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EFFECT OF ACTINOMYCIN D ON THE UPTAKE OF RADIOIODINE BY THYROIDAL AND EXTRATHYROIDAL TISSUES—I

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Abstract—Actinomycin D at a concentration of 15 μg/30 g mice markedly reduces the renal excretion and impairs the motility of stomach and thereby indirectly affects the iodide metabolism by sequestering a large amount of iodide in the stomach. It is possible that there is an enhancement of the uptake of radioiodine by the thyroid gland but it is evidenced only when the stomach releases iodine.

SEVERAL reports concerning the effect of Actinomycin D on the metabolism of the iodine in the thyroid gland have appeared[1–6] but none of them are conclusive enough. In fact, some of them are contradictory to each other. Actinomycin D was reported by Dumont *et al.*[7] to stimulate the uptake of radioiodine by the thyroid gland 3 hr after the administration of radioiodine. It was postulated by them that the enhanced uptake was due to a reversal of existing iodinated peptidic inhibitors on the basis of Halmi's[8] work along these lines. However, later investigations by the same authors revealed that the enhanced uptake was not due to any specific effect of Act. D but was due to reduced food intake of the mice pretreated with Act. D.[9]

Halmi[1] had failed to show an increase in thyroid uptake of radioiodine in rats. The work in our laboratory has also failed to demonstrate an increase in thyroid uptake of radioiodine in mice. It was shown in our earlier publication that Act. D treated animals showed a significant sequestration of iodine in the stomach.[10]

The present investigations were undertaken to study the effects of Act. D on iodine metabolism *in vivo* in mice and explore the causes of marked sequestration of iodine in the stomach.

MATERIALS AND METHODS

Carrier free radioactive $Na^{131}I$ and high specific activity (15 mc/mg) ^{51}Cr (sodium chromate) were supplied by Isotope Division, Bhabha Atomic Research Centre, Bombay. Actinomycin D was a product of Merck Sharp and Dhome Co. Pan.

Male Swiss mice weighing 25–30 g were injected intraperitoneally (i.p.) with freshly prepared Actinomycin D (Act. D) in a dose of 15 μg/30 g 17 hr prior to i.p. administration of 3–4 μc $Na^{131}I$. All the mice used in various experiments were fed colony diet until the injection of Act. D. Control group of animals received an

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equivalent volume of normal saline i.p. The control and the experimental animals were allowed water *ad libitum* throughout the period of the experiments.

A mention should be made here regarding the stability of Act. D in solution. It was felt that the drug lost its potency even if it was stored in the refrigerator. With a freshly prepared vial when a dose of 20 μg/30 g was injected i.p. all the injected animals died within 30 hr, while the injection from the same vial in the same dose after 1 week storage failed to kill any of the animals even after 40 hr.

Profile or linear scanning of the animals was performed by means of a slit collimator attached to a 2 in. sodium iodide scintillation detector according to the method described by Ramanathan *et al.*[11] The individual organs after dissection were counted with a flat field collimator attached to the scintillation probe described above.

Sodium chromate (^{51}Cr) was used as an unabsorbable marker to study the rate of its passage through gastrointestinal tract. Animals were injected with Act. D (15 μg/30 g) i.p. Seventeen hours later both control and Act. D treated groups were fed 25 μc $NaCrO_4$ orally. Linear scans were then recorded at various time intervals. After 24 hr, aliquot of the blood was counted for the radioactivity content to check if Na-chromate was absorbed through gastrointestinal tract.

Barium meal was given to the animals to study the motility of the gastrointestinal tract. Control and Act. D treated animals were fed barium 17 hr after i.p. administration of Act. D. X-rays were then taken at various time intervals to locate the passage of barium through gastrointestinal tract.

Total iodide retention studies were done by whole body counting of the animals by a 4 in. $\times$ 4 in. NaI scintillation detector. The distance between the animals and the detector was 15 cm.

