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1 TOXICOLOGICAL STUDIES OF CERTAIN
THORIUM SALTS

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A B S T R A C T

This manuscript presents the ^{1/}results of studies designed to determine the toxic manifestations following the administration of soluble thorium salts by various routes. ^{0.5-100}Experiments on methods for the detection of microgram quantities of thorium in biological material are described.

The acute toxicology of ^{11(100%)₄ 34120 J.H.G.}~~thorium nitrate~~ or ~~thorium chloride~~ following oral, intraperitoneal, intravenous, or ^{1.11.10-34.34.0}subcutaneous administration is given. The results of feeding thorium ~~nitrate~~ to rats during most of their life span are presented.

The distribution of thorium in the body as determined by the use of the ²³⁰~~thorium~~ isotope ^{UX₁} is indicated. A tolerance figure for thorium in man is estimated.

BIOLOGY

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TOXICOLOGICAL STUDIES OF CERTAIN THORIUM SALTS

Paul A. Mattis

Except for the use of thorium dioxide as a contrast medium in radiology there has been little therapeutic use of thorium compounds. The toxicologist has been principally concerned in the industrial applications of thorium with the hazards resulting from inhalation of thoron and the possible deleterious effects of the alpha particles emitted by this gas. With the advent of thorium as a source for fissionable elements it became more important to check the toxicology of this metal.

A search of the literature reveals a scarcity of information concerning the toxicity and metabolism of the soluble thorium salts. Sollman and Brown (1), Chace and Gies (2) studied the pharmacological actions following the administration of thorium nitrate, the citrate complex of thorium and thorium chloride. They did not study the results of chronic administration. Frouin (3) in 1910, as a part of his investigations on thyroidectomized rats, fed thorium chloride to normal animals for 42 days without disclosing toxic manifestations. Three years later, Hevesy and Wagner (4) reported on the distribution of thorium following subcutaneous injections of thorium nitrate into mice, utilizing for their experiments tracer techniques. Adami (5) administered thorium nitrate to rats and guinea pigs in small doses (all less than 0.2 mg.) for periods of 45 to 100 days and noted moderate liver and renal changes.

Robertson et al. (6) injected thorium oleate subcutaneously into white mice at varying intervals for 400 days. They observed a depression of the growth rate and a decreased life span in the thorium treated animals when compared to control mice that received oleic acid alone. These authors also reported on the distribution of thorium as determined by gravimetric and radiographic means. More recently Patrick (7) published the results of his toxicological investigations of thorium nitrate.

Other publications which have appeared sporadically have been concerned for the most part with the effects of thorium on isolated organs (1, 2, 8, 9), on specific cells or structures (9, 10, 11, 12, 13), on certain enzyme systems (14, 15, 16, 16a, 17, 18, 19), or on plant life (20, 21, 22, 23, 24).

The studies to be reported here have been confined mainly to the following phases:

- (1) The determination of acutely toxic doses.
- (2) The effects of administration of thorium in the diet of animals during a major portion of their life span.
- (3) The distribution of thorium in the animal body following various routes of administration.

- (4) Pathological changes following the use of thorium.
- (5) Attempts to devise a suitable chemical method for the determination of microgram quantities of thorium in animal tissues or body fluids.

Some of the results must be considered as preliminary and should be supplemented with additional data, but, in general, sufficient information is available to arrive at certain conclusions concerning the toxicological behavior of thorium. Thus, it would appear that large doses of soluble thorium salts would have to be ingested to produce severe toxicity and that these manifestations can be attributed largely to the acidity and hypertonicity of such solutions.

Although parenteral administration of thorium salts results in toxicity it appears unlikely that amounts sufficient to produce severe toxic manifestations would be introduced into humans except under very unusual circumstances.

ACUTE TOXICITY STUDIES

Methods: Female albino mice (CF₁) and male albino rats (Carworth Farms) were utilized for the acute toxicity studies. All animals were kept on a diet of commercial pellets (Friskies) and water until an hour or two before administration of the compound being studied. All animals were weighed prior to the test; doses were calculated on the basis of the animal weight. Solutions of thorium nitrate (Ames-purified) or thorium chloride (Ames) were made before each test, the concentrations utilized ranging from 1 per cent to 100 per cent, with pH values of from 0.6 to 2.69.

Intravenous injections were made in a tail vein, using a 24 or 26 gauge needle at a constant rate of 1 cc. per minute. Oral intubations were made by means of suitably modified needles, a 20 gauge, 1-1/2 inch needle for mice and an 18 gauge, 2-1/2 inch needle for rats.

Only those deaths occurring in a 24 hour period following dosage were used in calculating the LD₅₀ values, although all surviving animals were observed daily for at least 7 days and any late deaths were recorded. An exception should be noted in the case of the intraperitoneal toxicity data in which the 48 hour mortality was used for determination of the LD₅₀. The LD₅₀ values were calculated by the Litchfield-Wilcoxon method (25) unless otherwise noted.

The LD₅₀ for thorium nitrate injected intraperitoneally, as an aqueous solution, into white rats was found to be 2 grams per kilogram with 19/20 limits of 1.5 to 2.4 g/kg. These data should be regarded as indicative only since examination of Table 1 will reveal the heterogeneity of the results. This value does not differ significantly from that reported by Hodge et al. (26). It is probable that the tonicity and pH of the solutions used played a considerable role in the mortality since NaNO₃ adjusted to the same pH value (1.8-2.0) with HNO₃ and with the same molarity with regard to nitrate, possessed an LD₅₀ (approximate) of 1.5 grams per

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TABLE 1

THE INTRAPERITONEAL TOXICITY OF THORIUM NITRATE FOR RATS

DOSE	CONCENTRATION of SOLUTION	<i>NUMBER DEAD</i> TOTAL NUMBER	CUMULATIVE MORTALITY	LD ₅₀ and Method of Calculation
GM/KG	%		%	
0.32	50	0/5	0	
0.64	50	0/5	0	
0.89	15	0/9	0	
1.16	15	0/9	0	
1.51	30	6/9	18	1.9 (1.5 TO 2.4) GM/KG (Wilcoxon-Litchfield) **
1.97	30	5/9	55	
2.56	30	7/9	78	
3.32	40	6/9	89	
5.20	40	3/3	100	

** -- Reference 17

kilogram.

Rats that died following intraperitoneal injections of thorium nitrate frequently revealed extreme abdominal distention and rigidity. Bloody or milky fluid ranging in amount from 3 cc. to 7 cc. were removed at autopsy. The viscera were adherent, markedly congested and often revealed patches of white amorphous matter adhering to the liver, or intestines. Histopathological examination revealed, in general, the findings incident to chemical peritonitis. It is likely that the thorium nitrate was precipitated from solution in the peritoneal cavity and much remained there as the relatively insoluble thorium hydroxide. Three rats given 1.23 g/kg. of a suspension of thorium hydroxide, intraperitoneally, were sacrificed at intervals after the injection. All showed the presence of the material in the peritoneal cavity, without the intense inflammatory reaction which was seen with $\text{Th}(\text{NO}_3)_4$. It would be of interest to inject higher doses of this compound in order to determine its toxicity with relation to the more soluble nitrate.

A few animals (sixteen) were injected with thorium chloride as a 25 percent aqueous solution (pH 2.0). The LD₅₀ (Behrens method) was found to approximate 1.9 grams per kilogram. Autopsy of these rats revealed essentially similar changes in the peritoneal cavity.

The injection of solutions of thorium nitrate subcutaneously resulted in severe pain, necrosis and sloughing, therefore no attempt was made to determine an acutely toxic dose by this mode of administration. Since, however, there exists the possibility of workmen sustaining lacerating injuries into which thorium salts might be introduced, the following experiment was instituted. Ten normal male rats were etherized and the hair was removed from an area of approximately 4 square cm. over the shoulder region. The following day all animals were anesthetized (sodium pentobarbital, 35 mg/kg., intraperitoneally) and in each animal an incision, approximately 3 cm. long, was made through the skin and subcutaneous tissue to the superficial fascia. In six of these animals, thorium nitrate powder, as $\text{Th}(\text{NO}_3)_4 \cdot 4\text{H}_2\text{O}$, was carefully introduced into the wounds in an amount of 50 mg/kg.; the wounds were closed without the addition of the chemical. Each animal was given a subcutaneous injection of a penicillin preparation (0.3 cc. of 'Duracillin' equivalent to 100,000 units) in the dorsal region just cephalad to the tail.

The animals were observed daily for two weeks; the wound clips were removed on the seventh day. Three deaths occurred following the operation, two controls and one treated animal. No deaths occurred in the surviving animals during a three month period. Except for mild inflammatory reactions at the site of the operation which were more severe in the thorium treated animals than in the controls no other untoward reactions were noted. Approximately 2 months after the operation two of the thorium rats were sacrificed and sections of representative

tissues taken for histological examination. The site of the operation appeared normal with no superficial scar visible. One animal (#2) showed a small amount of yellowish-brown tissue which seemed fatty in nature. An insignificant deposit, brownish-black in color, resembling detritus was also seen in the subcutaneous tissue. The other animal appeared normal.

Microscopic examination of sections from these animals revealed essentially normal tissues in both rats except for the sections taken from the site of implantation of the thorium nitrate in animal #2. The subcutaneous area was the site of an intense reaction, consisting of coarse amorphous masses of acidophilic material or small masses of colorless crystalline substance, surrounded by giant cells, mesenchymal cells and fibroblasts. Many of the cells, particularly the fibroblasts showed intense acidophilic changes. The lesion is probably a granuloma resulting from the thorium nitrate deposition at that site.

Although the dosage implanted was quite small, it was felt that it fell within the range of quantities which might accidentally be acquired by humans. Certainly it had no observable effect on the animals. It is recognized that this experiment must be extended to check the mobilization of such deposits and the possible effects of larger dosages.

The possibility of thorium salts entering the blood stream also exists from causes similar in nature to those considered with respect to subcutaneous ingress. Consequently the intravenous toxicity of thorium nitrate and thorium chloride was investigated in mice and rats. Table 2 presents a summary of these experiments. It will be noted that in terms of thorium content there is little difference in the acute toxicity of these compounds for the two species investigated, except in the case of Th Cl_4 for mice. With respect to the acute toxicity of these thorium salts for mice the reliability of the determinations are in doubt because of the heterogeneity of the data.

Death following intravenous injection of these salts occurred immediately, being preceded in many of the animals by convulsions and Cheyne-Stokes respiration. In all deaths respiration ceased before the heart stopped. It seems likely that death following these injections may be attributed to emboli formed by the action of the compound on the cells or by precipitation since these salts are insoluble at the pH values encountered in the vascular system.

