teroides and *S. aureus* infected animals whose survival times were significantly prolonged. *In vitro,* however, 4-chlorotestosterone did not inhibit development of the infecting strains. According to these data the particular anti-infective action of 4-chlorotestosterone is not attributed to a direct chemotherapeutic effect but rather to a stimulating action of the steroid on organic reactive systems; particularly those inhibited by cortisone and conditioning the organic response to allergic or infective stimuli.

Summary. 4-chlorotestosterone is an anabolic steroid comparable to testosterone, but with less androgenic potential. It enhances the intensity of anaphylactic shock in guinea pigs, prolongs survival time of mice infected with *Nocardia asteroides,* or *Staphylococcus aureus,* hastens healing of cutaneous nocardosis in rabbits, and nullifies the proinfective action of cortisone.

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Received February 17, 1958. P.S.E.B.M., 1958, v97.

Comparison of Porphyrin Producing Enzyme Activities in Harderian Glands of Mice and Other Rodents.* (23877)

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It was observed that the Harderian glands of rats, hamsters, and most mice fluoresced red, while the Harderian glands of rabbits and guinea pigs did not(1-5). It had been assumed, and was later found, that the red fluorescent Harderian glands contained relatively greater quantities of porphyrin than those that did not fluoresce red. The enzymes for producing porphyrin must, however, be present in all Harderian glands because free protoporphyrin has been extracted from Harderian glands that were not red fluorescent. It was, therefore, of interest to determine the quantitative aspects of porphyrin producing enzymes in Harderian glands of various rodents. It was especially desirable to compare amounts of enzyme activity present in rat and hamster, where the glands are usually red fluorescent, with the amount of enzyme in Harderian glands in guinea pigs and rabbits, where these glands are usually not red fluorescent. In some rodents, where Harderian glands are white fluorescent, the porphyrin is present in such small quantities that its red fluorescence is masked by presence of blue-green fluorescent material. The blue-green and red fluorescence spectra are complimentary and the glands appear to be white fluorescent. In the rabbit, the Harderian gland consists of a dark and a white fluorescent portion. The white fluorescent portion contains by far the most porphyrin, but not nearly as much as the Harderian glands of rats and hamsters.

The results of these experiments on quantitative aspects of porphyrin producing enzymes in Harderian glands of rodents indicate that while these enzymes were present in rabbit and guinea pig Harderian glands, the enzyme activity in the rabbit gland was less than half that of the rat or hamster. In the guinea pig the enzyme activity was only 20%

* This work was supported by grants from Anna Fuller Fund, Am. Cancer Soc., Md. Division, and N.I.H., P.H.S. (CY3580).

† Submitted in partial fulfillment of requirements for degree of Doctor of Philosophy.

of that in the Harderian glands of rats and hamsters.

Materials and methods. The Harderian gland tissues used were obtained from commercially available male albino rats, rabbits, guinea pigs, and brown hamsters. The animals were housed in metal cages and given Purina Laboratory Chow and water without restrictions. Prior to removal of Harderian glands, the animals were anesthetized with ether and tissues dissected out in a cold room (6°C). The glands were removed as rapidly as possible, care being taken to exclude extraneous tissues. One hundred mg of Harderian gland tissue was weighed and placed in a mortar with small amount of coarse grinding sand and 2 cc of 50:50 buffer-saline solution. The gland tissue was homogenized with pestle and rinsed from the mortar with 3 cc of buffer-saline solution. The sand and coarse material were eliminated by centrifugation at 2500 rpm for 15 minutes. The supernatant material was used as the enzyme solution. The same procedure was followed to prepared enzyme solutions of brain, liver, and muscle. To each of 24 Klett tubes (6 for each tissue to be tested) 1.4 cc of 0.01 M δ-aminolevulinic acid in buffer-saline solution was added as the substrate. The buffer-saline solution consisted of equal volumes of isotonic sodium chloride solution and buffer solution pH 7.2 (0.029 molar potassium dihydrogen phosphate: 0.013 molar sodium tetraborate). The amounts of enzyme solution from each tissue to be tested were pipetted into the 6 tubes as follows: Tube No. 1—none (control); Tube 2—0.1 cc; Tube 3—0.2 cc; Tube 4—0.4 cc; Tube 5—0.8 cc; Tube 6—1.6 cc. Buffer-saline was added to each tube to bring volume to 3 cc. The tubes were then stoppered with cellophane covered corks and incubated at 37° for 24 hours. At the end of this period, 6.5 cc of concentrated HCl was added to each tube to stop the reaction and to clear the solution. This produced a 25% HCl solution. The tubes then stood overnight in cold room (6°C). A solution of hematoporphyrin hydrochloride in 25% HCl was prepared as standard to be used in a Klett-Summerson filter photometer to determine amounts of porphyrin formed. These experiments were repeated twice on 2 sets of animals.

