Summary. Succinic (SDH) and lactic (LDH) dehydrogenase activity was studied histochemically in the regenerating forelimb of adult Triturus. During the stages of wound healing and of blastemal cell accumulation, SDH activity decreased in the tissues underlying the wound epidermis while LDH increased. During stages of rapid growth of the regenerate, SDH was not histochemically demonstrable in the mesenchymatous tissue; but LDH activity was high. As the tissue differentiated in later stages of regeneration, SDH activity increased while LDH in general declined. The wound epithelium showed both LDH and SDH activity throughout regeneration with variations in intensity less striking than in underlying parts. The results of our work and of others indicate that in the stages of appearance, accumulation and proliferation of the blastema, metabolism *via* the Krebs cycle is replaced by glycolytic and/or the pentose pathway metabolism.

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Received February 24, 1964. P.S.E.B.M., 1964, v117.

Changes of Tissue Catecholamines in Thiazide-Fed Rats. (29489)

P. A. SERRANO AND L. B. CHÁVEZ (Introduced by W. Raab)
Department of Endocrinology, Instituto Nacional de Cardiologia, Mexico City

The hypotensive action of thiazides has been previously established both in the experimental and therapeutic fields. No satisfactory explanation has been found to account for the prolonged hypotensive mechanism of action of these compounds(1).

During the initial period of treatment with thiazides, the decrease in blood pressure can be explained on the basis of the known change in electrolytes(1-4), as well as on the observed diminution of plasma volume and cardiac output caused by these substances(5); the hypotensive effect after long-term treatment cannot be explained on this basis, as the lowering of blood pressure persists even after the electrolyte changes, the decrease of cardiac output and the decrease of plasma volume have disappeared or have been compensated(5-9).

A possible connection between the hypotensive effect of these drugs and the action of amines has been suspected. It has been shown that administration of the thiazides decreases the hypertensive response to infusion of epinephrine(10-11). Hollander and co-workers(12), found that the diminished reactivity to intravenous administration of noradrenaline disappeared after 20 days treatment with these drugs. Previous communications from our department(13-14) reported a diminution of urinary catecholamine excretion in a group of hypertensive patients who had been treated with thiazides. Diazoxide, a compound with hypotensive effect, which on several trials displayed the ability to retain sodium and water(15-17), clearly shows the dissociation between natriuretic and hypotensive effects of thiazides.

TABLE I. Changes of Tissue Catecholamines in Thiazide-Fed Rats.

	Heart		Kidney		Brain		Adrenal glands	
	Control group	Treated group	Control group	Treated group	Control group	Treated group	Control group	Treated group
Adrenaline								
N	10	10	10	10			9	9
Mean value	.126	.096	.150	.110	+	+	.422	.255
± S.E.	.009	.009	.010	.011			.033	.028
"P"		<.05		<.05				<.05
Noradrenaline								
N	10	10	10	10	10	10	9	9
Mean value	.328	.413	.118	.081	.224	.174	.097	.092
± S.E.	.025	.033	.008	.008	.013	.014	.014	.016
"P"		>.05		<.05		<.05		>.05
Dopamine								
N					10	10		
Mean value	+	+	+	+	.247	.136	+	+
± S.E.					.018	.030		
"P"						<.05		

Values are given in μg/g of tissue except for adrenal gland, which is expressed in mg/g.
N = No. of animals in each group.
± S.E. = ± Standard error of mean.
+ = Undetectable amounts.

The present work was planned with the aim of studying the possible effects of thiazides on catecholamine metabolism, by measuring tissue levels of adrenaline, noradrenaline and dopamine before and after thiazide feeding to rats.

Methods. Wistar rats, weighing 140 to 160 g with a random distribution of homogeneous groups, were used in this experiment. Quinethazone was the first compound to be tested and the present report concerns the effect of this drug on tissue catecholamines. The drug was uniformly mixed with a normal diet and administered during 20 consecutive days. This thiazide was studied in amounts similar to the therapeutic dosage used in humans, namely 600 μg/kg/day; 10 animals each were used in the treated and control groups.

At the end of the study, the animals were sacrificed by decapitation without anesthesia and the heart, kidney, brain and adrenal glands were placed in the deep-freeze until analysis. Adrenaline, noradrenaline and dopamine were measured after the homogenization of each organ, using a fluorimetric differential technique as described by Sourkes *et al*(18).

Results. Noradrenaline was detected in all the above listed organs. Adrenaline was detected in all of these organs except brain tissue. Dopamine was not detected except in brain tissue (Table I).

A tendency toward a decrease of catecholamine concentration was evident in almost all organs. Adrenaline decreased in heart, kidney and adrenal gland with a "p" value <0.05 in each group. Concentration of noradrenaline was found to decrease in the kidney, ($p<0.05$) and also in the adrenal gland, but in this last organ the change was not found statistically significant when compared to the control values ($p>0.05$). In brain tissue, the concentration of noradrenaline and dopamine was also found to decrease significantly. The mean value of noradrenaline concentration of the heart was higher in the treated group than in the control animals, but this difference gave a "p" value >0.05.

Discussion. Our results have shown that catecholamine concentration is decreased in some tissues of the rat after thiazide feeding.

Administration of the drug was continued over a 20-day period, because it has been found that after this time the changes of cardiac output and plasma volume have almost disappeared(5-9). This number of days was also selected on the basis of results of

previous experiments with other thiazides in which the decrease of catecholamines was evident after this interval(20).