RESULTS AND DISCUSSION

Distribution of radioiodine in various tissues of mice

The animals were injected with Act. D and $Na^{131}I$ as described earlier. Linear scans of the animals were recorded at various time intervals (2, 4, 5·5, 7·5, 10, 24 and 30 hr) following the injection of radioiodine. Figure 1 depicts a linear scan of a control animal as compared to Act. D pretreated animals as a function of time. The thyroid of both the groups pick up radioiodine, however, Act. D treated animal shows less amount of radioiodine in the beginning (Fig. 1A). Gradually the thyroid of Act. D treated animals pick up more radioactivity, and at the end of 5–6 hr the concentration in the thyroid is identical in both the groups. Stomach, however, in both the cases concentrates a large amount of radioiodine initially. At about 6–7 hr, the gastrointestinal tract of the control animal contains very little radioactivity (Fig. 1C) whereas, large amount of radioiodine is seen in the stomach of Act. D treated animals even after 24 hr (Fig. 1D).

In order to quantitate the radioactivity in various tissues, the animals were killed at the end of 1, 5 and 24 hr. Various organs such as thyroid, stomach, liver, intestine, kidney and carcass were removed and counted as described earlier. Total retention of radioiodine was then calculated by summing up the radioactivity in all the organs and expressing as percentage of the administered dose. The percentage of radioiodine distribution is shown in Table 1. It is apparent from Table 1 that the stomach of Act. D treated mice concentrates more radioiodine than that of the control animals at any given time. Almost all the radioactivity in the stomach was always found to be in its

contents in the lumen. Initially, at 1 hr thyroidal radioiodine uptake of Act. D treated animals is low. However, it approaches the same value at about 5 hr and then increases significantly as compared to the thyroid uptake of control animal.

Kidney, liver and intestines had comparatively negligible amount of radioactivity. Since none of the organs were perfused, part of the radioactivity in these organs may be due to circulating iodide. Carcass of the Act. D treated mice showed more radioactivity than control animal, at the end of 1 and 24 hr.

It is evident that there is more of the circulating radioiodine in Act. D treated animals as compared to the control one. This may be the result of either the delayed emptying of the stomach or impaired renal clearance.

Motility and emptying of stomach

(i) Isotopic studies with ^{51}Cr labelled sodium chromate.

Orally fed ^{51}Cr salts are unabsorbed through its passage through the gastrointestinal tract.[12] In order to study the motility of the gastrointestinal tract, ^{51}Cr labelled sodium chromate was fed to Act. D treated and control mice as described earlier. Serial linear scanning was carried out at various time intervals (Fig. 2). The scans show that after 4·5 hr the control animals show significant decrease in ^{51}Cr radioactivity and is reduced to almost zero at the end of 22 hr. However, Act. D treated mice had considerable amount of radioactivity in the abdominal region even at 24 hr, suggesting

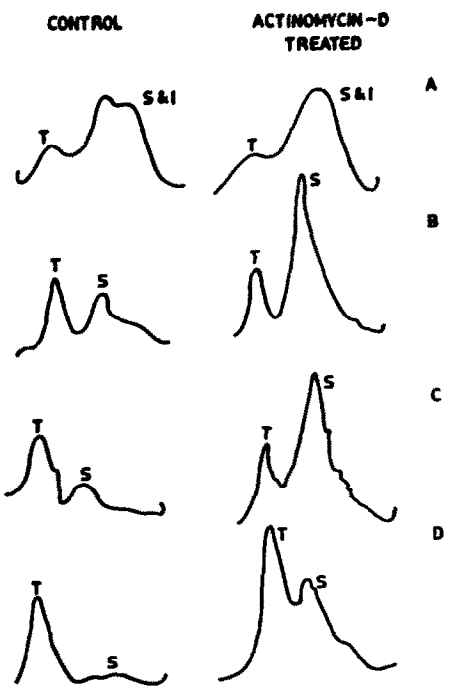

FIG. 1. Linear scan of the control and Act. D treated mice. Linear scan showing the distribution pattern of radioiodine injected i.p. in mice treated with normal saline (control) and Act. D at a dose of 15 μg/30 g. A—0·5 hr, B—3 hr, C—7 hr and D—24 hr later. Note the build up of ^{131}I in the stomach (S) and the delayed uptake by the thyroid (T) in Act. D treated group as compared to the controls.