Certain animals which were examined following intravenous injections of toxic doses of thorium nitrate appeared to show an increased clotting time of the blood. Independent investigations by Scott (27) at Rochester indicated that there might be a reduction in the number of circulating thrombocytes. Accordingly,

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TABLE 2

THE INTRAVENOUS TOXICITY OF THORIUM NITRATE AND THORIUM CHLORIDE FOR MICE AND RATS

Species Strain Sex	No. of Animals Used	Compound	LD ₅₀ (with 19/20 confidence limits) in mg./Kg.	
			Salt	In terms of Thorium
Mice CF ₁ Female	110	TH(NO ₃) ₄	39 (33 to 50) †	19 (16 to 24)
	100	TH Cl ₄	52 (43 to 63) †	32 (27 to 39)
Rats Carworth Farms Male	50	TH(NO ₃) ₄	42 (39 to 45)	20 (18 to 22)
	50	TH Cl ₄	34 (31 to 37)	21 (19 to 23)

† Data for these determinations are significantly heterogeneous and the value for the LD₅₀ should be considered provisional.

we administered thorium nitrate to nine rabbits weighing between 1.6 and 2.9 kg. The drug was given intravenously in a dose of 10 mg/kg. of body weight in a concentration of 1.096 g. per liter. This represented a dose of 9.1 cc. per kg. given at a rate of 3 cc. per minute. At 1, 2, 4, 8 and 24 hours following the administration the following determinations were made, erythrocyte count, platelet count and prothrombin time (50% dilution). The platelet counts were made by the cover slip smear method using a 1:1 dilution of whole blood with Rees-Ecker diluting fluid. The same technician always carried out the platelet counts in order to reduce the errors to a minimum. In order to control the effect of the cation injection the rabbits were allowed to recover for 10 days at which time they were reinjected with NaNO_3 in a dose of 7.1 mg/kg. This represents a dose of 5.2 mg/kg. as nitrate which is equivalent to that given with the 10 mg/kg. dose of $\text{Th}(\text{NO}_3)_4$. The volume of the dose of NaNO_3 was only 1 cc/kg.

From examination of Table 3 it may be seen that there is a noticeable decrease in the hemoglobin concentration following the injection of both thorium nitrate and sodium nitrate. Similarly, there is a moderate, concomitant decrease in the number of erythrocytes, although these values, except for the fourth hour following thorium nitrate, can hardly be called statistically significant, which is not true with respect to the hemoglobin values. The platelet counts and prothrombin times are not significantly altered.

From these results it appears that such small doses of thorium nitrate, administered intravenously, have little effect upon either the prothrombin time or the number of circulating platelets. Long continued administration or considerably greater dosage might effect these values, but it is likely that other more serious toxic manifestations would supervene. Although there is a definite decrease in hemoglobin and a less well defined decrease in erythrocytes one can not attribute these changes to thorium per se, since sodium nitrate caused similar though less marked effects. We have no adequate explanation for these effects which appear to be due to the nitrate anion. We do not believe that it is due to interference with the method used for the determination of hemoglobin (acid hematin method). Normal blood, when NaNO_3 is added in amounts similar to those encountered in the above experiment, gives hemoglobin values either greater or similar to the untreated samples of the same blood.

The acute toxicity of thorium nitrate and thorium chloride, following oral administration has been determined in two species - mice and rats. In Table 4 are summarized the data from such experiments. The LD_{50} of thorium nitrate for white mice (CF_1) was found to be 1.5 g/kg. with 19/20 confidence limits of 1. to 2.1 g/kg. This compares favorably with the data reported by Patrick (7) which, when calculated in a similar manner, reveals an LD_{50} of 1.8 with 19/20 limits of 1.5 to 2.0. Patrick used a 14 day mortality, whereas we have used only 24 hour deaths in arriving

TABLE 3

THE EFFECT OF THE INTRAVENOUS ADMINISTRATION OF THORIUM NITRATE (10 mg./Kg.) AND OF SODIUM NITRATE (7.1 mg./Kg) ON CERTAIN HEMATOLOGICAL VALUES OF RABBITS.

COMPOUND	HR	HEMOGLOBIN gm./100 cc		ERYTHROCYTE per cu. mm		PLATELETS per cu. mm.		PROTHROMBIN TIME Secs - 50% dilution	
		Avg*	SD	Avg*	SD	Avg*	SD	Avg*	SD
TH(NO ₃) ₄	0	13.9	1.64	5,762,000	623,000	591,000	295,000	9.2	0.7
	1	11.6	0.96	5,221,000	884,000	400,000	215,000	11.8	1.5
	2	11.3	0.68	5,090,000	555,000	418,000	133,000	10.1	1.2
	4	10.9	0.53	4,801,000	680,000	513,000	132,000	9.8	0.4
	8	11.0	0.74	4,971,000	710,000	499,000	189,000	8.8	0.7
	24	11.6	1.10	5,300,000	656,000	633,000	148,000	8.1	0.3
NaNO ₃		Avg**	SD	Avg**	SD	Avg**	SD	Avg**	SD
	0	13.9	0.6	5,969,000	540,000	535,000	186,000	9.1	0.7
	1	12.1	.25	5,319,000	264,000	611,000	172,000	9.3	0.6
	2	11.8	0.6	5,397,000	249,000	557,000	246,000	9.3	0.8
	4	12.2	0.85	5,451,000	224,000	650,000	200,000	9.0	0.7
	8	11.6	0.59	5,471,000	314,000	776,000	241,000	8.3	0.6
	24	12.0	.71	5,349,000	214,000	571,000	143,500	8.4	0.9

* -Average values and standard deviations for nine animals.

** - Average values and standard deviations for seven animals.

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TABLE 4

THE ORAL TOXICITY OF THORIUM NITRATE AND THORIUM CHLORIDE FOR MICE & RATS

SPECIES STRAIN SEX	No. of Animals Used	COMPOUND	LD ₅₀ (with 19/20 Confidence Limits) in g./Kg.	
			Salt	In Terms of Thorium
Mice CF ₁ Female	125	TH(NO ₃) ₄	1.45 (1. to 2.1)	0.7 (0.5 to 1.0)
	150	TH Cl ₄	8.0 (6. to 10)*	
Rats Carworth Farms Male	115	TH(NO ₃) ₄	3.7 (3.1 to 4.3)	1.8 (1.5 to 2.1)
	100	TH Cl ₄	8.0 (6.3 to 10.2)	3.9 (3.0 to 5.0)

* This value should be considered provisional since the data are significantly heterogeneous.

at an LD₅₀ value. Since an insignificant number of late deaths occurred our LD₅₀ would probably not be altered by this longer period of observation.

The majority of those mice showing toxic manifestations were hyperactive, demonstrated hyperesthesia and labored respiration. A large number died in convulsive seizures. Gross autopsy immediately following death of mice receiving very high doses of thorium nitrate (8.0 g/kg.) revealed moderate distention of the stomach and upper intestine, widespread venous congestion and extreme distention of the right auricle, superior and inferior vena cava. Mice dying on lower doses sometimes revealed similar but less marked gross changes or occasionally no observable gross pathological effects. Many showed evidence of mucosal changes.

The LD₅₀ for male rats (Carworth Farms) was found to be 3.7 g/kg. with 19/20 confidence limits of 3.1 to 4.3 g/kg. Those animals examined after death showed essentially similar changes to those described for mice.

In another experiment rats were administered thorium nitrate in a dose of 2 g/kg. per day for 14 days after which time the survivors were sacrificed. Only six of twenty rats so treated survived the two week period. Histological examination of tissues from these rats revealed the following changes. The kidneys were usually fairly well preserved, but uniformly showed moderate nephrotic changes. The glomeruli and vascular bed were not significantly altered. The lungs were usually the seat of slight atelectasis of the focal type, some emphysema and in some sections edema, hyperemia and slight hemorrhage were seen. The most consistent lesion was a nodular focus of bronchopneumonia, peculiar in that it consisted of a variety of cells including monocytes, lymphocytes and a few polymorphonuclear leucocytes. Frequently the lesion gave the impression of being granulomatous although no fibrous cell or giant cell formation was noted. In many instances the peribronchial lymphoid tissue aggregates appeared unusually abundant and hyperplastic. It is unlikely that these changes in the lung are due to thorium since one sees similar lesions in control rats.

The gastrointestinal tract revealed the most marked changes, these for the most part being limited to the glandular portion of the stomach and the small intestines. The stomach almost uniformly showed an extensive lesion consisting of large, coarse, acidophilic masses deposited superficially along the mucosa and associated with degenerative and necrotic changes in the adjacent glands. Similar masses were occasionally seen in the lumens of more deeply situated glands or even in the base of crypts. There was distinct necrosis of the lining cells of the acini accompanied by desquamation. These changes were associated with a polymorphonuclear cell exudate but were rarely accompanied by ulceration. In addition, the stomach showed a submucosal

lesion chiefly of the exudative type with a diffuse cellular infiltration consisting of lymphocytes, monocytes or polymorphonuclear cells. Occasionally there was edema or mild hemorrhage. Such lesions sometimes extended into the adjacent muscularis mucosae. These lesions were occasionally observed in the duodenum or ileum, but were not seen in the large intestine.

The other tissues examined revealed no significant changes with the exception of spleen and bone marrow. The spleen in most animals showed a depletion of the red pulp and an increased prominence of the white pulp. The germinal centers appeared markedly hyperplastic often extending as cords between sheaths of hyperplastic reticular cells to form an anastomosing pattern between adjacent follicles. The red pulp was depleted of lymphocytes but seemed to show somewhat increased ectopic hematopoiesis. A somewhat similar picture obtained to some extent in the lymph nodes and in other lymphoid tissue aggregates, i.e., there appeared to be moderate hyperplasia and somewhat more follicular necrosis than would ordinarily be seen.

The cause of death in these animals may be attributed for the most part to the destructive action of the thorium nitrate on the gastrointestinal mucosa and the resulting shock.

In order to further clarify our impression of the toxic manifestations caused by the oral administration of thorium nitrate we initiated the following short term experiment. A group of 15 rats were given thorium nitrate orally in a dosage of 4 g/kg. (as a 57.4 per cent solution of $\text{Th}(\text{NO}_3)_4 \cdot 4\text{H}_2\text{O}$), after which water was withheld for 24 hours. A second group of 11 rats, similarly treated, were allowed to drink freely and, in addition, were given physiological saline (1 cc.) intraperitoneally initially, and two and four hours later. A group of 10 rats, sham treated, acted as controls. The percentage of hemoglobin, volume of packed red cells, numbers of erythrocytes and leucocytes, the differential leucocytic count and the total protein were determined before and at 2, 4, 8 and 24 hours after thorium administration.

