

which keeps the total isolated contractile protein concentration unchanged. It is also notable that no measureable effects on the ability of the muscle to develop tension were associated with these changes in chemical characteristics. These matters deserve further consideration.

Summary. The viscosimetric response of rat myocardial actomyosin solutions to addition of ATP (ATP sensitivity) varies with ventricular weight. This suggests quantitative or qualitative changes in the components of the contractile system with cardiac growth. Total contractile protein concentration is unchanged and maximum isometric tension development by surviving muscles did not vary.

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Effect of Estradiol on Albumin- I^{131} in the Skin of Mice Following Intravenous Injection.* (30180)

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It was previously observed that clearance of albumin- I^{131} from plasma and apparent transvascular passage of this substance into the myocardium of male dogs decreased with age(1). It was suggested that age changes in the connective tissue may play a role in this phenomenon(2). To gain further insight into this matter the passage of albumin- I^{131} into connective tissue-rich organs was investigated. During this study it was observed that estradiol treatment resulted in increased bound I^{131} in the skin after the latter was injected intravenously.

Method. Male mice weighing 25-30 g were obtained from commercial sources. Hormones were injected daily in 0.1 cc sesame oil or aqueous suspension subcutaneously for 5 days. Control animals received only oil. Following the last injection 0.1 cc (original activity 1 μ c/0.1 cc) of human albumin- I^{131} †

was injected into a tail vein. Eight hours after intravenous injection the animals were killed by cervical fracture. The tail, head, and limbs were removed, the hair was shaved off, and the fat was scraped from the underside of the pelt. The skin was cut into small pieces and put into 15 ml of 90% formic acid in a screw-cap tube. The tubes were autoclaved at 15 lb pressure, 250°F for exactly one hour. After cooling, the contents of the tube were shaken and 5 ml were placed over a "silver saddle" overnight. Triplicate samples of 1 ml were placed in plastic planchettes, the formic acid was evaporated to dryness and I^{131} was counted in a gas-flow apparatus. The recovery of bound I^{131} exceeded 90% by this procedure.

Results. Results are shown in Table I. Four minutes after injection the cpm in the skin of the estradiol-dipropionate-treated mice did not differ from that of the controls.

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† Volk Radiochemical Co., Skokie, Ill.

TABLE I. Albumin- I^{131} in Skin of Mice Treated with Estradiol, Testosterone, and Cortisone.

Substance	Dose/day	Time*	Group				Exp/control (%)
			Experimental		Control		
			No.	cpm†	No.	cpm	
Estradiol dipropionate ¹	5 μg	4 min	13	312 ± 69‡	12	358 ± 81	—12.8
<i>Idem</i>	.2 "	8 hr	13	1658 ± 196	13	1547 ± 158	+ 7.2
"	1 "	"	13	1911 ± 230	"	"	+23.5
"	5 "	"	15	1096§ ± 103	15	812 ± 40	+35.0
"	25 "	"	15	867 ± 109	"	"	+ 6.8
Testosterone propionate ²	1.25 mg	"	13	493 ± 29	12	501 ± 46	— 1.6
Cortisone acetate ³	1.25 "	"	12	424 ± 24	"	"	—15.4

* After I.V. injection of albumin- I^{131} .

† cpm (counts/min) for 1/15 total skin—control values differ because of difference in activities of injected solutions.

‡ Standard error of mean.

§ $P = < .02$.¹ Ovocyclin dipropionate, Ciba Pharmaceutical Products, Inc. U.S.P., E. Lilly & Co.² Testosterone propionate, Cortone acetate, Merck Sharp & Dohme.

Since this period corresponds to the circulation time the finding suggests that blood volume of the skin was not affected by estrogen. The skin of estrogen-treated animals exhibited higher levels of albumin- I^{131} 8 hours after intravenous injection of this substance. Following a total dose of 25 μ g an increase of 35% was observed. With a total dose of 125 μ g the mean value was not much greater than that of the controls. Testosterone propionate produced no effect, and cortisone acetate treatment resulted in a statistically insignificant decrease in albumin- I^{131} content with the doses which were administered.

Discussion. Bound I^{131} in the skin was increased 35% in animals which received 5 μ g of estradiol dipropionate daily for 5 days as compared with their controls. This effect was not observed when cortisone or testosterone was given. These dose levels were used in previous metabolic studies(3). Failure to observe the effect following a daily dose of 25 μ g of estradiol dipropionate may have been due to a toxic action.