TABLE I. Porphyrin Production by Aqueous Extracts of Harderian Gland and Other Rodent Tissue Homogenates.

		Enzyme extract conc. μg of porphyrin/3 cc of reaction mixture/24 hr				
Rodent	Tissue	0.1	0.2	0.4	0.8	1.6
Mouse†	Hard.	7.1	12.8	32.3	49.5	100.7
Rat	gland	6.6	11.5	29.4	48.7	100.0
Hamster		5.2	9.8	27.7	46.7	97.8
Rabbit (R)		.8	1.7	4.4	8.2	16.3
" (W)		2.4	5.5	14.5	19.3	48.3
Guinea pig		.9	1.9	4.9	8.3	18.8
Mouse†	Liver	5.5	9.5	28.5	34.6	73.4
Rat		5.2	9.0	28.7	34.2	71.0
Hamster		4.7	8.3	27.8	33.4	69.4
Rabbit		5.7	8.3	27.8	33.9	69.2
Guinea pig		5.7	8.0	26.1	32.4	69.8
Mouse†	Brain	.8	2.5	4.4	5.2	9.7
Rat		.5	2.4	4.0	5.1	8.8
Hamster		.1	2.0	3.6	5.2	8.7
Rabbit		.3	1.8	4.1	4.8	8.8
Guinea pig		.1	1.8	4.5	5.0	8.2
Mouse†	Muscle	1.8*	3.4*	6.7*	8.7*	16.1*
Rat		0	0	.4	1.2	3.1
Hamster		0	0	.3	.8	2.5
Rabbit		0	.1	.6	1.3	2.8
Guinea pig		0	.2	.8	1.7	2.9

R = postero-ventral lobe of Harderian gland.
W = antero-dorsal " " " " .
* Muscle plus bone and bone marrow.
† See reference (7).

Results. Variation in amount of porphyrin produced by different tissue enzyme preparations is shown in Table I. It may be seen that the amount of porphyrin produced by Harderian glands of different rodents did vary considerably. The amount of porphyrin produced in tubes containing extracts of the Harderian gland homogenates from the rat and hamster was far greater than that in rabbit and guinea pig. This corresponds to the relatively intense red fluorescence seen in rat and hamster and lack of appreciable red fluorescence in rabbit and guinea pig Harderian glands.

The Harderian gland of the rabbit consists of 2 parts; (W) the white antero-dorsal lobe and (R) the grey-reddish, postero-ventral lobe(6). The white (also white fluorescent) antero-dorsal lobe when tested alone possessed a greater amount of enzyme activity than did the grey-reddish postero-ventral lobe when tested by itself.

Liver, brain, and muscle extracts of the

same animals also exhibited a definite ability to convert δ-aminolevulinic acid to porphyrin. The porphyrin producing potentialities of liver tissue extract were, however, less than that of Harderian gland in rat and hamster, but more than in rabbit (both lobes) and guinea pig. The Harderian gland and liver enzyme extracts showed greater activity than brain and muscle in all instances.

In contrast to the observations on Harderian glands, enzyme activities of liver, brain, and muscle of all rodents tested were approximately the same when corresponding tissues were compared in the different rodents.

Discussion. It is of interest that porphyrin producing enzymes in the extracts of homogenates of liver, brain, and muscle did not vary appreciably in porphyrin producing enzyme activity in different rodents. In contrast, a great variation in enzyme activity of Harderian glands was found. The variation in amount of enzyme activity in the Harderian glands of various rodents parallels the degree of red fluorescence observed. This is related to the fact that there is more porphyrin in the rat and hamster Harderian gland than in rabbit and guinea pig. These observations, however, still give no clue as to the possible function of the porphyrin produced by Harderian glands. For example, it is not clear why rats and hamsters and mice should need a Harderian gland that produces more porphyrin than rabbit and guinea pig. An age study in mice revealed that there was a close parallelism between development of sex maturity and appearance and accumulation of porphyrin producing enzymes in the Harderian glands(8). It has been pointed out earlier that animals with relatively large amounts of porphyrin in the Harderian glands were quite susceptible to cancer(4,5). That is, mice, rats, and hamsters are quite susceptible, and other animals seem to be less susceptible. The hypothesis that there is a relationship between porphyrin metabolism and cancer susceptibility is still attractive, but no mechanism which would explain such a relationship has been discovered.