As might be anticipated, the mortality following such a dosage was high, so that of the group given thorium alone 4 of the 15 animals were dead at two hours, 5 at four hours, 8 at the eighth hour and only 2 survived for twenty-four hours. Of those given thorium and physiological saline, 2 of the 11 were dead at two hours, 3 at the fourth hour, three at the eighth hour and 3 of the 11 survived for twenty-four hours. Four of the 10 control rats died between the eighth and twenty-fourth hour.

It will be noted (Figures 1 and 2) that all of the hematological values for the thorium treated rats increased during the eight hours following administration. The injection of physiological saline and free access to water was only partially successful in preventing the occurrence of these blood changes and in reducing the mortality. In general, all twenty-four hour determinations fell within the normal range or somewhat below

HEMATOLOGICAL CHANGES IN RATS FOLLOWING ORAL ADMINISTRATION OF
THORIUM NITRATE (4.0 GRAMS / KG.)
FIG. 1A

FIG. 1B

DIFFERENTIAL LEUCOCYTIC COUNTS IN RATS FOLLOWING ORAL ADMINISTRATION
OF THORIUM NITRATE (4 GRAMS/KG)

FIG. 2

LYMPHOCYTES —————
MONOCYTES - - - - -
POLYNUCLEARLYMPHOCYTES — · — · — · —

this range, with the exception of the leucocyte counts for the control rats, which were increased. Thus the data supports the picture of anhydremia consequent to the osmotic effects produced by the ingestion of the hypertonic solution of thorium nitrate.

Histological examination of tissues from some of these animals revealed changes essentially similar to those described previously for animals that had received half the dosage daily for 14 days. The damage to the gastrointestinal mucosa differed only in amount and extent. The kidneys, however, showed somewhat more severe nephrosis than in the former group.

In connection with another phase of the thorium problem we had utilized the insoluble thorium fluoride and so felt it might be of interest to ascertain the consequences following the ingestion of this compound. Only limited data on a small number of mice (30) are available but they indicate a complete lack of toxic manifestations in this species. A dose of 10 grams per kg., the maximum which we could administer, was well tolerated.

CHRONIC TOXICITY:

In order to determine the effects of the absorption of very small amounts of thorium over a long period the following feeding experiment was instituted. Five groups of 30 rats each were selected and placed on the following dietary regimen.

Group I	Control diet alone (Table 5).
Group II	Control diet with the addition of 4 per cent thorium nitrate.
Group III	Control diet with the addition of 1 per cent thorium nitrate.
Group IV	Control diet plus aluminum chloride in such an amount that the chloride concentration was molecularly equivalent to the nitrate concentration in the 4 per cent thorium nitrate diet.
Group V	Control diet plus aluminum nitrate in such an amount that the nitrate concentration was equivalent to the nitrate in the 4 per cent thorium nitrate diet.

It should be noted that the animals in Group V were not placed on the special diet until two months after the other groups were on test. Rats from the same shipment (of the same age) were utilized but were allowed to remain on the control diet during the two month interval. It is obvious that this group should not be compared too closely with the other four sets.

All rats were weighed at weekly intervals for the first five months and at four week intervals thereafter. Determinations of the total number of erythrocytes, leucocytes, the differential

TABLE 5
COMPOSITION OF DIET FOR RATS

<u>Control Diet</u>	
	<u>Per Kg.</u>
"Klim" brand powdered whole milk	750 g.
Primex	100 g.
Dextrin	130 g.
Cellu-flour	20 g.
Mineral and Vitamin supplement	1 g.
Choline chloride	1 g.

<u>Mineral and Vitamin Supplement</u>	
	<u>Per 100 grams</u>
Fe SO ₄ 7H ₂ O	25 g.
Cu SO ₄ 5H ₂ O	8 g.
Thiamine chloride	0.8 g.
Pyridoxine hydrochloride	0.8 g.
Riboflavin	1.6 g.
Nicotinic Acid	4.0 g.
Calcium pantothenate	4.4 g.
Inositol	54. g.
Synthetic K	1.4 g.

<u>Final Composition of Control Diet</u>	
	<u>Percent</u>
Butter fat	21
Hydrogenated cotton seed oil	10
Milk proteins	20
Lactose	28.5
Dextrin	13.
Cellu-flour	2.
Minerals	4.5
Moisture	1.

leucocytic counts, the amount of hemoglobin and the nonprotein nitrogen and total protein of the blood serum were determined monthly for the first three months and at three month intervals until the completion of the experiment.

With the attainment of the two year period of this test it was thought desirable to terminate the experiment since the number of surviving animals was so few. In addition to the usual hematological determinations it was decided to determine what changes might have occurred in some of the more important enzyme systems of the body, namely, the alkaline phosphatase of the liver, kidney, spleen, bone marrow and serum, the succinic oxidase of liver and kidney and the adenosinetriphosphatase in liver, kidney and spleen. Accordingly animals were sacrificed, usually two at a time, samples of tissue removed for the biochemical studies and the remaining tissues taken for histological examinations.

In Figure 3 are presented the average growth curves for these animals (see also Tables 6, 7, 8, 9 and 10). One may observe that those rats fed the diets containing thorium show a depression in the growth rate. This lag in growth became apparent early in the experiment and continued throughout the period. The animals given the diet with aluminum chloride added grew at a rate intermediate between those fed the thorium nitrate diets and the control animals. The rats on the diet with aluminum nitrate appeared to grow at a rate exceeding that of the control animals. However, it should be noted that these rats were placed on the experimental diet two months later than the other rats and hence are not strictly comparable. If one takes into account the initial weight and adjusts the curve accordingly there appears to be little difference between the two groups. Although both groups of animals given thorium in their food showed a lag in their growth rate we felt that it might be advisable to examine the data statistically in an effort to determine whether the differences were significant. Accordingly the reciprocal of the age in weeks was plotted against weight in grams on semilog paper, after the method of Zucker et al. (28). There was some doubt as to the exact age of these animals at the initiation of the experiment although the records indicated 9 weeks as the approximate age. By appropriate methods we were able to determine the best age at onset to be nine weeks.

A line of best fit was drawn for each animal and the k value or slope of this line determined. This was calculated by the formula

$$k = \frac{\log W_2 - \log W_1}{1/t_1 - 1/t_2}$$

In general terms the greater the k value the greater the increment in growth rate for that animal.

FIGURE 3

GROWTH OF RATS INGESTING DIETS CONTAINING THORIUM NITRATE, ALUMINUM CHLORIDE
OR ALUMINUM NITRATE

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TABLE NO. 6

THE WEIGHT (in grams) OF RATS RECEIVING THE BASAL DIET

RAT		WEEKS																								At Time of	
NO	0	4	8	12	16	20	24	28	32	36	40	44	48	52	56	60	64	68	72	76	80	84	88	92	96	100	Sacrifice
1	173	180	231	262	266	262	270	220	D*																		
2	127	143	D																								
3	142	180	D																								
4	110	134	D																								
5	171	208	D																								
6	144	198	221	255	268	266	280	291	296	300	311	320	328	326	330	320	334	348	370	363	366	366	358	354	262	350	350
7	146	181	217	256	265	272	280	276	275	286	310	324	326	334	350	352	354	370	389	396	378	378	391	D			
8	143	187	195	261	271	270	278	280	288	308	324	348	360	358	362	340	352	352	387	384	375	380	376	349	344	336	K +
9	126	161	194	217	233	232	230	234	240	D																	
10	124	161	186	226	241	244	254	263	268	268	288	244	D														
11	119	154	187	219	198	224	223	239	248	266	264	292	260	288	310	304	318	304	309	283	D						
12	93	123	133	151	160	168	178	190	193	200	210	222	236	250	244	240	246	224	D								
13	106	142	156	182	162	196	194	206	210	226	234	246	246	250	258	240	263	260	285	290	268	266	239	305	326	295	D
14	107	139	139	168	181	178	174	200	200	196	202	210	208	214	218	220	224	226	223	245	268						
15	122	134	147	169	182	192	180	187	190	190	188	190	202	204	210	204	221	214	232	232	232	212	236	231	230	242	243
16	136	172	186	238	249	262	280	298	316	324	330	354	362	372	384	364	376	375	408	396	406	380	356	348	262	D	
17	101	148	188	209	230	240	246	246	246	232	D																
18	100	148	134	223	232	178	198	214	230	252	276	274	292	266	310	280	300	288	330	334	326	318	332	336	326	228	240
19	132	174	226	270	290	298	178	D																			
20	161	189	212	278	288	294	308	320	328	350	362	378	390	390	396	380	374	366	341	298	258	230	D				
21	128	162	199	238	263	272	288	286	295	304	318	320	330	324	320	300	304	286	311	295	275	D					
22	140	168	198	239	260	267	278	300	318	322	327	342	340	342	342	308	336	334	356	373	361	D					
23	130	178	222	227	210	198	176	169	174	160	166	202	218	220	D												
24	165	195	242	302	320	328	340	369	377	K																	
25	122	175	180	229	249	260	290	310	310	320	340	360	370	255	D												
26	124	185	153	248	276	284	284	284	275	292	314	334	356	370	384	358	378	400	410	404	409	411	417	432	448	434	397
27	142	209	266	D																							
28	110	162	222	230	256	262	282	296	277	300	246	216	D														
29	129	167	186	248	246	284	280	293	292	296	310	324	327	325	328	308	323	314	333	336	312	310	312	D			
30	138	170	184	207	222	236	230	239	244	262	270	264	270	278	276	266	D										

