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EFFECT OF ACTINOMYCIND ON THE UPTAKE OF RADIOIODINE BY THYROIDAL AND EXTRATHYROIDAL TISSUES—II

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Abstract—Actinomycin D, *in vivo*, in mice markedly reduces the sodium, potassium and iodide ion excretion indicating an impaired renal clearance. Histopathological examination of kidney of mice injected with the drug reveal distinct degenerative changes in the distal tubule region. The degree of damage to the kidney parallels the amount of the drug injected.

The rates of the emptying time of iodide from the stomach to the intestine and excretion through the kidney are significantly lowered in the experimental animals.

The increased thyroidal radioiodine uptake after 5 hr is due to a longer availability of the radioiodine caused by the impaired renal clearance and a delay in emptying time of stomach. Histological findings do not show any evidence of stimulation of thyroid gland.

REPORTS concerning the action of actinomycin D, an inhibitor of nucleic acid and protein synthesis, on the thyroidal iodide metabolism are in disagreement.[1–11] Dumont *et al.*[6, 8] reported a stimulatory effect of the drug on the uptake of radioiodine by the thyroid gland, 3 hr after the administration of radioiodine. However, Halmi and his associates[7] have not been able to confirm these findings in rats. We, in our laboratory, have also failed to demonstrate 3 hr increase in thyroidal radioiodine uptake in mice.[9]

In our previous publication, we have reported that actinomycin D, *in vivo*, in mice, increases the iodide retention which may be a result of either impaired renal clearance or a delay in emptying time of stomach.[11] In the present communication, we have further elucidated the mode of action of actinomycin D on the iodide metabolism in mice, *in vivo*.

MATERIALS AND METHODS

Actinomycin D was obtained from Merck Sharp and Dhome Co. $Na^{131}I$ was supplied by Isotope Division, Bhabha Atomic Research Centre, Bombay. Male Swiss mice weighing 25–30 g fed on a colony diet were used in all experiments. The antibiotic was injected intraperitoneally (i.p.) 17 hr prior to the administration of 3–4 μc of radioiodine. In all the experiments, both the control and the act D treated group consisted of six animals.

Total iodide retention studies were done by the whole body counting of the animals by a 4′ × 4″ NaI scintillation detector, at a distance of 15 cm from the detector.

Sodium and potassium estimations were done on 24-hr urine sample by flame photometer.

Effect of graded dose of act D

The animals were injected with 5, 10 and 15 μg actinomycin D 17 hr prior to administration of 3–4 μc of radioiodine. Control group of animals received an equivalent volume of saline. Total body retention studies were performed at the interval of 3, 5, 7, 10, 24 and 72 hr as mentioned earlier.[11]

Effect of acute and divided dose of act D

Three groups of animals were administered a total dose of 5, 5 + 5 and 5 + 5 + 5 μg of act D i.p. respectively. Initially all the animals received 5 μg of act D 17 hr prior to 3–4 μc of Na ^{131}I i.p. Each additional 5 μg was injected into the respective groups of mice receiving 5 + 5 and 5 + 5 + 5 μg at the interval of 48 hr. Control groups received an equivalent amount of saline. Two additional batches of animals received 10 and 15 μg act D in one single dose. Total body counting was followed at the interval of 4, 24, 48, 72, 96 and 144 hr from the time radioiodine was administered. At the end of the experiment, the radioiodine distribution in various organs was studied by counting radioiodine content in various organs such as thyroid, stomach, kidney and carcass.

Renal function

Control and experimental animals (act D; i.p.; 10 μg/mice) were placed in metabolic cages and the sodium and potassium estimation were done on 24 hr pooled urine sample.