TABLE 1. DISTRIBUTION OF RADIOIODINE IN THE VARIOUS ORGANS OF CONTROL AND ACT. D TREATED MICE AS PERCENTAGE OF ADMINISTERED DOSE

Time (hr)	1 hr		5 hr		24 hr	
	Control	Act D	Control	Act D	Control	Act D
No. of animals	4	4	4	4	12	10
Thyroid	9·2 (± 3·89)	5·5 (± 0·23)	19·8 (± 6·26)	16·0 (± 4·37)	18·0 (± 6·22)	26·0 (± 7·76)
Stomach	13·0 (± 3·36)	20·0 (± 3·94)	4·9 (± 1·32)	32·0 (± 5·82)	1·6 (± 0·01)	12·0 (± 4·18)
Liver	2·65 (± 0·05)	3·8 (± 2·08)	1·33 (± 0·68)	2·7 (± 1·11)	1·13 (± 0·16)	2·48 (± 1·08)
Intestine	3·70 (± 2·4)	5·9 (± 2·51)	2·25 (± 0·92)	5·25 (± 1·64)	2·82 (± 0·46)	5·64 (± 3·24)
Kidney	1·15 (± 0·7)	1·2 (± 0·74)	0·55 (± 0·2)	0·95 (± 0·011)	1·1 (± 0·14)	0·84 (± 0·35)
Carcass	44·0 (± 10·8)	59·0 (± 3·53)	25·0 (± 6·52)	28·0 (± 6·1)	6·3 (± 2·32)	24·0 (± 8·66)
					3·39* (± 0·97)	15·62* (± 5·92)
Retention	73 (± 7·5)	95 (± 4·74)	54 (± 8·66)	88 (± 6·12)	31 (± 6·63)	66 (± 11·25)
Thyroid:stomach	0·746 (± 0·0328)	0·276 (± 0·114)	4·09 (± 0·962)	0·554 (± 0·334)	11·68 (± 4·4)	2·17 (± 1·23)
					26* (± 8·9)	3·01* (± 1·76)

Control and Act. D treated group were administered saline and Act. D i.p. 17 hr prior to i.p. injection of 3–4 μc of $Na^{131}I$. At the interval of 1, 5 and 24 hr, thyroid, stomach, liver, kidney, intestine and carcass were counted. Total retention was then calculated by adding counts of each organ and determining its percentage of administered dose.

* Another experiment where animals were sacrificed after 24 hr.

Numbers in parentheses indicate the standard deviation.

probably, a delay in the emptying of stomach. No radioactivity was found in the blood confirming that Na-chromate was nonabsorbable.

(ii) Radiographic studies with barium

The motility of the gastrointestinal tract was further studied by barium meal in control and Act. D treated animals. Figure 3 shows typical photographs of animals after 4 and 7·5 hr after the barium ingestion, which was 17 hr later to i.p. administration of Act. D. The pictures show very clearly that while some barium had already passed in the colon in the control animal, most of the barium was seen in the stomach of experimental animal.

Iodide retention

The control and the experimental animals were injected as described before and the whole body counting was done at 1, 2, 3, 4, 5, 7, and 24 hr. Figure 4 shows the percentage retention of radioiodine in control and experimental animals. It is evident that Act. D treated animal retains much more radioactivity than control group at any given time. It is conceivable that very large doses of Act. D can damage the renal parenchyma and impair renal clearance of iodide. Histological examination of the Act. D treated animals reveal the damage to the distal tubules of kidney. In rats,