* D - Died

† K - Killed during bleeding

20

TABLE NO. 7

THE WEIGHT (in grams) OF RATS RECEIVING THE BASAL DIET WITH THE ADDITION 1 PER CENT THORIUM NITRATE

RAT		WEEKS																								At Time of	
NO	0	4	8	12	16	20	24	28	32	36	40	44	48	52	56	60	64	68	72	76	80	84	88	92	96	100	Sacrifice
1	126	148	148	177	175	184	142	D*																			
2	134	146	153	180	188	181	180	195	198	208	208	210	220	218	212	204	196	209									
3	106	144	K+																								
4	150	175	184	207	208	206	207	217	214	224	228	224	246	240	250	234	248	260	262	258	261	284	281	284	289	284	294
5	119	134	134	154	154	156	159	170	D																		
6	147	174	201	216	229	236	228	251	250	260	268	270	278	276	272	274	278	267	300	288	283	307	312	321	334	316	306
7	104	138	169	183	202	200	190	204	218	218	222	230	234	242	242	224	212	240	285	297	277	282	D				
8	135	153	161	174	170	180	181	177	182	182	184	186	192	190	192	200	215	202	208	204	239	211	207	210	206	254	213
9	141	210	278	322	348	332	356	362	372	392	422	416	450	442	470	454	426	390	404	403	360	394	370	425	438	436	400
10	129	153	150	176	179	184	183	186	186	190	202	210	220	220	212	216	222	218	216	202	195	177	172	D			
11	122	166	189	188	209	208	219																				
12	112	129	140	164	175	175	178	184	188	196	204	208	222	220	222	222	229	228	242	246	270	271	196	164	154	148	D
13	112	140	156	176	179	180	175	190	192	196	198	199	204	200	188	194	181	182	189	194	200	208	206	200	D		
14	130	146	146	176	175	183	188	197	193	200	200	185	194	202	194	D											
15	107	142	167	193	194	204	207	218	215	220	226	200	224	202	202	182	138	D									
16	140	172	189	206	210	210	208	212	220	216	222	240	252	250	268	224	235	254	275	276	280	312	288	310	317	287	243
17	125	161	178	195	208	212	210	236	242	250	256	272	294	300	304	272	260	282	316	320	D						
18	105	138	160	174	181	190	185	190	190	202	K																
19	158	201	232	258	276	280	302	322	348	346																	
20	129	169	166	230	248	254	250	278	268	280	282	288	300	302	308	248	261	304	355	366	343	350	361	417	420	406	399
21	119	136	134	160	166	175	175	178	184	190	192	202	202	200	204	202	214	208	216	210	223	226	236	234	246	241	254
22	105	131	130	173	125	140	169	168	190	194	178	194	216	202	218	212	218	223	225	220	224	234	226	228	226	186	D
23	108	136	134	163	169	166	153	175	176	174	182	184	192	196	198	212	208	D									
24	132	166	177	201	228	214	232	231	234	232	234	220	204	214	172	D											
25	122	153	175	181	200	194	198	209	218	212	222	208	238	224	244	240	248	255	285	D							
26	126	148	164	175	106	D																					
27	130	151	164	189	198	198	186	207	204	228																	
28	128	170	196	218	K																						
29	156	193	220	256	264	266	270	288	310	304	316	280	320	336	340	336	313	316	365	353	345	354	330	378	366	348	315
30	149	198	217	266	274	288	286	298	315	324	354	356	388	322	D												

* D - Died

+ K - Killed during bleeding

21

TABLE NO. 8

THE WEIGHT (in grams) OF RATS RECEIVING THE BASAL DIET WITH THE ADDITION OF 4 PER CENT THORIUM NITRATE

RAT NO	WEEKS																								At Time of Sacrifice		
	0	4	8	12	16	20	24	28	32	36	40	44	48	52	56	60	64	68	72	76	80	84	88	92		96	100
1	170	170	187	173	176	D*																					
2	112	130	143	168	179	186	190	197	202	212	222	232	234	215	232	234	236	224	234	D							
3	152	183	K**																								
4	150	153	180	168	D																						
5	130	145	161	193	116	D																					
6	125	163	K																								
7	134	138	160	220	240	262	272	274	297	302	306	322	338	305	330	306	300	293	297	315	276	315	276	227			
8	150	179	192	225	248	260	258	250	258	288	300	318	262	307	336	306	320	308	317	310	292	286	282	316	312	315	300
9	146	153	174	188	182	D																					
10	124	162	160	200	219	222	217	205	228	242	230	250	252	238	242	236	242	232	235	221	218	D					
11	136	166	192	248	260	288	296	321	330	354	380	400	410	386	418	386	400	385	405	410	401	D					
12	104	148	165	181	194	194	192	195	196	208	214	220	218	211	220	220	234	224	231	233	243	231	216	246	240	208	D
13	146	127	138	154	162	158	158	165	170	174	184	184	188	168	190	190	190	190	D								
14	111	184	K																								
15	120	160	166	188	190	204	204	206	205	D																	
16	127	150	147	168	174	178	180	188	188	200	194	214	214	192	198	210	230	212	223	217	204	224	187	212	217	184	D
17	130	K																									
18	126	158	163	181	186	199	194	197	202	208	217	224	232	214	232	204	214	210	207	167	252	D					
19	125	144	180	199	203	210	D																				
20	123	149	151	164	164	175	168	184	159	D																	
21	96	130	140	153	164	170	168	167	170	173	180	200	192	186	196	200	210	204	217	209	224	224	210	206	219	223	192
22	117	160	167	203	205	212	208	231	203	228	238	242	220	210	D												
23	146	169	173	203	K																						
24	135	131	149	209	233	170	D																				
25	124	139	164	194	210	240	237	234	258	272	284	296	230	165	D												
26	138	170	195	244	282	293	290	306	294	330	336	342	360	332	370	330	208	330	361	356	345	332	D				
27	141	157	206	226	D																						
28	135	152	161	194	200	208	200	216	233	244	244	254	264	254	260	224	211	243	245	D							
29	126	152	158	205	220	238	230	240	246	242	270	280	298	290	306	284	274	291	290	287	270	D					
30	128	163	158	202	223	240	236	256	258	264	306	306	328	314	350	330	342	336	356	350	338	320	253	232	238	215	184

*D-Died

**K-Killed During Bleeding

TABLE NO. 9

THE WEIGHT (in grams) OF RATS RECEIVING THE BASAL DIET WITH THE ADDITION OF ALUMINUM CHLORIDE*

22

RAT NO	WEEKS																								At Time of Sacrifice			
	0	4	8	12	16	20	24	28	32	36	40	44	48	52	56	60	64	68	72	76	80	84	88	92		96	100	
1	102	141	173	210	230	235	242	253	248	254	262	276	302	298	308	322	324	297	336	300	275	D						
2	144	184	207	278	284	288	265	330	344	360	392	390	414	402	412	404	410	377	414	431	402	374	342	387	411	390	362	
3	180	212	221	260	260	256	253	282	291	300	316	324	324	320	334	322	340	298	355	349	338	315	D					
4	123	155	192	222	240	222	233	218	212	200	D																	
5	160	185	222	246	255	256	266	K																				
6	124	156	202	238	258	261	262	276	272	280	294	322	338	338	362	362	370	355	398	384	400	413	360	D				
7	183	214	264	300	328	332	324	360	344	368	406	424	392	370	368	362	352	317	D									
8	152	152	206	272	293	296	292	324	330	338	352	374	392	394	402	408	400	375	427	432	430	435	444	452	452	436	432	
9	112	153	176	192	199	182	212																					
10	110	134	167	184	203	192	205	222	222	236	228	242	250	251	244	254	244	251	285	275	295	303	306	319	306	D		
11	104	140	154	169	175	153	187	187	187	178	192	196	188	194	190	184	187	162	K									
12	100	137	128	164	176	164	179	201	203	212	214	224	240	246	238	234	257	238	281	277	271	D						
13	88	92	D																									
14	139	149	163	177	181	163	190	189	186	184	188	194	198	200	198	192	201	184	209	205	225	208	206	213	216	224	220	
15	108	156	157	184	197	173	203	202	202	176	D																	
16	112	139	121	187	198	202	200	226	225	224	234	248	292	274	288	286	289	272	298	313	300	318	317	334	352	340	351	
17	139	154	167	175	174	180	195	185	183	190	194	202	208	214	190	204	214	195	216	228	249	224	230	246	258	262	258	
18	134	156	170	170	195	192	196	186	194	184	190	164	142	D														
19	114	135	110	177	175	184	184	200	200	204	180	186	210	200	206	178	D											
20	135	161	162	183	189	188	185	191	188	212	200	202	210	210	204	214	232	220	242	256	273	268	258	269	278	250	201	
21	106	140	160	186	193	187	193	205	200	202	190	214	216	214	200	174	D											
22	128	136	155	173	179	175	186	D																				
23	100	122	--	150	151	154	154	162	164	176	180	186	192	194	192	194	194	188	206	206	217	218	217	216	218	228	226	
24	142	180	213	276	271	300	297	314	324	320	340	337	364	314	362	350	352	322	343	364	328	340	358	350	352	337	315	
25	124	158	185	236	248	270	259	290	287	284	300	294	280	266	D													
26	128	179	212	247	260	268	268	288	291	294	318	338	356	352	348	358	294	350	297	397	400	405	406	409	410	402	415	
27	134	166	186	246	254	268	265	299	293	324	338	360	370	354	362	364	375	363	417	407	398	380	407	--				
28	140	164	211	256	271	228	216	D																				
29	112	156	210	260	262	282	281	D																				
30	122	139	182	190	196	222	225	249	246	252	D																	

* Aluminum chloride added to the basal diet in an amount of 27.5 g/Kg which is equivalent in chloride concentration to the nitrate concentration in the 4 per cent thorium nitrate diet.

