The Metabolism of Perchlorate in the Rat

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ABSTRACT. The disposal of $K^{36}ClO_4$ has been studied in rats maintained on a low iodine (Remington) diet. After intraperitoneal administration the radionuclide reached its maximal concentration in the thyroid at about 4 hr and at a level of approximately 2.5% of dose per g gland. All the radionuclide in the thyroid was trichloroacetic acid soluble. No significant concentration was found in kidney, liver, brain or spleen. The rates of disappearance of the radionuclide from the plasma and thyroid and rate of appearance in the urine were exponential, with half times of approximately 20 hr. These rates were closely similar to disposal rates of ^{131}I found by others (6). (*Endocrinology* 92: 1536, 1973)

A NUMBER of anions, such as thiocyanate and perchlorate, competitively inhibit uptake of iodine by the thyroid of both rat and man (1–3). They also discharge iodide already accumulated but not covalently bound in organic form. Similar results were found with ReO_4^-, TcO_4^-, BF_4^-, and NO_3^- (3,4) among others. Anbar *et al.* (5) proposed that like charge and similar ionic radius are the basis for these findings. Using perchlorate labeled with ^{36}Cl they showed that the ion is selectively concentrated by the rat thyroid, but undergoes no chemical change between injection and excretion.

Anbar and his associates limited their observations to periods of time less than 12 hr. Their data do not permit estimation of radiation dose to the thyroid or whole body from the radiolabeled perchlorate. The present studies were undertaken in order to determine the fate of perchlorate in the rat over a longer period of time. The findings indicate only a small accumulation of perchlorate in the thyroid, no significant retention in other structures, and a half-life of retention for the whole body and for the thyroid of about 20 hr.

Experimental

Two groups of male Sprague-Dawley rats were maintained on Remington diet[1] prior to the time of administration of labeled perchlorate, the first for 5 weeks, the second for 4½ weeks.

The first group consisted of 24 rats weighing 200–250 g and was divided into 4 sub-groups of 6 rats each. Each animal was injected ip with 0.1 μCi of $K^{36}ClO_4$[2] having a specific activity of 240 μCi/mmole in 1 ml saline solution (approximately 40 μg stable perchlorate per injection). Animals were given the diet and water *ad libitum*. Sub-groups were killed by cardiac exsanguination at 2, 8, 24, and 48 hr.

The second group of rats, weighing 300–350 g, was divided into 4 sub-groups of 5 rats each. Three of these underwent the same procedures as group one and were killed at 2, 4, and 8 hr. The fourth subgroup was treated the same way except that each animal was given 0.2 μCi labeled perchlorate and the animals were killed at 96 hr.

In all animals both lobes of the thyroid were removed and weighed. One lobe was solubilized in NCS.[3] The second lobe was homogenized in distilled water, repeatedly precipitated with 10% trichloroacetic acid (TCA), and then solubilized in NCS. Blood samples were obtained by cardiac puncture. Serum was solubilized in NCS.

From the 96-hr sub-group 10–20 mg samples of selected tissues were removed and solubilized in NCS. Urine samples were collected in metabolic cages from this group at 24-hr intervals.

All samples were dissolved in 10 ml of Aquasol[4] and counted in a Beckman CPM-100 liquid scintillation detector.

Results

The radionuclide retained in the thyroid at various time intervals appears in Table 1. It was taken up by the thyroid, reached a peak around 4 hr, and then fell to approximately 5% of this peak value within 96 hr. The data are plotted in Fig. 1 and appear to indicate an exponential function with a half-life of 20 hr. The level of radioactivity detected in the TCA precipitates of the thyroid homogenate was not significantly different from background.

The levels of radioactivity in the serum and the urinary excretion data are plotted in Fig. 1. In order to compare the disposal of the administered radionuclide with that of iodide, the data of Albert and Johnson (6) for rats with low levels of thyroidal radioiodide retention are also plotted in Fig. 1. It appears that the disappearance rates after administration of labeled perchlorate from the plasma and thyroid and the appearance rate in the urine correspond closely with those of iodide in the rats of Albert and Johnson, and that the disposal of both substances follows an exponential function with a half-life of approximately 20 hr.

The retention of the label after administration of perchlorate in selected tissues at 96 hr appears in Table 2. The levels at that time were less than 4% of the maximal levels found in the thyroid. The relatively high value for kidney tissue presumably reflects the fact that most of the perchlorate is excreted in the urine.

Discussion

Since perchlorate was first observed to displace iodide from the rat thyroid (1), it has been widely used in studies on the thyroid to block entry of iodide into the gland or to cause discharge of previously accumulated but non covalently linked iodide. It has been used in the therapy of Graves' disease (7). Perchlorate causes a discharge of iodide from the thyroid of patients with Pendred's syndrome (8), and evidence for an abnormally prolonged effect of perchlorate in this disorder has been presented (9).