Determination of rate of radioiodine uptake in various tissue of mice

In order to study the rate at which the iodine localises in various organs of mice, a group of one hundred animals was taken of which fifty animals were injected with with 10 μg act D whereas the remaining received an equivalent amount of saline, 17 hr prior to 3–4 μc of radioiodine administration. At the end of 2, 4, 6, 9, 12, 15, 18 and 24 hr, six animals from each group were sacrificed. Thyroid, stomach, kidney, intestine and carcass were removed and counted for their radioiodine content. Percentage distribution of the administered dose of radioiodine in each of these tissues was then calculated and the data was analysed on an analogue computer model No. EAC 62.

Histopathological studies

In almost all the studies the thyroids and kidneys were removed at the end of the experiment and were examined histologically.

RESULTS

Effect of graded doses of antibiotics

With increasing doses of the antibiotic, increasing retention of radioiodine was observed in mice (Fig. 1). This is possible if the degree of damage to the kidney or inhibition of the motility of the stomach is a function of the dose of the antibiotic. Histological examination of kidneys from animals injected with 5 μg did not reveal any significant damage. Animals injected with 10 μg showed minimal degenerative

changes in the renal tubules, while the animals injected with 15 μg showed distinct damage to the distal tubules resulting in necrosis at places (Fig. 2).

Effect of divided and single large dose of act D

The animals receiving a divided dose of act D showed a decrease in the percentage of radioiodine retention as compared to those which received an equivalent amount of the drug in one single dose (Table 1). Animals injected with 5 μg did not show any retention of radioiodine. There is an increasing retention of iodide as the antibiotic is

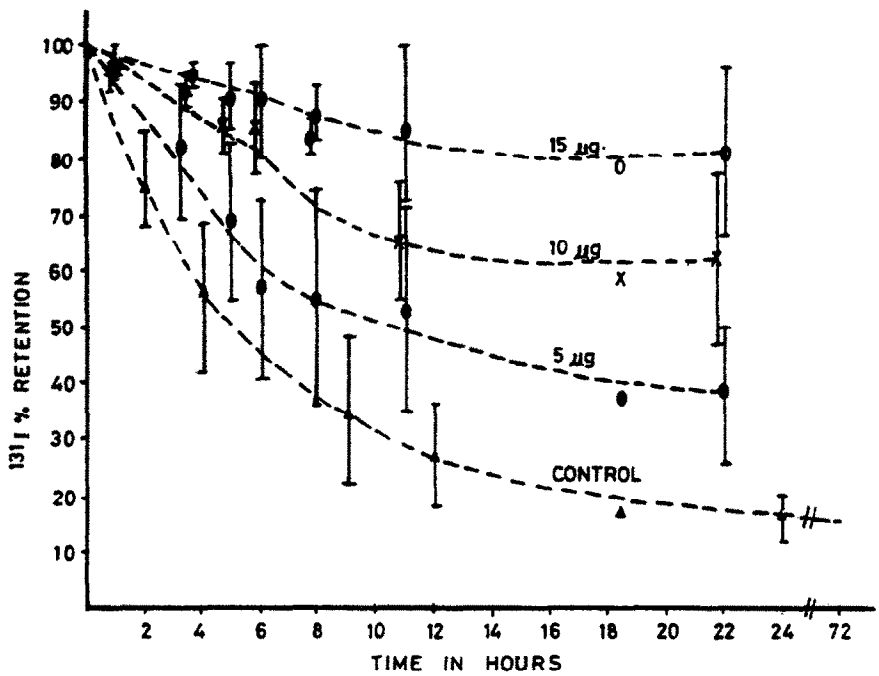

FIG. 1. The animals were injected with 5, 10 and 15 μg actinomycin D 17 hr prior to administration of 3–4 μc of radioiodine. Control group of animals received an equivalent volume of saline. Total body retention was performed at the interval of 3, 5, 7, 10, 24 and 72 hr.

injected for more number of days. However, 5 + 5 and 10μg have equal amount of the radioiodine retained at the end of 144 hr though animals injected with 10 μg as a single dose showed larger retention of radioiodine in the initial stages. Fifteen μg dose was found toxic to the animals as all the animals were dead in this group.