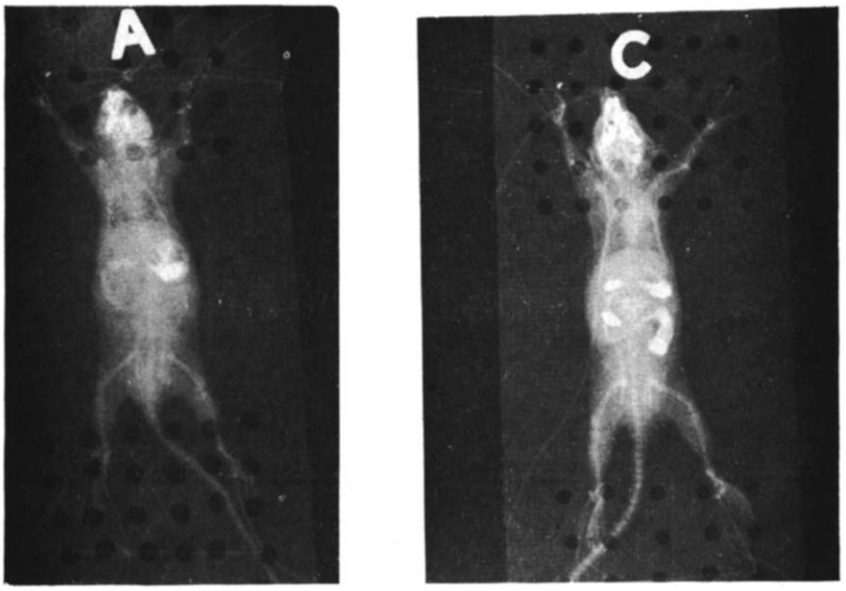

FIG. 3. I. Act. D treated animal (left).

A. Barium given (0·5 ml) 17 hr after i.p. actinomycin D 15 μg. Film taken 3 hr after barium instillation in the stomach. Most of the barium is seen in the stomach. A small amount of barium has passed into the proximal loops of small intestine.

C. Control animal: (right) 0·5 ml barium instilled in the stomach. Film taken at 3 hr.

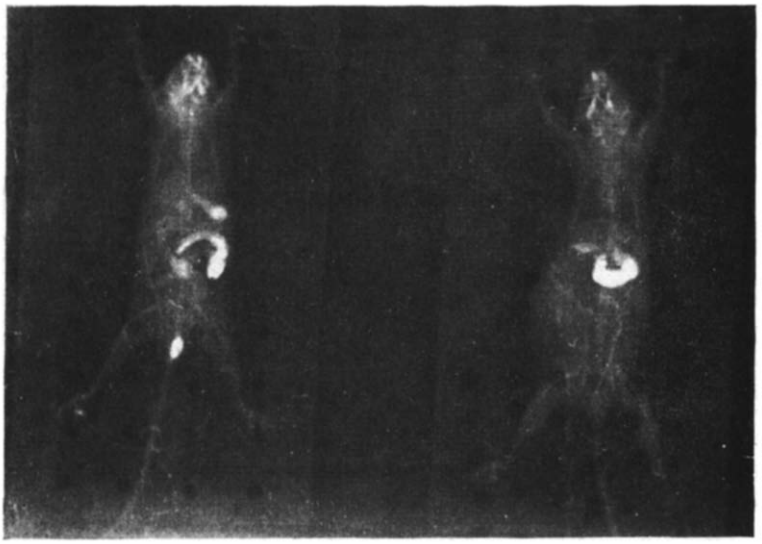

FIG. 3. II.

A. Act. D treated animal (right) 8 hr after barium instillation. Practically all the barium is seen in the stomach. A very small amount is seen in the small intestine.

C. Control animal (left) 8 hr film-practically all the barium in small intestine and large gut.

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kidney shows the maximum concentration of radioactivity 15 min after an i.v. injection of ^{14}C Act. D.[13]

The behaviour of thyroid uptakes could be explained when both impaired renal clearance and stomach motility are taken into account. Actinomycin D treated animal has more circulating iodide due to impaired renal function. Stomach, an extrathyroidal iodide concentrating tissue, receives more iodide, as more is available from circulation. Due to the impaired stomach motility, stomach holds the iodide for a longer period. Such an initial pooling of iodide in the stomach would take less amount of iodide available to the thyroid, and therefore the initial thyroid uptakes of the experimental group is low.