† D - Died

‡ K - Killed during bleeding

TABLE NO. 10

THE WEIGHT (in grams) OF RATS RECEIVING THE BASAL DIET WITH THE ADDITION OF ALUMINUM NITRATE*

RAT NO	WEEKS																				At Time of						
	0	4	8	12	16	20	24	28	32	36	40	44	48	52	56	60	64	68	72	76	80	84	88	92	96	100	Sacrifice
1																											
2	175	222	238	248	254	261	D†																				
3	178	189	185	D																							
4	195	256	261	269	270	281	282	302	318	346	356	338	347	310	334	345	355	356	350	355	363	380	351	378	376	377	
5	131	211	238	244	260	266	268	300	340	358	380	367	390	360	364	351	364	362	342	335	346	370	338	330	292	303	
6	154	205	243	247	258	263	260	294	328	352	352	352	382	376	375	370	415	393	419	430	451	475	439	450	442	D	
7	111	D																									
8	148	212	264	270	298	313	309	326	372	384	398	380	410	376	366	361	320	360	335	350	347	402	377	375	336	328	
9	136	187	210	-																							
10	203	278	308	308	310	320	339	356	D																		
11	167	186	222	238	252	258	258	276	314	338	362	362	374	360	368	368	363	378	356	360	372	401	376	414	398	385	
12	137	193	246	204	210	218	210	228	254	268	294	292	304	300	300	308	319	315	323	320	320	330	300	300	296	283	
13	195	243	277	286	294	298	306	322	362	376	382	392	404	392	381	398	412	390	375	375	395	400	369	403	400	434	
14	149	227	232	267	288	309	327	340	358	374	374	372	396	380	382	380	381	385	330	364	386	425	398	434	438	447	
15	190	229	270	252	286	300	302	320	342	358	366	346	384	350	359	363	373	377	349	345	369	392	364	363	370	396	
16	108	133	189	212	248	287	290	324	356	374	400	368	384	364	376	366	366	377	345	355	346	386	338	294	206	D	
17	146	230	242	286	302	328	330	348	370	396	412	418	444	410	412	415	387	405	366	406	403	464	430	434	355	D	
18	144	211	224	250	248	270	282	312	334	342	372	342	372	358	373	386	384	390	365	355	312	320	299	D			
19	160	226	246	228	266	232	170	D																			
20	128	193	241	242	250	278	283	306	328	344	370	348	382	374	367	390	396	385	370	374	392	386	368	388	382	389	
21	107	173	224	242	270	280	276	290	318	328	354	344	362	336	323	343	359	347	328	288	335	326	298	310	D		
22	144	203	254	252	276	295	308	318	338	360	380	366	380	380	362	388	402	404	394	406	415	384	358	384	380	369	
23	101	212	236	248	254	242	250	262	296	304	334	326	344	334	342	356	363	342	343	358	344	350	318	326	326	366	
24	--																										
25	129	183	200	203	194	203	209																				
26	124	176	189	195	194	204	216	222	218	220	238	216	230	196	214	223	217	227	210	230	228	212	208	246	225	233	
27	146	190	207	227	218	249	248	254	236	220	260	259	274	260	270	276	278	D									
28	135	177	190	197	200	198	205	222	212	226	236	212	220	222	215	234	231	228	230	235	233	223	219	200	174	227	
29	--																										
30	109	164	180	192	194	193	194	212	220	224	240	228	234	240	237	250	262	252	220	166	D						
31	151	244	284	288	308	317	307	346	370	386	406	396	406	410	400	400	421	404	374	412	408	410	392	384	353	392	
32	124	188	209	201	224	250	248	268	260	260	276	296	286	D													
33	134	193	220	210	204	207	232	240	252	258	250	252	266	264	268	224	230	250	258	275	275	282	254	270	282	257	
34	124	159	173	177	172	185	178	190	194	202	208	202	206	204	213	209	211	210	235	224	213	220	199	158	D		

* Aluminum nitrate ($\text{Al}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$) was added to the basal diet in an amount of 43.55 g/Kg of the basal diet.

† D - Died

In determining the k values those animals that died in the early stages of the experiment or for which a good line could not be fitted were discarded. For purposes of comparison we selected data from 23 control animals, 23 from the 4 per cent thorium group, 27 from the 1 per cent thorium fed group, 29 of the aluminum chloride animals and 28 of the rats fed aluminum nitrate. The frequency distribution of k values with their means, sigmas and chi-square values are presented in Table 11. It will be noted that the distribution of k values is reasonably normal for the control animals, the animals given 1 per cent thorium nitrate and the aluminum nitrate group. The distributions of k values for the other two groups are unsymmetrical. Although we have determined whether significant differences between the mean k values for these groups exist we are aware of the fact that the means do not adequately describe the distributions of these values and thus our results are open to criticism. A second test of significance was applied, the chi-square value. The chi-square tests indicated that the difference between the distribution of the k values for the control group and the 1 per cent thorium fed group would have occurred by chance less than once in one thousand tests and thus the difference is highly significant. With respect to the comparison of the distributions of k values for the remaining experimental groups with the control group, none of the differences is significant. Likewise, only the difference between the mean k values for the control and 1 per cent group is highly significant (4.67 sigmas). We feel that further treatment of the growth data is indicated, since we are not satisfied that the k value adequately describes the growth curve. A formula descriptive of the growth curve is being developed and if satisfactory the slope and intercept for each curve can then be determined and comparisons made by the usual methods.

Although these results would appear to indicate a greater depression of growth rate from a diet containing one per cent thorium nitrate than from a 4 per cent regimen it is likely that an explanation can be made on the basis of the number of animals and of sampling errors. If we could assume that those animals eating a 1 per cent thorium diet ate twice as much food as those on the 4 per cent diet, an assumption which is hardly consistent with our observations, the rats of the 1 per cent group would ingest one half the amount of thorium. Thus, unless the absorption is markedly different for the lower percentage diet, one cannot explain the difference in growth rate on either dietary intake or thorium toxicity. One explanation for the apparent difference appears more logical to us. The mortality rate (Figure 4) for those rats on a 4 per cent thorium regimen exceeded that for the lower percentage (1 per cent) diet in the early stages of the experiment. It is entirely possible that the surviving animals were the stronger, healthier, more resistant animals.

With respect to mortality during this long term feeding experiment (Figure 4) there is obviously little difference between the over-all lethal rates except for those animals receiving

FIGURE 4
CUMULATIVE MORTALITY (IN PERCENT) IN RATS ON DIETS
CONTAINING THORIUM AND ALUMINUM SALTS

TABLE 11
FREQUENCY DISTRIBUTION OF k^* VALUES

k	GROUPS				
	CONTROL	4% Thorium Nitrate	1% Thorium Nitrate	Aluminum Chloride	Aluminum Nitrate
1.0	f* -	f -	f -	f -	f -
1.5	-	2	2	3	1
2.0	-	-	3	1	4
2.5	2	2	6	2	2
3.0	3	7	5	7	4
3.5	4	3	9	3	7
4.0	6	2	1	2	6
4.5	4	2	-	3	3
5.0	2	1	1	4	-
5.5	2	3	-	3	-
6.0	-	1	-	-	-
6.5	-	-	-	1	1
N=	23	23	27	29	28
Mean-	4.216	3.944	3.156	4.004	3.606
P(Chi Square)		.135	.001	.082	.184

$$K = \frac{\log W_2 - \log W_1}{1/t_1 - 1/t_2}$$

f- frequency number

aluminum nitrate. If one plots cumulative death rates on semilog paper, one may calculate the time in months for the mean lethal rate, its fiduciary limits and determine whether significant differences between reaction times occur. By such a method the mean reaction times (ET50) with 19/20 limits are as follows:

Controls	- 18 months (10 to 28 months)
1% Th(NO ₃) ₄	- 21 months (17 to 26 months)
4% Th(NO ₃) ₄	- 16 months (7 to 24 months)

The reaction times for these groups are not significantly different and the mortality curves established are parallel with one another within the limits of experimental error.

The hematological values for these animals are presented in a series of graphs (Figures 5 to 11 inclusive) so that they may be readily compared. The most striking thing which becomes apparent on examination of these graphs is the lack of any significant change in the hematological findings. While there is a trend toward an increase in the total number of erythrocytes this is true for all groups. The total protein and nonprotein nitrogen show moderate increases at the 84th week of the experiment, but these again are not related to the experimental diets.

Histological examinations were made of the tissues from animals sacrificed at the completion of the experiment, as well as from rats that died during the test. In the case of the latter animals, examinations were made only in the event that they were secured at the time of death or shortly thereafter. The detailed results of the histological examinations will be published as a supplementary report. Essentially the findings are negative. One finds little difference in the tissues whether taken from rats fed aluminum salts or thorium nitrate. There are varying degrees of nephrosis in the kidneys of all rats, one of the few consistent observations. One gains the impression that the amount of kidney damage is somewhat greater in those sections taken from rats receiving one per cent thorium nitrate, but in no case is this damage severe enough to indicate irreversibility.

A detailed discussion of the methods utilized and the results obtained in the studies of enzyme concentrations in various tissues from rats after two years on these diets has been presented by Lavik et al. (29). In summary, their results indicated that no significant reductions in the concentrations of alkaline phosphatase, succinic dehydrogenase, or adenosinetriphosphatase could be detected by the methods used.

Correlation of the data from this long term feeding experiment leads to the conclusion that thorium nitrate in the amounts fed causes no very startling toxic manifestations. There is a depression in growth rate, but it cannot be concluded from the data that the effect is due to thorium per se.

FIGURE 5
CERTAIN HEMATOLOGICAL VALUES OF RATS ON CHRONIC
TOXICITY EXPERIMENT

FIGURE 7
CERTAIN HEMATOLOGICAL VALUES OF RATS ON CHRONIC TOXICITY EXPERIMENT

FIGURE 8
CERTAIN HEMATOLOGICAL VALUES OF RATS ON CHRONIC
TOXICITY EXPERIMENT

FIGURE 9
CERTAIN HEMATOLOGICAL VALUES OF RATS ON CHRONIC
TOXICITY EXPERIMENT

FIGURE 10
CERTAIN HEMATOLOGICAL VALUES OF RATS ON CHRONIC TOXICITY
EXPERIMENT

FIGURE II
CERTAIN HEMATOLOGICAL VALUES OF RATS ON CHRONIC
TOXICITY EXPERIMENT

Observations made during the experiment led us to the belief that the depression of growth rate was due fundamentally to a lowered caloric intake, since it was obvious from the behavior of the animals that the diet was unpalatable. Accordingly we initiated a short term paired feeding experiment on a limited number of rats. Seven litters of rats were selected at weaning and isolated. At an appropriate age, these were arranged so that one brother or sister was in each of seven groups. Thus, animal A₁ of group A, was from the same litter as B₁ of group B, C₁ of C, E₁ of E, etc. Unfortunately since some deaths had occurred before the test began there were unequal numbers of animals in the groups. The dietary regimen as setup was as follows:

- Group A - (Animals A₁ to A₇) - Control diet*
- Group B - (Animals B₁ to B₅) - Control diet to which was added 0.5 per cent thorium nitrate.
- Group C - (Animals C₁ to C₅) - Control diet
- Group D - (Animals D₁, D₂, D₃, D₄, D₆) - Control diet to which was added 1.5 per cent thorium nitrate.
- Group E - (Animals E₁, E₂, E₃, E₄, E₆) - Control diet.
- Group F - (Animals F₁, F₂, F₃, F₄, F₆) - Control diet to which was added 4.5 per cent thorium nitrate.
- Group G - (Animals G₁, G₂, G₃, G₄, G₆) - Control diet.

The animals of groups A, B, D and F were allowed to eat their respective diets ad libitum; their food consumption was recorded daily. The rats in groups C, E and G were given the unsupplemented control diet in the same amount as their litter mate of the preceding group (so-called paired mate) consumed on the previous day. Thus, animal C₁ was given access to plain diet in the same amount as rat B₁ ingested of the 0.5 per cent thorium nitrate diet the previous day. Food consumption of the pair fed rats were also recorded.

The weights of all animals were taken initially and at weekly intervals thereafter (Table 12). Certain hematological studies (Table 13) were also made at the beginning of the experiment and one month later.

Those animals on the 0.5 per cent and 1.5 per cent thorium nitrate diets grew at a rate not markedly different from the control animals (Figures 12 and 13). As may be noted from the food consumption chart (Figure 12), the pair fed controls of the 0.5 per cent group did not eat as well as their mates for approximately two weeks. When they began to eat a similar quantity they began to grow at a rate paralleling their paired mates. It was noted that the animals did not like the restricted

*Control Diet - Powdered commercial chow (Friskies').