Further exploration of this last phenomenon has required the use of radio-labeled perchlorate in these patients, but existing data have not permitted radiation dose calculations.

Our data indicate a low level of thyroidal uptake of radio-labeled perchlorate at the specific activity levels used, no evidence of covalent bonding of the radionuclide by the thyroid, no selective retention in the tissues tested, and exponential disappearance from the body with a half time of approximately 20 hr. Comparison of our data with those of Albert and Johnson (6), who studied the disposal of iodide in rats with low levels of iodide retention, shows a similarity

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[1] General Biochemical Inc., Chagrin Falls, Ohio.

[2] The Radiochemical Centre, Amersham, England.

[3] Nuclear Chicago Co., Des Plaines, Illinois.

[4] New England Nuclear Corp., Boston, Massachusetts.

TABLE 1. Percent dose of ^{36}Cl per g tissue of rat thyroid after administration of $K^{36}ClO_4$

Time (hr)	% Dose/g tissue Group 1	Group 2
2	2.42 ± 0.9*	2.52 ± 0.1
4		3.16 ± 0.5
8	1.73 ± 1.3	2.68 ± 0.4
24	1.68 ± 0.9	
48	0.755 ± 0.2	
96		0.142 ± 0.1

The number of animals represented in each figure is 6 for Group 1 and 5 for Group 2.

* SD.

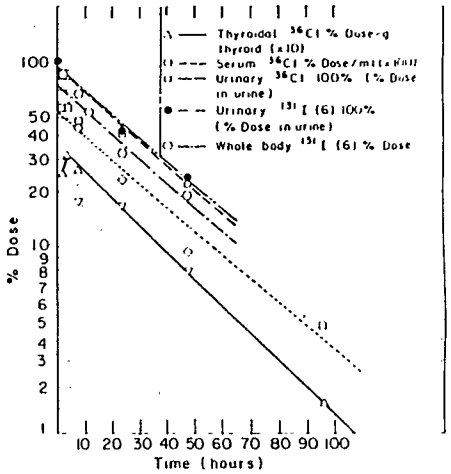

FIG. 1. The disposal of $^{36}ClO_4^-$ in rats maintained on a low iodine diet compared with disposal of ^{131}I in rats with low thyroidal iodide retention taken from the studies of Johnson and Albert (6). All lines were drawn with the use of least-squares regression. The urine values are cumulative.

TABLE 2. Percent dose of ^{36}Cl per g tissue at 96 hr

Organ	% Dose/g tissue
Thyroid	0.142 ± 0.1*
Kidney	0.125 ± 0.09
Spleen	0.098 ± 0.03
Liver	0.048 ± 0.01
Brain	Background

Each figure represents 5 animals.

* SD.

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in rates between the two ions. This may be taken as evidence for closely similar pathways of metabolism apart from organification of iodide. Thus it would appear reasonable to base radiation dosage calculations for radio-labeled perchlorate administered to man on the assumption that there is no organic binding of the radionuclide and that its retention and disposal are closely similar to iodide when covalent binding of the latter is blocked. It is, of course, possible that a different pattern of metabolism might emerge at substantially lower doses of stable perchlorate than were given in these experiments, because of a saturation effect. In earlier experiments (2) on propyl thiouracil blocked rats 50 μg stable iodide, a dose equivalent to the perchlorate used in the group 1 rats of the present experiment, reduced the thyroid to serum ratio of ^{131}I to approximately 50% of control values, and 20 μg to approximately 70%. The present experiments were done at the lowest dose of radio-perchlorate consistent with adequate counting rates and with the highest available specific activity $K^{36}ClO_4$.

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Induction of a LATS-like Response by Thyroglobulin in the McKenzie Assay System

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ABSTRACT. In a modified McKenzie bioassay system, significant, and sometimes striking, stimulatory activity resembling that of LATS was induced by soluble supernatants of bovine thyroid tissue, by purified bovine thyroglobulin (TG), either obtained commercially or freshly-prepared by DEAE cellulose chromatography, and by the soluble supernatant of a human thyroid gland. Stimulatory activity, as evidenced by an increase in blood ^{131}I, was manifest at 2 hr after administration of TG and was maximum between 11 and 20 hr. Varying doses of TG elicited a typical log dose response curve, the minimum effective dose being 0.1 mg. As indicated by direct analysis, increased blood radioactivity elicited by TG was comprised mainly of ^{131}I-thyroxine. The effect of TG could not be explained by a retarded disappearance of ^{131}I-thyroxine from the blood, since very large doses of TG failed to retard the disappearance of exogenous labeled hormone. (*Endocrinology* **92**: 1538, 1973)

DURING experiments to study the neutralization of LATS in serum by thyroid particulate fractions (1-7), we sometimes observed either appearance or enhancement of thyroid stimulatory activity. Experiments conducted to elucidate this phenomenon strongly suggested that stimulation was due to thyroglobulin (TG) contaminating the particulate fraction and that, in appropriate dosage, TG is itself a stimulator of thyroxine (T_4) release in the McKenzie bioassay system. The findings have been reported in part in abstract form (8). Subsequently, a report

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Morgan and co-workers has provided confirmation of these findings and has suggested that the effect may be due to inhibition of thyroidal phosphodiesterase (9).