Summary and conclusions. 1. A quantitative study was made of porphyrin producing enzyme activities of Harderian glands, livers, brains, and muscles of mice, rats, hamsters, rabbits, and guinea pigs. 2. The tissues used were obtained from commercially available male albino rats, rabbits, guinea pigs, and brown hamsters, and strong A strain mice. 3. A buffer-saline solution or extract was made from a weighed quantity of each homogenized tissue mentioned. Measured amounts of these extracts were mixed with 0.01 M solution of δ-aminolevulinic acid and incubated at 37° for 24 hours. 4. Harderian gland extracts of mice and rats showed greatest amount of porphyrin producing enzyme activity, while the same tissue from the hamster was only slightly lower in enzyme activity. The 2 lobes of the Harderian gland of rabbit and guinea pig contained far less porphyrin producing enzyme. The white fluorescent antero-dorsal lobe of the rabbit contained considerably greater quantities of porphyrin producing enzymes than the grey-reddish postero-ventral lobe. 5. Liver, brain, and muscle extracts of the same rodent revealed amounts of porphyrin producing enzyme, uniform for tissue in various rodents and these values compared favorably with previous values determined for same tissues in the mouse. 6. Because of great variation in amounts of enzyme in various Harderian glands, the liver homogenate extract from rat and hamster contained less porphyrin enzyme than the Harderian gland. The reverse, however, was true in rabbit and guinea pig, for liver tissue contained more porphyrin producing enzyme than the Harderian glands of these rodents. It may be concluded that porphyrin produced by Harderian glands may have some function. This comparative study failed to give any clue as to what the function may be.

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Received November 26, 1957. P.S.E.B.M., 1958, v97.

Pharmacologic Characterization of Reserpine Responses in Rats Pre-Treated with Iproniazid.* (23878)

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Reserpine is known primarily for its hypotensive and sedative activities(1). It causes also liberation of pressor amines, notably epinephrine, norepinephrine and serotonin(2). Pressor activity consequent on release of these substances has been unmasked in anesthetized dogs and cats(3-5) by pre-treatment with iproniazid, a mono-amine oxidase inhibitor and attributed to impaired degradation of norepinephrine(4). In conscious mice, rats and rabbits, pre-treatment with iproniazid altered the response to reserpine from sedation to excitation(3,6); this change was attributed primarily to release of serotonin and its delayed inactivation(7,8). The present report concerns effects of reserpine on arterial pressure and behavior of anesthetized and conscious rats pre-treated with iproniazid and attempts to characterize the factors involved in these responses.

Methods. Experiments were done in male Sprague-Dawley rats weighing 175-255 g each. Except when indicated, anesthesia was induced with sodium amytal (9 mg/100 g body weight intraperitoneally) and vascular reflexes were inhibited with pentolinium bitartrate (5 mg solution in 20% polyvinylpyrrolidone subcutaneously); blood pressure was recorded following carotid cannulation (9). When given, iproniazid phosphate (25 mg intraperitoneally) was injected 18-24 hours before the test. Free reserpine dissolved in glycol vehicle was administered intraperitoneally in a dose of 5 mg/kg after arterial pressure had stabilized. Drugs used in evaluating the nature of responses were chlorpromazine hydrochloride (10 mg/kg intraperitoneally), lysergic diethylamide (to 250 μg/kg intravenously) and phenoxybenzamine hydrochloride (to 5 mg/kg intravenously). These were given 30-90 minutes before injection of reserpine. Parallel studies of symptomatic effects were done in conscious animals.

Results. 1. *Pressor effects of reserpine.* In rats treated with pentolinium but not with iproniazid, reserpine produced a small, transient initial decrease in arterial pressure followed by gradual rise to maximum of about 20 mm Hg above control levels; this pressor effect persisted for 90 minutes (Fig. 1A). In rats given both iproniazid and pentolinium, the response to reserpine consisted, as above, of initial small fall in pressure, followed by sharp rise which reached a maximum between 50 and 100 mm Hg within a half hour after injection (Fig. 1B) and remained at this level to 90 minutes. In rats given iproniazid but not pentolinium, reserpine was substantially as pressor as in rats given iproniazid and pentolinium; since post-iproniazid responses were presumably maximal, we could not demonstrate pressor sensitizing effect of the ganglion blocking agent.

2. *Effects of blocking agents on responses to reserpine.* While LSD had no definite effect, chlorpromazine inhibited but did not wholly prevent pressor activity of reserpine in iproniazid treated animals (Fig. 2A): under the same conditions, the same dose of chlorpromazine inhibited almost completely pressor effects of serotonin (1 μg). Phenoxybenzamine, an adrenergic blocking agent, reversed the effect of reserpine in iproniazid treated

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