FIGURE 12

AVERAGE DAILY FOOD CONSUMPTION AND INCREASE IN WEIGHT OF RATS
ON A DIET CONTAINING 0.5 PERCENT THORIUM NITRATE

TABLE NO. 12

AVERAGE WEIGHT(in grams) OF RATS ON A PAIRED FEEDING EXPERIMENT

GROUP	DIETARY REGIMEN	No. of ANIMALS	DAYS						
			0	7	14	21	28	35	42
A	DIET C*	7	109	132	157	193	218	237	247
B	DIET C + 0.5% TH(NO ₃) ₄	5	103	123	149	174	193	210	222
C	DIET C Consumption restricted to the same amount/rat/ day as in Group B	5	94	111	121	157	179	191	191
D	DIET C + 1.5% TH(NO ₃) ₄	5	108	137	163	187	209	228	237
E	DIET C Consumption restricted to same amount/rat/ day as in Group D	5	113	132	144	166	185	198	202
F	DIET C + 4.5% TH(NO ₃) ₄	3	105	107	125	140	154	162	167
G	DIET C Consumption restricted to same amount/rat/ day as in Group F	3	105	97	118	141	154	164	167

* Diet C- Control diet which consisted of a powdered commercial chow ('Friskies')

dietary intake and frequently wasted food. The curves depicting average weight gain for the controls and the 1.5 per cent thorium fed rats are quite similar (Figure 13) and are matched by the essentially similar consumption of food. Here again their pair fed mates usually refused to eat the food offered and as a result grew at an appreciably slower rate.

In the case of the animals fed the 4.5 per cent thorium nitrate regimen we have quite a different situation. It will be noted (Figure 14) that their food consumption was consistently less (about 4 g/rat/day) than that of the control animals. After an initial lag the pair fed mates ate approximately all of the food furnished them. The rate of growth of these animals mirror the lowered caloric intake, being significantly different than the control group.

Those changes in the hematological values (Table 13) that were found are entirely consistent with any group of animals placed on such a dietary regimen. There are no changes which can be attributed to the presence of thorium.

This experiment, though admittedly limited as to the number of animals used and length of time that they were fed, aids in the interpretation of the data on the long term feeding study. It seems entirely probable that the decreased growth rate of animals fed thorium nitrate in concentrations of 4.5 per cent or less are due to the self-imposed restriction in food intake. This is in contradistinction to the results of Hodge et al. (26) who found rats markedly depressed by a 10 per cent thorium nitrate diet, whereas pair fed controls grew well.

DISTRIBUTION STUDIES:

Limited data are available pertaining to the absorption characteristics and the distribution of absorbed thorium following intraperitoneal and oral administration.

A group of five rats were given a diet, similar to that of Group II of the chronic feeding experiment, containing in addition to four per cent thorium nitrate, UX_1 in the amount of 2.4×10^4 d/m/gram. The total intake for the animals was calculated by the formula:

$$C \times 10 \times T \times f = \text{total intake per rat.}$$

C = the concentration of UX_1 in disintegrations per minute per gram of diet.
 10 = grams of food eaten daily by each rat.
 T = time in days during which the diet was fed.
 f = decay factor, calculated by taking the number of days from the initial feeding of the diet to the day of autopsy.

These animals were sacrificed after twenty-eight days on the diet.

FIGURE 13
AVERAGE DAILY FOOD CONSUMPTION AND INCREASE IN WEIGHT
OF RATS ON A DIET CONTAINING 1.5% THORIUM NITRATE

FIGURE 14

AVERAGE DAILY FOOD CONSUMPTION AND INCREASE IN WEIGHT OF RATS ON A DIET CONTAINING 4.5% THORIUM NITRATE

TABLE NO. 13

EFFECT OF VARIOUS DIETARY CONCENTRATIONS OF THORIUM NITRATE ON CERTAIN HEMATOLOGICAL VALUES

HEMATOLOGICAL VALUES	GROUPS													
	A		B		C		D		E		F		G	
	DIET C		DIET C		DIET C		DIET C		DIET C		DIET C		DIET C	
	(ad lib) *		±10.5% TH(NO ₃) ₄ (ad lib.)		Consumption restricted to same amount/ rat/day as in Group B		± 1.5% TH(NO ₃) ₄ (ad lib.)		Consumption restricted to same amount/ rat/day as in Group D		± 4.5% TH(NO ₃) ₄ (ad lib.)		Consumption restricted to same amount/ rat/day as in Group F	
	Initial	30 day	Initial	30 day	Initial	30 day	Initial	30 day	Initial	30 day	Initial	30 day	Initial	30 day
HEMOGLOBIN (grams per 100 cc)	15.1	18.5	17.8	16.7	17.3	17.5	15.2	16.6	15.3	17.2	16.2	17.2	15.9	17.3
ERYTHROCYTES (x 10 ⁶ per cu. mm)	7.55	9.85	8.50	9.59	8.40	9.65	7.98	9.23	7.79	9.39	7.68	10.15	7.90	9.29
HEMATOCRIT (per cent)	47.9	52.1	51.1	51.0	51.9	51.4	51.4	46.9	49.4	49.7	55.5	51.3	52.8	51.6
CLOTTING TIME † (seconds)	30	13.3	17	17.5	20	14	16	22	16	17	15	18	15	20
LEUCOCYTES (thousands)	14960	15200	11270	12560	11600	12420	13610	13575	12730	10920	13270	14930	10090	11800
DIFFERENTIAL LEUCOCYTIC COUNT (%)														
Neutrophils	24	20	19	17	16	20	23	14	21	17	26	15	28	18
Lymphocytes	74	77	80	81	83	79	75	84	77	82	72	83	71	80
Monocytes	1	2.0	0.5	1.0	0.5	1.0	1.0	1.0	1.0	0.5	1.0	1.0	1.0	1.0
Eosinophils	1	1.0	0.5	1.0	0.5	1.0	1.0	1.0	1.0	0.5	1.0	1.0		1.0

* Diet C- Control diet which consisted of a powdered commercial chow ('Friskies')

† Determined by capillary tube method.

Samples of liver, spleen, heart, lung, muscle, kidney and femur were removed, weighed, ashed and counted. The skin, paws, tail and gastrointestinal tract were discarded, the remainder of the carcass was weighed, homogenized and then treated in the same manner as the other samples. Particular care was taken to prevent contamination during autopsy.

Table 14 presents the actual counts recovered at autopsy of these animals. It will be noted that only the femur and carcass show any considerable concentration of UX_1 . In all cases the other tissues show absorption of less than 0.01 per cent of the dose administered. The number of disintegrations recovered from the carcass is in all determinations less than would be expected from a consideration of the femur content. Thus, calculations of the total amount in the skeleton gave values exceeding the carcass counts except for animal #28. The carcass was ground, reduced to a liquid homogenate, distributed in dishes, dried and then redissolved in nitric acid before distributing in crucibles for ashing and counting. Since as many as 10 crucibles were sometimes needed for the distribution of material from one carcass the possibilities for loss and errors were multiplied.

Using a method outlined in a previous report (30) we have calculated from the concentration in the femur that approximately 4 per cent (4.07%) of the ingested UX_1 could be accounted for in the skeleton, whereas the average percentage of the total dose recovered was 3.8 per cent. The agreement would appear to lie within the limits of the accuracy of our methods. Examination of the data discloses the fact that whatever may have been the actual absorption of thorium and UX_1 in this experiment, the major portion of the metal has been deposited in the skeleton.

Since these results indicate a considerable concentration of thorium with its accompanying radiation in the skeletal tissues, it is of importance to establish a tolerance level for Th^{232} . We have estimated the tolerance amount of Th^{232} (one year old) for a 70 kilogram man to be 490 mg. or 0.054 μc (See Appendix A).

It is necessary to reach an estimated tolerance value for the rat. Utilizing the tolerance value for a 70 kilogram man we may calculate by simple proportion that a 200 gram rat would have a tolerance value of 1.4 mg. or 0.0002 μc . Since the majority of the thorium is deposited in the skeleton, a similar calculation based on relative skeletal weights might be made. Assuming the skeleton of a 70 kilo man to weigh 10 kilo and that of the 250 gram rat to weigh 15 grams, the tolerance amount for rats would approximate 0.74 mg.

On the basis of this tolerance figure the rats in this experiment received some 400 times the tolerance radiation. If the absorption figure is correct those animals receiving a 4 per

cent thorium nitrate diet for 84 weeks would have shown some very definitive changes in the hematological values as well as histological changes in the bone marrow. Since such changes were not observed we must conclude that either the absorption figure as found in the UX_1 feeding experiment is incorrect or that the rats tolerance for thorium is far greater than we have assumed. Experiments in progress indicate that the former is true; absorption following oral ingestion of thorium nitrate is more probably in the order of 0.01 per cent. Values similar to this have been reported by others (7,26,31).

The relatively low concentrations in most tissues following the continued ingestion of thorium lies in contradistinction to the uptake following intraperitoneal administration. Rats were given single intraperitoneal injections of thorium nitrate (40 mg/kg.) with UX_1 added in an amount equivalent to 2.56×10^5 d/m/kg. At 2, 4, 8, 16 and 32 days after the injection, animals were sacrificed and tissues removed for UX_1 determinations. In general, the only tissues showing significant amounts of UX_1 were liver, spleen and the gastrointestinal tract (Table 15). The fact that thorium is precipitated upon injection into the peritoneal cavity and probably exists largely as the insoluble thorium hydroxide would prevent any considerable amount of absorption. The relatively high concentrations in the liver and spleen may be in part accounted for on the basis of the reticulo-endothelial cells which have taken up the particulate material. The counts recovered from the samples of liver and spleen include both internal and external portions of the organ. Some of the activity of these organs can undoubtedly be attributed to material which was adsorbed on or had adhered to the capsules, even though the organs were carefully washed with water and, if considered necessary, dipped in a solution of nitric acid. An attempt was made to determine how much of a role this played in the determinations by collecting internal portions of liver and comparing with the usual samples from the same organ. Although the individual differences were extremely varied, two of eight liver samples treated in this manner had lower counts in the interior than from the sections including the capsule. This is an indication that adsorption and adhesion played a role in the counts obtained from the abdominal viscera.

The percentage uptake in the liver appears to increase on succeeding periods up to 16 days: the 32nd day shows a decrease. Previous findings at Rochester (26) indicated secretion following intravenous administration largely by way of the gastrointestinal tract. Thus, one might expect an increasing concentration in the intestine as the thorium is mobilized and excreted. The data (Table 15) are not consistent with such a disposition of thorium following intraperitoneal administration, since the concentration in the intestine remains relatively constant at 2, 4 and 8 days, decreases somewhat on the sixteenth day and shows an increase on the thirty second day. It is possible that this final increase may be related to the decrease in the concentration in the liver.