The distribution of radioiodine in various organs of the mice at 144 hr showed an increase in thyroid uptake in the experimental group, Table 2. The concentration of radioiodine in the stomach was six times higher than that in the normal animals. However, the histological findings do not show any change in the thyroid gland while as reported above, kidneys showed evidence of damage to the distal tubules.

TABLE 1. EFFECT OF ACTINOMYCIN D ON WHOLE BODY PERCENTAGE RETENTION OF ^{131}I WITH GRADED AND SINGLE DOSES

Time in hr	Saline	Act D (5 μg)	Act D (5 + 5 μg)	Act D (5 + 5 + 5 μg)	Act D (10 μg)
4	41·52 (± 20·1p)	40·32 (± 15·8p)	52·27 (± 13·7)	53·22 (± 12·1)	66·7 (± 9·1)
24	22·05 (± 5·8p)	20·96 (± 11·6)	26·8 (± 5·9)	29·8 (± 11·5)	57·1 (± 16·4)
48	15·52 (± 3·26)	17·74 (± 3·13)	20·02 (± 2·97)	23·9 (± 7·2)	29·77 (± 16·8)
72	11·3 (± 2·36)	13·2 (± 1·61)	13·86 (± 1·94)	18·8 (± 5·86)	21·47 (± 4·2)
96	9·65 (± 2·08)	11·91 (± 1·41)	11·55 (± 2·76)	19·44 (± 5·95)	17·87 (± 0·22)
144	8·56 (± 1·94)	9·46 (± 3·28)	13·5 (± 2·46)	16·45 (± 8·1)	13·75 (± 1·63)

Animals received 5, 5 + 5, 5 + 5 + 5, 10 and 15 μg of actinomycin D 17 hr prior to 3–4 μc of radioiodine. Each additional 5 μg of actinomycin D was injected i.p. at 48 hr. interval. The control groups of mice received an equivalent amount of saline. The whole body counting was carried out as indicated in text.

Figures in the parenthesis indicate standard deviation.

TABLE 2. PERCENTAGE DISTRIBUTION OF ^{131}I IN DIFFERENT ORGANS OF MICE AT 144 hr

	% ret.	Stomach	Thyroid	Kidney	Carcass
Saline					
Experiment I	9·30 (± 2·75)	0·15 (± 0·10)	11·08 (± 4·39)	0·48 (± 0·12)	5·36 (± 1·09)
Experiment II	14·28 (± 4·23)	0·203 (± 0·158)	17·0 (± 6·7)	0·76 (± 0·20)	8·21 (± 1·70)
Act D 5 μg					
Experiment I	12·21 (± 4·82)	0·77 (± 0·09)	13·44 (± 4·78)	0·49 (± 0·15)	8·1 (± 1·81)
Experiment II	18·75 (± 7·39)	1·18 (± 0·14)	20·7 (± 6·58)	0·44 (± 0·23)	12·5 (± 2·8)
Act D 10 μg					
Experiment I	14·92 (± 2·35)	0·95 (± 0·89)	16·99 (± 4·82)	0·28 (± 0·15)	8·5 (± 1·5)
Experiment II	22·96 (± 3·46)	1·45 (± 1·38)	26·1 (± 7·4)	0·98 (± 0·39)	13·03 (± 2·30)

The animals received 5 μg and 5 + 5 μg actinomycin D i.p. (5 μg was administered every 48 hr) 17 hr prior to 3–4 μc radioiodine i.p. administration. At the end of 144 hr, the animals were excised and the radioiodine content of stomach, thyroid, kidney and carcass were calculated as percentage of administered dose.

Figures in the parenthesis indicate standard deviation.