The kinetics of albumin transfer from the blood into the skin involves transvascular passage into the interstitial-ground substance space and return to the circulation *via* the lymphatics. During the 8-hour interval considerable metabolism of albumin- I^{131} probably occurred(4); however, there is no evidence that cells in the skin can metabolize this substance. The blood volume in the skin was apparently not increased in the estrogen-

treated animals. The explanation must lie either with increased rate of vascular passage, increase in volume for dispersion of plasma albumin in the ground substance, decreased lymphatic return, or a combination of these.

The second possibility, at least, may operate by virtue of marked changes which occur in the mucopolysaccharides of the skin following estrogen treatment(5). Davidson(5) has reported that estrogen treatment increases the amount of hyaluronic acid in the skin. This would increase its water-holding capacity. The quantity of albumin in the skin would depend in part on the molecular sieving action of hyaluronic acid(6) and on its molecular weight. Testosterone also increases the amount of hyaluronic acid in the skin, but albumin- I^{131} was not increased as compared with that of the controls in the experiment described above. The findings raise the question of whether molecules of similar size to albumin may also increase in connective tissue following estrogen treatment.

Summary. Albumin- I^{131} was injected intravenously into mice. Eight hours later the skin was removed and assayed for bound I^{131} . In animals treated with 5 μ g of estradiol dipropionate daily for 5 days, a significant increase in bound I^{131} was observed. This effect was not found in testosterone- or cortisone-treated mice.

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Myrosinase Activity in Bacteria as Demonstrated by the Conversion of Progoitrin to Goitrin.* (30181)

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Progoitrin is a thioglycoside present in high concentration in the seeds of most Brassicaceous plants and in the edible parts of some, particularly rutabaga and turnip. It is hydrolyzed by enzymes (myrosinases) in these plants to yield goitrin, a potent anti-thyroid compound(1,2,3) (Fig. 1).

Although it had been assumed for many years that hydrolysis of thioglycosides similar to progoitrin could be accomplished only through the action of myrosinase of plant origin, recent studies have indicated that this view is incorrect. Reese *et al*(4) reported that the fungus *Aspergillus sydowi* produced a β -thioglycosidase (termed "sinigrinase") active on the mustard oil thioglycosides sinigrin, sinalbin, and progoitrin; however, the enzyme could not be obtained from other fungal genera or from the bacteria, *Bacillus subtilis* or *Pseudomonas aeruginosa*, which also were found to "consume" sinigrin on prolonged (14-day) incubation with this compound. Thioglycosidase activity hydrolyzing several purinyl thioglycosides has been reported by Goodman *et al*(5) to be widespread in mammalian species, and to occur in the microorganisms *Tetrahymena pyriformis* and *Escherichia coli*.

Because of the possible importance of goitrin derived from commonly ingested vegetables in the production of human non-toxic goiter, studies were undertaken of the metabolic fate of pure crystalline progoitrin in man after oral ingestion. It was found that in such cases goitrin could be demonstrated

in blood and urine(6). These results suggested that the conversion of progoitrin to goitrin might have been carried out by thioglycosidase activity of bacteria in the gastrointestinal tract. This hypothesis was reinforced by the finding that incubation of rat or human feces with progoitrin resulted in goitrin formation(3). This myrosinase activity in feces was destroyed by boiling, filtration, or sonoration, and was markedly reduced by prior oral administration of Amphotericin B and Neomycin(3), a regimen which has been shown to effect a striking decrease in viable fecal bacterial flora(7).

The data reported here concern experiments on the isolation and identification of fecal bacteria with myrosinase activity, comparison of such organisms with stock strains of various bacterial species, and conditions necessary for formation and activity of bacterial myrosinase.

Materials and methods. Progoitrin was prepared from rutabaga seed by the method of Greer(2). Bacterial cell suspensions were obtained by growing the organisms, unless otherwise stated, in nutrient broth containing 1% glucose for 18 hours at 37°C without shaking. The cells were harvested by centrifugation, washed once with water, and resuspended in water at a concentration between 1.0 and 2.0 mg bacterial nitrogen per ml. The cell suspensions were incubated with progoitrin and buffer at concentrations noted in the legends, in a total volume of 5.0 ml contained in 50 ml Erlenmeyer flasks which were shaken in a water bath at 37°C. Length

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