44

44

TABLE 14

RECOVERY OF UX_1 FOLLOWING ADMINISTRATION IN THE DIET OF RATS.

Animal No.	Counts in Tissues (d./m.)*								Percent of Total Dose Recovered	Percent of Total Dose in Femur
	Liver	Spleen	Heart	Lung	Kidney	Femur	Carcass	Total Recovery		
25	821	159	—†	302	124	9300	132,194	142,900	4.73	0.31
26	2009	194	237	252	254	4326	46,671	53,943	1.79	0.14
27	250	127	—	156	1157	6922	85,549	94,161	3.11	0.23
28	920	—	—	—	200	4851	132,943	138,914	4.60	0.16
29	1357	139	105	129	140	7084	135,330	144,284	4.79	0.23

* - Disintegrations per minute corrected to t_0 .

—† Count obtained was not significant.

Total dose - 3.02×10^6 d./m.

TABLE 15

AVERAGE PERCENTAGE RECOVERY OF UX_1 FROM TISSUES OF RATS GIVEN A SINGLE INTRAPERITONEAL INJECTION OF THORIUM NITRATE (40 mg./Kg.) AND UX_1 (2.56×10^5 d./m./Kg.)

ORGAN	Number of days after injection recovery determined				
	2	4	8	16	32
	%	%	%	%	%
Liver	9.6	13.8	15.6	21.2	15.3
Spleen	.9	2.3	2.2	2.1	2.8
Stomach	1.6	1.8	1.2	0.7	2.6
Small Intestine	4.7	3.7	4.2	3.7	5.3
Heart	*	*	0.2	0.1	0.2
Lung	.1	0.2	0.3	0.1	*
Skeletal Muscle	.5	0.1	0.1	0.1	0.1
Brain	*	*	*	0.1	0.1
Kidney	.1	0.2	0.3	0.1	0.3
Femur	.2	0.2	0.3	0.1	0.1
Skin	.3	0.2	0.1	0.1	0.2
Large Intestine	1.5	2.5	1.6	1.2	1.7
Abdominal Muscle	.4	1.3	0.6	2.8	0.7
Total Recovery	19.9	26.	26.7	32.4	29.4
Number of Animals	5	5	5	4	4

* Count obtained was not significant.

The concentration in the femur is not markedly different than that found following the ingestion of thorium (Table 14). The percentage of the total dose in the skeleton was calculated from the concentration in the femur by the method previously cited. By this method one estimates a skeletal concentration of 3.8 percent on the second and fourth days, 5.7 percent on the eighth day and 1.9 percent on the sixteenth and thirty second days. The amount in the bone during the early period of this test does not differ significantly from that calculated for rats fed thorium for twenty eight days.

From the data obtained following ingestion of thorium nitrate as well as that from the intraperitoneal experiments it would appear that an amount not exceeding 5 percent of the administered dose is absorbed. It is possible to understand how this might occur following intraperitoneal administration through the translocation of the precipitated metallic hydroxide, but it is difficult to explain the absorption from the gastrointestinal tract, since here again the existing pH would not permit thorium nitrate to remain in solution. It also appears unlikely that there would be sufficient complexing action, by bicarbonate, citrate, etc. to explain the absorption.

Experiments are in progress to determine the absorption following single oral doses of thorium nitrate. Animals (rabbits) are given thorium nitrate plus UX_1 by stomach tube. A sample of the material used is taken from the stomach tube before and after administration to act as a standard in checking dosage and recovery. Rabbits are placed in an appropriate holder and an indwelling catheter is placed in the bladder. Blood and urine samples are collected at 1, 2, 4, 8 and 24 hours for UX_1 determination. All urine is collected. Feces and intestinal contents are discarded. At autopsy (24 hours after administration of the thorium) representative samples of organs and tissues are removed, weighed and the UX_1 content determined.

Two rabbits have been completed. In the first animal no UX_1 was recovered from the blood at any time; only the two and twenty-four hour urine samples had any activity and this was not significant. None of the organs examined had significant amounts of activity. The second animal showed some UX_1 in the spleen, insignificant amounts in the femoral marrow, kidney and adrenal; the remaining organs, with the exception of the gastrointestinal tract, were negative. Significant counts were obtained in the urine samples collected at 4 and 8 hours after administration of the chemical; counts obtained at other periods could not be considered significant although we have recorded them (Table 16) and used them in our calculations. Thus, a total of approximately 1800 d./m. were recovered from the urine samples collected during the 24 hour period. This represents about 1.2×10^{-5} percent of the administered dose. If we total the counts obtained from the organs other than the gastrointestinal tract we can account for less than 600 disintegrations per minute. None of the blood

TABLE 16

RECOVERY OF UX_1 IN THE URINE OF A RABBIT GIVEN AN ORAL DOSE OF THORIUM NITRATE
(100 mg./kg.) and UX_1 (4×10^7 d/m/kg.).*

SAMPLE	VOLUME cc	UX ₁ FOUND	
		d/m/cc	TOTAL d/m
1 hour	2.6	13†	34
2 hours	2.5	4†	10
4 hours	9.2	67	616
8 hours	7.6	47	357
24 hours	32.6	23†	750

TOTAL RECOVERY - 24 hours -

1767 d/m

* Total dose UX_1 - 1.46×10^8 d/m.

† Counts are not considered significant.

samples (3 cc.) taken gave significant counts. Thus this animal revealed essentially the same findings as the first rabbit; the amount of absorption in the 24 hours following oral intubation is extremely limited.

Our data on the absorption following intubation of rats with thorium nitrate plus UX_1 is too limited to present at this time. A comment may be made that the results appear to parallel those presented for rabbits although the absorption seems somewhat greater in rats. Dosages used in rats were considerably greater than in rabbits, being 2 grams/kg. of body weight.

A limited number of experiments on the distribution of thorium nitrate following intratracheal administration have been completed. Preliminary experiments were initiated using albino mice in an effort to determine the approximate dose of the nitrate which could be administered without causing serious toxic manifestations. Mice were lightly anesthetized with ether and thorium nitrate, as an 80 per cent aqueous solution (pH 0.5), was introduced via the trachea. The doses ranged from 0.032 cc. to 0.1 cc., equivalent to 1.2 to 3.7 g/kg. of body weight. It was found that a considerable portion of the material found its way into the gastrointestinal tract. This was checked by radioautographs of mice sacrificed one, two, four, eight and twenty-four hours following intubation with UX_1 . All mice showed the presence of UX_1 in the lungs at the one and two hour periods; at four hours and thereafter a major portion of the material was apparent in the stomach and other portions of the gastrointestinal tract. In none of these autographs was there apparent any evidence of distribution to other parts of the body.

Radioautographs have been made of rats given thorium nitrate plus UX_1 intratracheally, with and without tying off the esophagus. When the esophagus was ligated the autographs revealed the presence of the injected material only in the respiratory passages and lungs. When the esophagus was not ligated, the autographs were essentially the same as those of the mice; the thorium had found its way to the gastrointestinal tract.

Rats with a ligated esophagus tolerated doses of thorium nitrate of 250 mg/kg. of body weight. Doses exceeding this usually resulted in death. Examination of the lungs following administration of thorium nitrate solutions revealed moderate to severe hyperemia, and varying amounts of hemorrhage and fluid in the bronchi and bronchioles.

CHEMISTRY

There are numerous methods available in the literature for the determination of thorium. None of these is satisfactory for the determination of microgram quantities of the metal in animal tissues or tissue fluids. Use may be made, as has been done in some of our experimental procedures, of the thorium

isotope UX_1 , but it was felt that a straightforward chemical method might prove useful. Accordingly we attempted to develop a method for thorium in biological materials limiting our efforts, for the most part, to colorimetric or polaroscopic procedures. A titrametric method utilizing bromine or iodine was investigated; neither of these reagents proved sufficiently sensitive.

In the early phases of this research a considerable number of analytic reagents were tested. Of these ammonium molybdate appeared promising. Since ammonium molybdate precipitates thorium quantitatively and since there are several very sensitive tests for the molybdenum ion, this reagent was investigated thoroughly. It was found that the sensitivity and reproducibility attained by this method was too irregular to be useful.

A more promising reagent was 8-hydroxyquinoline. This compound forms colored precipitates with many metals which can be readily extracted with chloroform for spectrophotometric estimation. Since this reagent is sensitive to changes in pH, metals may be selectively precipitated by choosing the proper pH range.

It was found that complete precipitation of thorium could be secured between pH 4.6 and 5.0. In this range most of the other elements commonly present in animal tissues would remain in solution. This reaction was carried out at room temperature in a 0.1 M acetate buffer solution (equal parts of 0.1 M sodium acetate and 0.1 M acetic acid) at a pH approximating 4.7. The final buffer concentration should not exceed 0.1 M since stronger buffer concentrations produce erratic results.

The procedure followed in the determination of thorium samples was as follows: To a glass stoppered test tube containing 1.0 cc. of a buffered thorium solution was added 1.0 cc. of a 1 per cent solution of 8-hydroxyquinoline in 95 per cent ethyl alcohol. After standing at room temperature for 30 minutes exactly 2 cc. of chloroform were added. The resulting precipitate was extracted by vigorous shaking. After brief centrifugation, the chloroform layer was pipetted off into cuvettes and read on the spectrophotometer at 375 m μ . Reference to Figure 15 demonstrates that in plotting optical density against known concentrations of thorium, the method complies with the Beer-Lambert Law for concentrations ranging between 1 and 10 mg. per 100 cc. of thorium.

Tests were conducted to ascertain which metallic ions might interfere with this method. Of those examined, including Mg, Ca, Na, K and Cu, only Fe, in both the ferrous and ferric states, interfered. Several methods were tried in an effort to eliminate the interfering ion. Since the same reagents which complex iron also complex thorium this procedure was not fruitful. Extraction of iron with various commonly employed solvents, such as ether, amyl alcohol, etc., also removed some of the thorium.

50

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FIGURE 15
CALIBRATION CURVE FOR TOTAL THORIUM WITH "THORON"
(1-(O-ARSONOPHENYL-AZO)-2-NAPHTHOL-3,6-DISULFONIC ACID)

Since iron precipitates with oxine in a very wide pH range, attempts were made to precipitate this metal in a very acid solution, hoping to leave thorium behind. Here again some of the thorium was carried down with the iron.