Renal function

In order to assess the renal function, Na^+ and K^+ estimations were done on 24 hr pooled urine sample of the control and experimental animals. A significant reduction was observed in the excretion of both the electrolytes, Table 3. Sodium excretion was reduced by 70 per cent while potassium excretion was reduced by 60 per cent.

Determination of rate of radioiodine incorporation in various tissues

Table 4 shows the percentage distribution of radioiodine in various organs of mice.

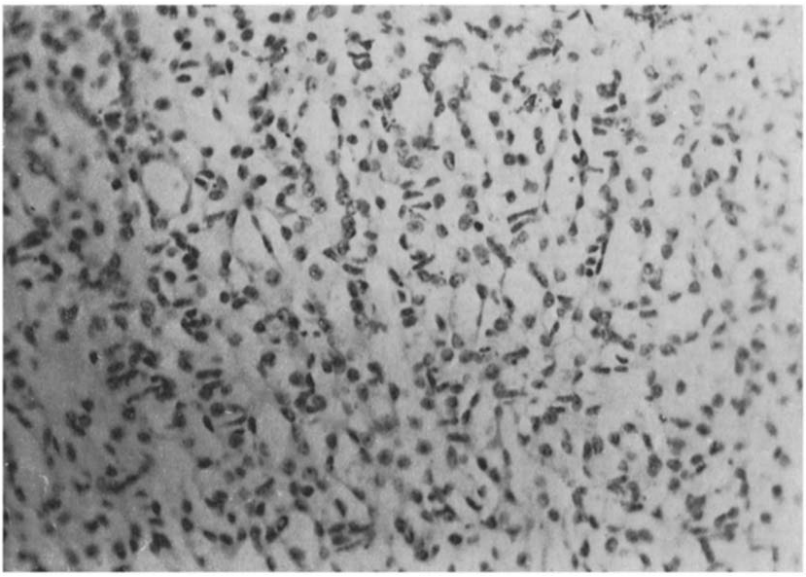

FIG. 2. A microphotograph of kidney slide showing degenerative changes of the tubules in mice injected with 10 μg of actinomycin D sacrificed at the end of 24 hr magnification $\times$ 280.

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TABLE 3. EFFECT OF ACTINOMYCIN D ON SODIUM AND POTASSIUM EXCRETION IN URINE

Expt.		Sodium m-equiv./l.	potassium m-equiv./l.
Control	Experiment I	0·068	0·03
	Experiment II	0·051	0·047
	Experiment III	0·075	0·040
Act D	Experiment I	0·032	0·02
	Experiment II	0·023	0·019
	Experiment III	0·020	0·015

Control and the experimental group of animals (actinomycin D i.p. 10 μg/mice) were put in metabolic cage and sodium and potassium ions were estimated in 24 hr urine collection

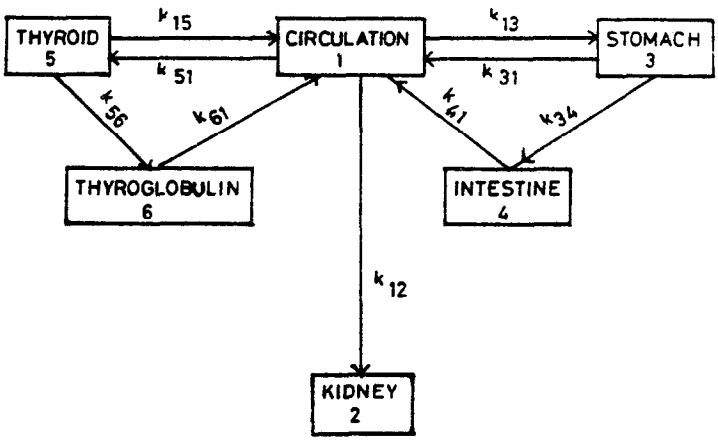

FIG. 3. A simplified iodide model pool system.

It is evident from the data that thyroid uptake is low initially, rises to a comparable value as that of the control animals at about 5 hr and then increases significantly above the control value whereas the stomach concentration in the experimental animals is always higher than that of the control animals.

