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Effect of Actinomycin-D on Calcium Homeostasis. (30343)

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Actinomycin-D, an inhibitor of DNA dependent synthesis of RNA(1), has been shown to block the actions of several hormones(2,3). Recent studies(4,5) have also clearly established the fact that this drug abolishes the hypercalcemic response of parathyroidectomized rats to exogenous parathyroid hormone. The hypothesis has been advanced that Actinomycin-D, by blocking protein synthesis, antagonizes the action of parathyroid hormone on calcium mobilization(4). If this hypothesis is valid, then the drug should produce a hypocalcemic response when given to normal animals by blocking the action of endogenous parathyroid hormone.

The following experiments were performed to determine if such a hypocalcemic effect could be produced in rats with intact parathyroid glands.

Methods. Male Sprague-Dawley rats weighing from 150 to 170 g were used. Actinomycin-D (Merck, Sharp and Dohme) was injected intraperitoneally. Control rats were injected with an equal volume of normal saline. The following groups of animals were used: 1) Normal, injected with Actinomycin-D, in a dose of 1 μg/g body weight; 2) Nephrectomized rats: bilateral nephrectomy was performed in these animals 24 hours prior to injection of Actinomycin-D, 1 μg/g body weight; 3) Adrenalectomized rats: bilateral adrenalectomy was done 48 hours prior to injection of Actinomycin-D, 0.5 μg/g body weight. These rats were allowed 0.9% saline for drinking. 4) In the last group of rats total thyro-parathyroidectomy was performed under ether anesthesia. The parathyroid glands were then transplanted into the gastrocnemius muscle. A week later blood was obtained through the retro-orbital venous plexus and serum calcium determined. Only those rats with serum calcium above 7 mg% indicating functional parathyroids were injected with Actinomycin-D, 1 μg/g body weight.

In all experiments, after administration of the drug, the rats were given no food, only drinking water, until they were anesthetized at the appropriate time interval and bled through the abdominal aorta. Serum calcium determination was performed by the Kingsley-Robnett method(6).

Results. In a preliminary study adrenalectomized rats appeared particularly sensitive to Actinomycin-D and did not tolerate a dose of 1 μg/g body weight. None of them survived the 24-hour period after injection. A mortality of approximately 20% occurred, however, under the conditions of this experiment, when a smaller dose of 0.5 μg/g body weight was used. Other groups of rats tolerated well a dose of 1 μg/g body weight.

Table I shows the average serum calcium of various groups of Actinomycin-D treated and saline injected control rats killed 24 hours after injections. The mean serum cal-

TABLE I. Serum Calcium in Rats, 24 Hours After Injection of Actinomycin-D or Normal Saline. Means and S.D.

Groups	No. of rats	Treatment	Serum calcium (mg %)
Normal rats	6	Saline inj	9.53 ± .46
" "	6	Actinomycin-D	7.83 ± .83*
Nephrectomized rats	5	Saline inj	9.48 ± .78
" "	5	Actinomycin-D	5.34 ± 1.01*
Adrenalectomized rats	6	Saline inj	9.52 ± .52
" "	6	Actinomycin-D	7.55 ± .64*
Thyroidectomized rats with functional parathyroid transplants	5	Before inj	8.68 ± 1.02
		After Actinomycin-D	5.42 ± 1.18*

* P <.001

cium of normal rats treated with the drug was 7.83 ± 0.83 mg%, significantly lower than that of the control rats, 9.53 ± 0.46 mg% (P<0.001).

Actinomycin-D treated nephrectomized rats had an average serum calcium of 5.38 ± 1.01 mg%, again significantly lower than the saline injected nephrectomized rats, whose serum calcium averaged 9.48 ± 0.78 mg% (P<0.001).

Bilateral adrenalectomized rats, when treated with Actinomycin-D, had an average serum calcium of 7.55 ± 0.64 mg%, significantly lower than the mean serum calcium of saline injected adrenalectomized rats, 9.52 ± 0.52 mg% (P<0.001).

Thyroidectomized rats, with functioning parathyroid transplants, had an average serum calcium of 8.68 ± 1.02 mg%. Twenty-four hours after injection of Actinomycin-D, the average serum calcium decreased significantly to 5.42 ± 1.18 mg% (P<0.001).