Since iron may be electroplated readily, it was thought feasible to remove iron by electrolytic means. A micro-electrolysis apparatus was designed and constructed so that a total volume of 5 to 7 cc. of solution per cell could be electrolyzed. Solutions containing known amounts of thorium and iron were electrolyzed for 5 minutes at a starting amperage not exceeding 5.8 volts. A mercury cathode was employed; the current was cut by running mercury out of the cell, never by cutting the current at the switch. Results using this procedure appear somewhat promising although erratic. Recoveries of added thorium were usually 97 per cent or better, but occasionally would fall below 80 per cent. Further work on this method is necessary before it could be used with confidence.

Concurrently with the electrolytic experiments the possibility of using a polarographic procedure was explored. Solutions containing (a) 0.5 mg. per cc. of iron, (b) 15 mg. per cc. of iron plus 5 mg. per cc. of thorium and (c) 5 mg. per cc. of thorium were tested polarographically, using 0.1 N tetra-N-butyl ammonium iodide as an electrolyte at 0.0 to -2.5 volts. It was found that thorium gave a distinguishable curve. Calcium, magnesium and certain other metals are also reducible at this voltage and may interfere. Since the method appeared neither sufficiently selective nor sensitive for practical use further work was dropped.

Kuznetsov (32) had published a method for the colorimetric determination of thorium using the dye 1-(o-arsonophenylazo)-2 naphthol-3, 6-disulfonic acid. A sample of this dye was made available for our use and has been given the name 'Thoron'. Recently Thomason et al (33) have published a method utilizing this dye. The procedure which we are using is as follows: An aqueous solution containing thorium is placed in a 10 cc. volumetric flask, five drops of concentrated HCl and 1 cc. of a 0.1 N aqueous solution of 'Thoron' are added and the solution made up to volume with water. This solution is read on the spectrophotometer against its own blank at 5480 Å. A standard curve for values between 10 µg and 100 µg of total thorium is presented in Figure 16. In this method only ferric ions interfere, but boiling with hydroxylamine converts the iron to the ferrous state which does not interfere.

Since this method appeared promising an attempt was made to recover thorium from whole blood containing a known amount of added thorium nitrate. The blood was digested with H_2O_2 in sulfuric acid until a clear solution was obtained. The

FIGURE 16

COMPLIANCE WITH THE BEER-LAMBERT LAW BY THORIUM
HYDROXYQUINOLINE

sulfuric acid was boiled off, the residue boiled with two portions of nitric acid and with hydroxylamine hydrochloride. Recovery of thorium following this procedure was unsatisfactory.

Paper chromatography was tried in an attempt to separate thorium from the interfering ions. The solvent employed was 90 per cent acetone - 10 per cent HCl. The strip was developed for 16 hours, using Whatman #1 filter paper. By this means thorium could be successfully separated from other cations such as iron. When digested blood with added thorium was chromatographed the recoveries were still low and erratic.

The poor recovery of thorium from blood led to a study of other ions which might interfere with the determination of thorium by either 'Thoron' or 8-hydroxyquinoline. It was found that phosphates, oxalates, an excess of sulfate, and probably all ions that complex thorium interfere in the determination. The interference by phosphate is probably the most serious since most of the remaining complexers are oxidized during the digestion procedure.

Preliminary investigations using a cation exchange column to separate thorium from interfering ions have been carried out. This work is still in progress and will be reported in the future.

DISCUSSION:

The results presented here indicate that very limited absorption occurs following the administration of thorium nitrate by any route. The fact that thorium nitrate (and this is equally true of the chloride and sulfate) is insoluble at the pH values usually encountered in vivo would certainly impede absorption. Thus, when thorium nitrate in solution comes in contact with tissue having pH values of 6.8 to 7.4 the salt will be precipitated as the hydroxide. Complex salts of thorium which are ultra filtrable can be formed but the amount of complexing which might occur in the body is limited by the necessity for a high concentration of the complexer. A 0.2 M bicarbonate concentration is necessary to complex a 0.005 M thorium nitrate solution (29). Concentrations of bicarbonate lower than this do not permit complex formation and thus prevent ultrafiltration.

The amount of thorium retained in the body following continued feeding of a diet containing a thorium isotope (UX_1) appears to exceed what one would expect of such an inert substance. The continual absorption of small quantities coupled with a low rate of excretion could account for the accumulation of the material in the body. Since UX_1 has a relatively short half-life it is not the ideal isotope for use in long term feeding studies. Studies using a thorium isotope (Thorium 230) with a half-life of 8.3×10^4 years will be initiated. Experiments, which have not been reported here, indicate that the concentration of thorium which is attained

during the first month is not exceeded although administration is continued for a prolonged period. This could be due either to alterations in the gastrointestinal mucosa so that further absorption is prevented, or to an adaptation of the excretory mechanisms of the kidney so that the rate of excretion is increased, or perhaps to both. Experiments designed to elucidate these points are in progress.

SUMMARY:

Studies presented here indicate that ~~thorium nitrate~~ is a relatively innocuous compound. Thus, rats or mice must ingest from ~~one to two grams per kilogram~~ of body weight before severe toxic manifestations occur. Large doses of ~~thorium nitrate~~ given orally result in severe anhydremia.

Thorium nitrate solutions are not well tolerated when given intraperitoneally. Moderate to severe peritonitis results from the acidity and hypertonicity of such solutions.

~~Thorium nitrate~~ is considerably more toxic when administered intravenously. The hemolytic properties of thorium, its effect upon protein and the insolubility of ~~thorium nitrate~~ at the pH of the blood are important factors in the production of toxic reactions when the chemical is introduced into the blood stream.

Subcutaneous injections of thorium nitrate are extremely irritating and give rise to pain, necrosis and sloughing. Small amounts of the crystalline salt introduced subcutaneously appear to be reasonably well tolerated.

Rats fed a diet containing one and four per cent ~~thorium nitrate~~ for a long period show a depression of the growth rate. This depression in the growth rate does not appear to be due to ~~thorium~~ per se, but to a reduction in caloric intake. This has been verified by a paired feeding experiment, in which the growth rate of rats receiving a diet containing four and one half per cent thorium nitrate was indistinguishable from that of pair fed mates receiving the same caloric intake of unsupplemented diet.

The very limited absorption of ~~thorium nitrate~~ has been demonstrated by the use of the thorium isotope, UX_1 . Following oral administration, ~~the limited data available indicates that~~ less than 0.01 per cent of the administered dose is absorbed.

A greater percentage of the material is absorbed following intraperitoneal injection. Since the ~~thorium~~ appears largely in the liver, spleen and bone marrow, it seems logical to assume that the substance has been transported to these sites by cells of the reticuloendothelial system.

The concentration attained following prolonged feeding of thorium-nitrate exceeds that found after single oral doses. Further study is necessary to determine the factors responsible. The majority of the absorbed material can be found in the skeleton, with only insignificant amounts in the viscera, in particular, the liver and spleen.

The results of studies designed to determine microgram quantities of thorium in tissues or tissue fluids have been presented. Two reagents, 8-hydroxyquinoline and 1-(o-arsono-phenylazo) -2-naphthol-3, 6-disulfonic acid, ('Thoron'), have been utilized for the determination of thorium. Neither has given completely satisfactory recovery of thorium from tissues, although both are sufficiently sensitive to be used for assay purposes.

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Appendix ACalculation of Tolerance Amount of Th²³²*

Th²³² A rational calculation of the tolerance amount of Th²³² and its decay products can be made by a comparison with radium for which the accepted tolerance value is 0.1 microgram (0.1 microcurie) deposited in the normal (70 kilo) man. In order to make this calculation, the following assumptions were made:

1. Fifty per cent of the radon (and its decay products) formed by decay of radium is eliminated from the body.
2. The thorium used was separated one year previous to use.
3. RaTh and further decay products of one year old thorium have activities of seventy per cent of their equilibrium activities (34).
4. No loss of Th occurs.
5. The distribution in the body of thorium and radium is essentially similar.
6. The contribution to the biological effect by the β and γ radiation is negligible in comparison to the α radiation.

It is necessary to determine the energy dissipation in the body due to 0.1 microgram of radium. The amount of energy available per disintegration of radium was calculated using assumption 1 above and the following decay scheme. It was determined to be 14.373 Mev/disintegration of Ra.

* We are indebted to Mr. Jack S. Krohmer for the calculations appearing in Appendix A.

Isotope	Type of Radiation	Energy of α Radiation in Mev
Ra ²²⁶	α , γ	4.791
Rn ²²²	α	5.486
RaA	α	5.998
RaB	β , γ	-
RaC	α , β , γ	5.50 (0.02%) 5.44 (0.02%)
RaC'	α	7.68 (99.96%)
RaC''	β	-

Using this value, the energy liberated in ergs/day was calculated as follows:

$$\begin{aligned}
 \text{Energy in ergs per day} &= (\mu\text{c}) (\text{dis/min}/\mu\text{c}) (\text{min/day}) (\text{Mev/dis}) (\text{erg/Mev}) \\
 &= (0.1) (2.22 \times 10^6) (1440) (14.372) (1.6 \times 10^{-6}) \\
 &= 7.35 \times 10^3 \text{ erg/day}
 \end{aligned}$$

Using assumptions 2, 3 and 4 and the following decay scheme, the energy liberated per disintegration of Th²³² was calculated to be 26.386 Mev/disintegrations of Th²³².

Isotope	Type of Radiation	Energy of α Radiation in Mev
Th ²³²	α	3.98
MsThI	β	-
MsThII	β	-
RdTh	α	5.418 (83%) 5.333 (17%)
ThX	α	5.681
Tn	α	6.282
ThA	α	6.774
ThB	β	-
ThC	α , β , γ	6.081 (33.7%)

Isotope	Type of Radiation	Energy of α Radiation in Mev
ThC'	α	8.776 (66.3%)
ThC''	β, γ	-

Using this energy per disintegration, the activity of Th²³² in μc necessary to liberate 7.35×10^3 erg/day was calculated.

$$\begin{aligned}
 \mu\text{c} &= \frac{(\text{erg/day})}{(\text{dis/min}/\mu\text{c}) (\text{min/day}) (\text{Mev/dis}) (\text{erg/Mev})} \\
 &= \frac{7.35 \times 10^3}{(2.22 \times 10^6) (1440) (26.38\text{e}) (1.6 \times 10^{-6})} \\
 &= 0.0543 \mu\text{c Th}^{232} \text{ (equivalent to } 0.1 \mu\text{c Radium)}
 \end{aligned}$$

This activity of Th²³² weighs approximately 490 mgms. Thus we may say in view of assumption 5 that the tolerance amount of Th²³² (1 year old) for a 70 kilo man is 0.054 μc or 490 mgm.*

*All but an insignificant weight of the Thorium decay chain is due to Th²³².

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