Above data was analysed in an analogue computer, with a simplified iodide model pool system as shown in Fig. 3. The observed total body retention could be satisfied only when the parameters namely, renal clearance (k_{12}) and release from the stomach to intestine (k_{34}) were changed. As seen in Table 5, there is a marked decrease in the rate constants (k_{12}) and (k_{34}) in the experimental animals as compared to that of the control group of mice.

DISCUSSION

The data reported by various investigators on effect of actinomycin D on 4 hr thyroidal radioiodine uptake is at variance. Dumont *et al.*[6] reported a sevenfold increase in the uptake of radioiodine by the thyroid gland 3 hr after the administration of radioiodine in mice. However, not only that we fail to observe any such stimulation

TABLE 4. DISTRIBUTION OF RADIOIODINE IN THE VARIOUS ORGANS OF CONTROL AND ACTINOMYCIN D-TREATED MICE AS PERCENTAGE OF ADMINISTERED DOSE

Time in hr	Retention		Thyroid		Intestine		Stomach		Carcass	
	Control 6	Act. D 6	Control 6	Act D 6	Control 6	Act D 6	Control 6	Act D 6	Control 6	Act D 6
2	75·96 (± 8·56)	87·6 (± 4·45)	7·62 (± 2·02)	4·74 (± 0·93)	4·3 (± 1·6)	5·02 (± 1·9)	7·41 (± 3·84)	14·75 (± 4·0)	34·6 (± 8·9)	40·0 (± 6·8)
4	55·12 (± 13·1)	80·15 (± 11·6)	9·6 (± 2·2)	7·6 (± 2·4)	3·3 (± 0·16)	3·1 (± 0·7)	5·0 (± 2·7)	15·6 (± 5·3)	28·8 (±17·8)	35·6 (± 6·02)
6	34·6 (± 11·1)	84·6 (± 10·7)	8·8 (± 1·6)	11·1 (± 3·8)	2·2 (± 0·54)	3·8 (± 2·2)	2·4 (±2·0)	26·3 (± 5·8)	11·6 (± 5·2)	28·2 (± 3·6)
9	35·2 (± 12·78)	69·43 (± 16·7)	10·95 (± 3·1)	12·7 (± 2·1)	2·7 (± 1·8)	2·5 (± 0·7)	3·4 (± 1·9)	18·6 (± 7·8)	11·4 (± 4·5)	22·5 (± 6·4)
12	27·0 (± 8·8)	60·4 (± 11·9)	10·3 (± 3·0)	17·8 (± 2·8)	1·1 (± 0·14)	2·1 (± 0·18)	1·6 (± 1·2)	11·9 (± 3·5)	6·2 (± 2·3)	17·6 (± 5·1)
15	26·2 (± 8·1)	46·6 (± 14·4)	11·5 (± 5·6)	17·8 (± 2·9)	1·52 (± 0·63)	1·84 (± 0·35)	1·64 (± 0·83)	11·04 (± 6·2)	6·0 (± 3·53)	13·43 (± 17·5)
19	29·0 (± 11·6)	53·7 (± 15·9)	11·7 (± 4·9)	20·3 (± 2·6)	1·8 (± 0·94)	1·8 (± 0·3)	2·34 (± 1·75)	10·9 (± 5·31)	9·55 (± 2·96)	13·9 (± 6·65)
23	35·3 (± 8·8)	56·9 (± 13·4)	16·0 (± 2·4)	23·4 (± 2·4)	1·93 (± 0·23)	1·93 (± 0·2)	3·43 (± 1·26)	9·82 (± 2·4)	12·2 (± 4·3)	10·64 (± 1·71)

Control and actinomycin D treated group were administered saline and actinomycin D 10 μg i.p. 17 hr prior to i.p. injection of 3–4 μc of I-131. At the interval of 2, 4, 6, 9, 12, 15, 19 and 23 hr, thyroid stomach kidney, intestine and carcass were counted. Figures in the parenthesis indicate standard deviation.