Iodides are not locked up in the stomach for an indefinite period. There is a gradual release of iodide from the stomach and its reabsorption through the intestine. As more radioiodine becomes available, the thyroid picks up more radioiodine. This would explain the gradual rise in the thyroid uptake of the Act. D treated animals. Why the thyroid should pick little more radioiodine at 24 hr in the Act. D treated animals is difficult to explain. Either Act. D enhanced the thyroid uptake or there is large amount of radioiodine still available to the thyroid gland at the end of 24 hr because of the damaged kidney function. Enhancement of the radioiodine uptake by the thyroid gland was not evidenced earlier because of the fact that a large proportion of

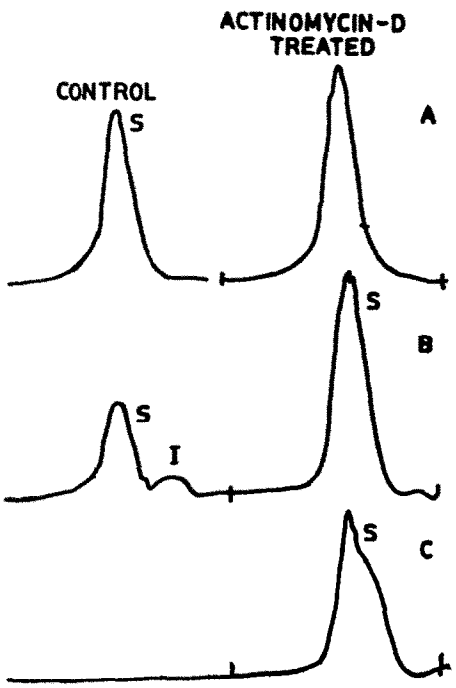

FIG. 2. Control and Act. D treated mice (15 μg/30 g) given 25 μc of ^{51}Cr-sodium chromate orally. A—1 hr, B—6 hr and C—24 hr later. Note that ^{51}Cr activity in the stomach (S) and Act. D treated animals continues to persist even at the end of 24 hr indicating delayed emptying of the stomach. During this time ^{51}Cr has been completely excreted in the control.

iodine was locked in the stomach and was unavailable to thyroid. Variations in the food intake cannot be the cause of an increased uptake because in our experiments the food was withheld from both the control and experimental groups.

It appears from our investigations that Act. D in toxic dose in mice markedly reduces the renal excretion and also impairs the motility of stomach resulting in the sequestration of a large amount of iodide in the stomach which indirectly affects the iodide metabolism. It is possible that there is an enhancement of the uptake of radioiodine by the thyroid gland but it is evidenced only when the stomach releases iodine. To confirm the above mentioned hypothesis, this data is being analyzed on an electronic analogue computer and will be communicated shortly.

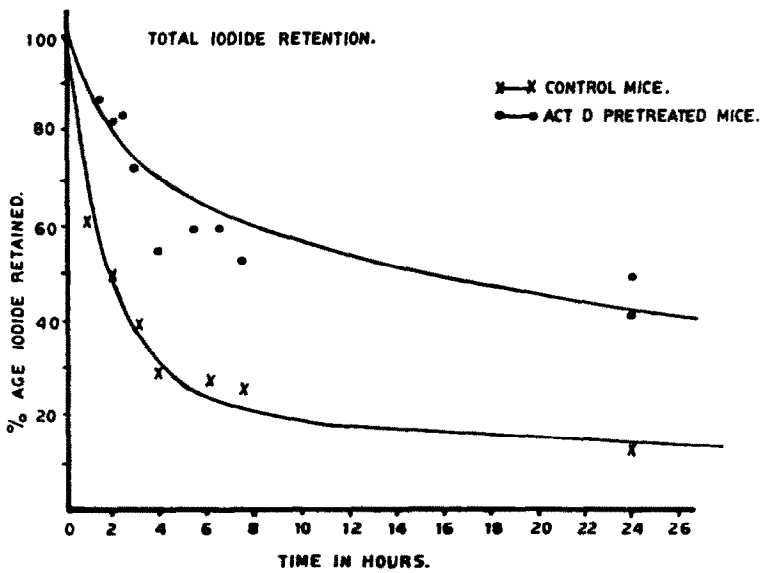

FIG. 4. Percentage of the administered ^{131}I dose retained in the control and actinomycin D treated animals. Animals received Act. D (15 μg/30 g) i.p. 17 hr prior to i.p. injection of 3–4 μc of $Na^{131}I$. An equivalent volume of saline was injected i.p. prior to i.p. administration of $Na^{131}I$, in the control group of mice. Whole body counting was then followed as indicated.

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