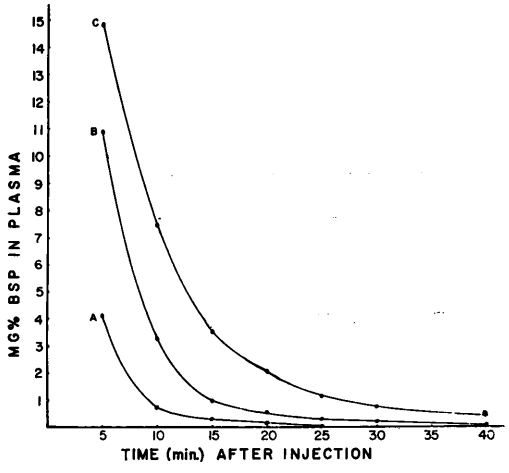

FIG. 1. Rate of disappearance of BSP from plasma in rabbits following: (1) 10 mg/kg dose—Curve A (normal); (2) 20 mg/kg dose—Curve B (normal) and Curve C (liver injury).

peared to have no effect. The activity range and periodic fluctuation of SGOT in normal animals must be ascertained before proper evaluation of these changes following drug administration can be made.

Summary. Using 20 mg of BSP per kg of body weight and determining the percent retention after 20 minutes, it was demonstrated that daily administration of 10 mg/kg for 6 days of methyltestosterone and norethandrolone to rabbits significantly impaired the removal of the dye from the blood stream. Less frequently and to a lesser degree the SGOT activity appeared to increase with drug administration.

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Specificity of Penicillin Amidases. (28559)

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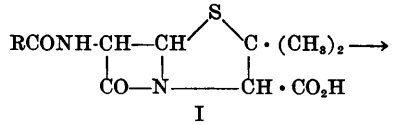
Numerous workers have reported the presence of a penicillin amidase or acylase of the following type among fungi, yeasts, actinomycetes, and bacteria(1-9). This enzyme catalyzes the hydrolysis of penicillins (I) into 6-aminopenicillanic acid (II) and acid corresponding to the side chain.



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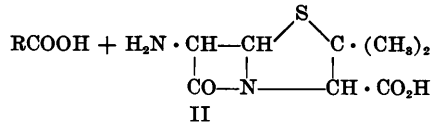

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The substrates for these enzyme studies have been, for the most part, naturally occurring penicillins.

Rolinson *et al.*(5) considered that 2 distinct types of enzyme activities were encountered, differing primarily in rates of splitting of particular types of penicillins. One type, widely distributed among the actinomycetes and filamentous fungi, readily hydrolyzes penicillins K, dihydro F and V but splits penicillin G very slowly. The second type, found in certain bacteria, splits penicillin G readily and penicillin V at a much slower rate.

Erickson and Bennett(8), using cells of *Penicillium chrysogenum,* and Cole and Rolinson(10), using cells of *Emericellopsis minima,* found activity of the first type in that penicillin V was split much more readily than G. Claridge *et al.*(1), Rolinson *et al.*(5) and Huang *et al.*(2) reported on penicillin amidases from bacteria which would fall into the second category in that penicillin G was split more readily than penicillins V, K, O, or phenethicillin. Recently Huang *et al.*(11) tested the hydrolysis of a series of biosyn-