TABLE 5.

Rate constants	Control	Actinomycin D
k_{12} = rate constant for excretion from plasma via kidney	0·182	0·064
k_{13} = rate constant for absorption by stomach.	0·184	0·182
k_{31} = rate constant for reabsorption from stomach.	0·086	0·132
k_{34} = rate constant for release from the stomach to intestine	0·842	0·256
k_{41} = rate constant for reabsorption from intestine	1·57	1·57
k_{51} = rate constant for uptake by thyroid	0·045	0·040
k_{15} = rate constant for release of iodide from thyroid	0·00	0·00
k_{56} = rate constant for synthesis of thyroxine	0·046	0·046
k_{61} = rate constant for release of thyroxine	0·493	0·493

of 3 hr thyroidal radioiodine uptake, but we find an initial lag in the uptake of radioiodine by the thyroid gland. Halmi and his associates also failed to observe any significant enhancement of the 4 hr uptake of ^{131}I by the thyroid gland in rats.[7] None of these workers have tried to pursue their studies long enough or correlate it with its effect on other iodide concentrating tissues.

In our previous report, we had shown that mice treated with actinomycin D retain a large amount of radioiodine as compared to that of the control group of animals.[9] The retention of radioiodine was attributed to either a delay in emptying time of stomach and/or impaired renal clearance. A delay in emptying time of stomach was visualised by the passage of barium through the G.I. tract.

Our data with graded and single dose of actinomycin D indicate that iodide retention increases paripassu with the amount of the drug injected. Histological examination of kidney slides of actinomycin D-treated animals show a progressive degenerative change in the region of distal convoluted tubule. Kidneys from animals injected with 5 + 5 μg show degenerative changes in tubules and kidneys exposed to 10 μg showed widespread degenerative changes in tubular region with necrosis at places. Glomerules were unaffected and the changes were distinct in the medullary region only. Both histological and iodide retention studies suggest damage to the kidney tubules, proportional to the amount of the drug injected. This effect though less marked but significant persists even after 144 hr (Table 2).

Impaired renal clearance was further evidenced by the significant amount of reduction of sodium and potassium in the 24-hr urine samples.

Percentage distribution of radioiodine in various organs of the control and experimental animal when analysed on the computer, revealed that the observed changes in total body retention can be brought about only by simultaneous affection of two parameters of iodine metabolism, namely renal clearance and secretion and reabsorption from G.I. tract. There is a marked reduction in emptying time of stomach (0·842–0·256) and in renal clearance (0·182–0·064) in experimental animals. Other parameters were not affected appreciably (Table 5).

Other investigators[7, 10] have measured the concentration of radioiodine in blood to check for the nephrotoxic effect of the drug. In their thyroidal radioiodine uptake studies they have removed thyroids 12 hr after the injection of radioiodine, which was given 4 hr after the administration of 5 mU of TSH. As seen from our data (Table 4) 12 hr is too late a period to observe the initial low uptake of iodide in the thyroid gland (control and the experimental animals have a comparable uptake at the end of 5 hr

after the administration of radioiodine after which the thyroidal iodide concentration increases in the experimental animals). Their failure to observe any nephrotoxic effect by measuring serum iodide concentration could be readily explained from our data, as most of the radioiodine is locked up in the stomach and hence the iodide level in the blood does not rise appreciably. A decreased food intake as explained by Dumont *et al.*[6] cannot be the cause of an increased thyroidal uptake as in our studies the food was withheld in both the control and experimental animals.

Histological examination of the thyroid gland also, does not show any change. The observed increased thyroidal radioiodine uptake in the actinomycin-pretreated animals is explained by a longer availability of radioiodine because of impaired renal clearance and delayed emptying of the stomach.

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