The serum calcium of normal rats killed at 0, 3, 6, 10, 12, 15, 18, 21 and 24 hours after injection of a drug was also determined. A curve constructed from the data obtained is presented in Fig. 1. As can be seen, serum calcium remains close to the normal 9 mg% for about 10 hours after administration of the drug. Then a gradual fall begins until a low level of approximately 7 mg% is seen at 15 hours, after which it levels off.

Discussion. These results clearly demonstrate the hypocalcemic effect of Actinomycin-D in rats with intact parathyroid glands. That this effect is not mediated through renal excretion of calcium or hypocalcemic factors from the adrenals(7) or thyroids(8), is evident from the fact that it occurs even in the absence of kidneys, adrenals and thyroid glands (Table I).

It is interesting to compare the time course of the development of hypocalcemia after Actinomycin-D treatment (Fig. 1), with that reported by Munson for parathyroidectomized rats(9). In his study, the fall in serum calcium occurred almost immediately after parathyroidectomy and in 2 hours had reached the minimal value attained(9). In our study, the decline in serum calcium began about 10 hours after injection of Actinomycin-D and levelled off by 15 hours. This apparent difference in timing of the fall in serum calcium is striking and cannot now be explained.

Another interesting difference may be noted between effect of Actinomycin-D on

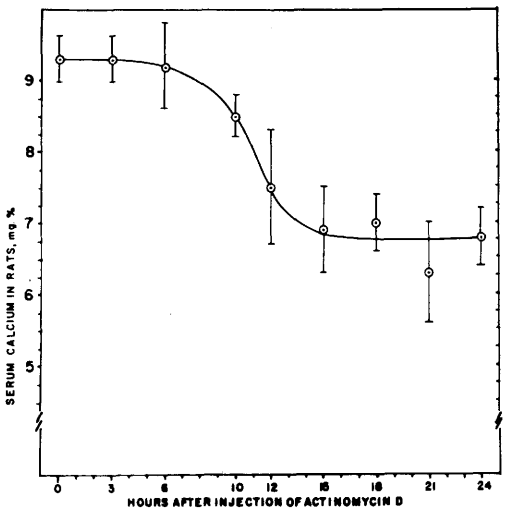

FIG. 1. Serum calcium of normal rats at 0, 3, 6, 10, 12, 15, 18, 21 and 24 hr after injection of Actinomycin-D, 1 μg/g of body weight. Five rats in a group. Means and S.D.

exogenous and endogenous parathyroid hormone. In Tashjian's studies(5), Actinomycin-treated rats were parathyroidectomized and given exogenous parathyroid hormone 3 hours after administration of the drug. Hypocalcemia was noted 7 hours after Actinomycin (4 hours after parathyroid hormone injections). In our studies, hypocalcemia did not occur until 10 hours after administration of the drug to rats with intact parathyroids. It must be noted also that Tashjian *et al*(5) did not obtain hypocalcemia 7 hours after administration of Actinomycin-D to rats with intact parathyroid glands. They were unable, as we are unable at the present time, to explain the difference between the effect of the drug on exogenous and endogenous parathyroid hormone.

From present available data, no definite conclusion may be reached as to the exact mechanism of action whereby Actinomycin-D produced hypocalcemia. It is possible that the drug, by antagonizing the calcium mobilizing effect of endogenous parathyroid hormone, causes a fall in serum calcium. However, another possibility must be considered. Since parathyroid hormone is a protein or polypeptide and by definition may be affected by agents blocking synthesis of proteins, does Actinomycin-D inhibit synthesis and/or secretion of parathyroid hormone, and by this mechanism affect calcium homeostasis? This question was not answered by our experiments. Further studies are indicated.

Summary. Actinomycin-D produces hypocalcemia in rats 10 to 15 hours after administration. This effect can be produced in the absence of kidneys, adrenals and thyroid glands. Possible mechanisms of action are discussed.

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Effect of Hemodialysis on Serum and Parotid Fluid Phosphate Levels. (30344)

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Mechanisms controlling the inorganic phosphate concentration of parotid fluid are not understood. Studies by Langley *et al*(1) have suggested that parotid fluid phosphate levels in the dog are influenced by alterations in serum values. A 7-fold increase in plasma phosphate doubled parotid fluid concentration. Studies with human subjects have produced conflicting results in this regard(2-10).

The high serum phosphate levels encountered in azotemic renal disease are subject to rapid readjustment when hemodialysis is employed. The present study was designed to determine the effect of decreasing abnormally high serum phosphate concentrations by hemodialysis on parotid fluid phosphate levels.