Materials and Methods

Thyroid tissue. Thyroid glands were obtained from steers immediately following slaughter, were placed immediately into plastic bags on ice, and were rushed to the laboratory. In one instance, a specimen of human colloid goiter was obtained at surgery. Within 1 hr the glands were trimmed, weighed, and then minced in 4 vol of either physiological saline or 0.25M sucrose. Minces were homogenized for 30 sec at high speed in a Waring blender. The homogenate was then either centrifuged at 4 C in a Spinco Ultracentrifuge[1] (Model L, No. 40 Rotor) at 100,000 × g to obtain particulate and soluble supernatant fractions or was differentially centrifuged according to the method of Ekholm and Strandberg to obtain a microsomal fraction (10).

Purified thyroglobulin. Several batches of TG were purchased from a commercial source.[2] Purified bovine TG was also prepared from fresh bovine thyroid glands by column chromatography on DEAE cellulose according to the method of Ingbar, Askonas, and Park (11). In the latter experiments, protein concentration of the effluent fractions was measured by the method of Lowry (12), and was increased if necessary by ultrafiltration, utilizing a Diaflow apparatus with a UM-10 membrane.[4]

Bioassay. Bioassay of LATS was performed according to the general method of Furth (13), with certain modifications. CD-1 female albino mice[5] were used and blood was drawn from the orbital venous sinus by means of disposable heparinized 100-μl capillary tubes[6] (14). When, as suggested by Furth, 2.5 μg injections of triiodothyronine (T_3) were used to suppress secretion of endogenous TSH, mice showed high concentrations of ^{131}I in the blood before injection of the test material. Therefore, the amount of T_3 administered was increased to 0.8 μg. This resulted in lower baseline values of blood radioactivity without diminishing the sensitivity of the assay.

In each bioassay, separate groups of animals were injected with normal human serum, with TSH (0.8 μU/mouse), and with either normal saline or 0.25M sucrose. Human serum to be used in assays was routinely absorbed with DEAE-Sephadex to decrease toxicity in the test animals, as recommended by Florsheim (15).

Analysis of bioassay results. Blood drawn at appropriate time intervals was counted in a well-type scintillation detector.[7] Except where otherwise indicated, the response index was calculated as the ratio of blood radioactivity at 20 and zero hr. The significance of differences between results obtained in the several experimental groups was assessed by analysis of variance, followed by Student's *t* test in those experiments in which the analysis of variance revealed significant differences to exist (16). Since logarithmic transformation of the raw data prior to application of the *t* test did not alter the significance of the differences observed, such transformations do not appear in the data to be presented.

T_4 determinations. Measurements of ^{131}I-T_4 and ^{125}I-T_4 concentrations in the sera of test mice were performed by a technique described in detail elsewhere (17). Briefly, serum was enriched with ^{125}I-T_4 to act as both a recovery standard and a source of labeled hormone for displacement analysis. Small aliquots of serum (10–20 μl) were extracted of T_4 on a Sephadex column at alkaline pH. Efficiency of extraction averaged 94.1 ± 2.4% (mean ± SD) in 20 analyses. After the Sephadex was washed with barbital buffer, pH 8.6, to remove iodide and possibly iodoproteins (18), the T_4 remaining on the column was counted. ^{127}I-T_4 was measured by binding displacement analysis of the extracted T_4.

Results

Strong thyroid stimulating activity could readily be demonstrated in soluble supernatants of bovine thyroid gland. Results of a typical experiment are shown in Fig. 1. A 60-ml homogenate in 0.25M sucrose was prepared from 4.0 g of tissue. After centrifugation, an aliquot of soluble supernatant was set aside and the particulate fraction was then washed repeatedly with 15 ml aliquots of 0.25M sucrose, each wash being saved. The initial supernatant proved highly stimulatory in that 0.1 ml produced a mean response index of approximately 11. Successive washes of the particulate fraction index were progressively less stimulatory, so that, by the sixth wash, even a dose of 0.5 ml failed to induce a significant response.

Stimulatory activity of soluble supernatants of

[1] Beckman Instruments, Belmont, California.
[2] Sigma Chemical Company, St. Louis, Missouri, Thyroglobulin, Bovine, Type I.
[3] Sephadex-D, Calbiochem, Los Angeles, California.
[4] Amicon Corp., Lexington, Massachusetts.
[5] Charles River Breeding Laboratories, Wilmington, Massachusetts.
[6] Drummond Scientific Co., Broomall, Pennsylvania.
[7] Nuclear-Chicago Corp., Chicago, Illinois.