Quinethazone is used in humans in doses from 50 to 150 mg/day which represents 842 to 2500 μg/kg for an adult patient of 60 kg. In a previous study(20), polythiazide was studied at 3 dose levels, from the equivalent of the human therapeutic dose per kg, to 10 and 100 times more in the 2 following groups. The tissue catecholamine-decreasing effect was observed in the 3 groups without a dose effect relationship. Quinethazone was studied in an amount per kg proportionate to the lowest maintenance dose used in human beings.

Adequate homogeneous controls were employed in this as in previous experiments in order to compare the usual variations of catecholamine concentration in the tissue of normal animals observed in different groups. These variations are due not only to the technique (which has been found to be reproducible, both with regard to recovery figures and standard curves) but also to changes in environment of the animals, such as seasonal variations, temperature and feeding.

Physical conditions and weight of the animals were controlled and no important alterations were observed. Blood pressure and electrolyte changes could not be controlled.

The change in tissue catecholamine concentration after thiazide feeding to rats is not as evident as that observed after reserpine or after administration of an monoamine-oxidase inhibitor(19). However, it is felt that after thiazide feeding to rats, the decrease in catecholamine concentration was evident in most of the organs studied and that it concerns the 3 types of amines detected by this technique: dopamine, adrenaline and noradrenaline. Adrenaline seems to be most markedly and most regularly decreased. Only in one category of tissue was a catecholamine increase observed, namely noradrenaline in the heart muscle. Similar results were obtained when polythiazide was used(19,20).

It appears that catecholamine metabolism is disturbed during thiazide feeding. This phenomenon might possibly be involved in the hypotensive effect of these drugs, as suggested by a previously observed decrease in catecholamine excretion in hypertensive patients treated with thiazides(13,14).

The hypotensive effect of thiazides is suspected as being due to an interference with the normal action of amines, because of the decreased vascular reactivity found after thiazide administration(10), and because of the difficulty of explaining the sustained hypotensive action as being caused only by electrolyte and hemodynamic changes(11). Furthermore, the alterations in catecholamine metabolism might be related to the observed persistence of decreased peripheral resistance under prolonged administration of thiazides (5,9).

Experiments will be carried out with Diazoxide, a compound which has proved to produce evident hypotensive effects without an increase of sodium and water excretion(15-17).

Summary. Tissue concentration of catecholamines in heart, kidney, brain and adrenal gland was measured in Wistar rats to which Quinethazone was orally administered at doses of 600 μg/kg/day, during 20 consecutive days. A tendency to decreased catecholamine concentration was evident. Adrenaline especially was observed to decrease in heart, kidney and adrenal gland to a degree which, by comparison to control animals, seems to be statistically significant. It is suggested that this alteration in catecholamine metabolism might possibly contribute to the decreased peripheral resistance and hypotensive effect of thiazides.

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Received March 16, 1964. P.S.E.B.M., 1964, v117.

Anti-Inflammatory Assay. An Improved Method Based on the Reversal of Endotoxin-Induced Lung Inflammation in Mice. (29490)

A. MELI, C. R. SMITH AND A. WOLFF (Introduced by W. M. Govier)
Department of Physiology, Warner-Lambert Research Institute, Morris Plains, N. J.

Inhalation of aerosolized bacterial endotoxin (S. Abortus Equi) induces in mice severe inflammation and increase in weight of the lung. The observation that these effects could be reduced by subsequent administration of anti-inflammatory agents led to the development of an anti-inflammatory assay (1).

In its original design, however, the aerosolization chamber presented some inconveniences: (a) tendency of the animals to crowd in a limited area of the chamber; (b) occasional deposition of feces in the open end of the nebulizer, resulting in occlusion of the nozzle, which required repetition of the test; (c) use of vaseline as a seal between the dome and the stand, and (d) the limited number of animals per each exposure.

With the apparatus described here, not only were these inconveniences eliminated, but it was observed that a concentration of endotoxin equal to that used previously(1) could produce a comparable degree of inflammation and increase in lung weight when administered to a double number of animals. This observation prompted us to investigate the effect of lower concentrations of endotoxin.

Materials and methods. The apparatus is shown in Fig. 1 and 2. The Black, Sivalls and Bryson regulator with pressure gauge, is attached directly to the pressurized air outlet. The DeVilbiss No. 40 nebulizer is connected to the regulator by Tygon tubing (18″ long, ¼″ i.d.) and attached to the stand *via* a ⅞″ rubber stopper with a ⅝″ hole in the center.

The plastic dome is 9″ high with an inner diameter of 10¼″. The outside diameter at the base is 11½″. The upper part of the dome begins to assume its concave appearance approximately 4½″ from the base. A rubber "half round" gasket (¼″ wide, ⅛″ thick) is glued to the peripheral edge of the base of the dome.

The round cage in which the animals stay during aerosolization consists of: (a) a 5/16″ square wire mesh platform (10″ i.d.) with a 3¼″ hole in the center, (b) 2 concentric walls, 4¼″ high, 10″ and 3¼″ i.d., respectively, made of galvanized sheet metal (27 gauge) and (c) a 5/16″ square wire mesh cover with a 3¼″ hole in the center, kept in place by 3 galvanized tabs bent at a 90° angle and soldered to the periphery of the cover. The inner part of the cage (Fig. 2) is divided into 4 sections, each one large enough to house 5 mice. The cage is sup-