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UCRL-2243
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UNIVERSITY OF CALIFORNIA

Radiation Laboratory

Contract No. W-7405-eng-48

MEDICAL AND HEALTH PHYSICS QUARTERLY REPORT

January, February, and March, 1953

June 11, 1953

Berkeley, California

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THE METABOLIC PROPERTIES OF VARIOUS MATERIALS

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Project 48A

TRACER STUDIES

THE RELATIVE BIOLOGICAL EFFECTS OF RADIOPOTASSIUM (K^{42}, K^{43}) AND ASTATINE^{211} IN RATS AND MONKEYS

J. G. Hamilton and K. G. Scott

The work presented here is a continuation of attempts to establish the relative biological effectiveness of beta-particles from radiopotassium and alpha-particles from astatine^{211}. The assumption was made that both of these radioelements are relative uniformly distributed through body tissue. Actually they are not; but the degree of inhomogeneity of distribution is not great, especially in astatine^{211}, which is by far the best choice for an alpha-particle radioelement.

There were four separate experiments set up employing rats and one using monkeys. In the rat experiments the distribution of radiosodium and the change in blood picture were employed as criteria of radiation effects. In the monkey experiments weight and the alterations in total differential leukocyte counts were taken as indicators of possible radiation injury. The monkeys received only astatine^{211}, the primary consideration being that the weight of the animals was too great to do radiation studies with K^{42}, K^{43}. In some of the rat experiments the data are not yet all available--in particular the differential white counts--and in the fourth rat experiment radiosodium distributions are not quite complete.

In the first experiment 10 control animals and 6 treated animals were used. Each of the 6 treated rats received 2.32 millicuries of carrier-free K^{42}, K^{43}. The animals weighed on the average 185 grams, and thus the dose delivered, neglecting for excretion, was calculated to be 12.5 microcuries per gram or 520 rep. The radiosodium tracer was given to all 16 rats at the time the radiopotassium was given to the test animals, by intraperitoneal injection. The blood counts were done one day before the administration of radiopotassium, and at the time of sacrifice five days later. It may be seen from Table I that there was not much difference between the relative distributions of radiosodium in tissue and organs taken from the treated and from the control groups. Mention should be made of the fact that it was necessary to keep the tissues several weeks after administration of radiopotassium so that there would not be enough of it left to introduce a significant error in the radiosodium counting.

In Table II it is evident that there was a marked leukopenia, though the ratio between lymphocytes and granulocytes is not greatly altered. In each of the 10 control and 6 treated animals 500 leukocytes were counted for

the differential count. The treated animals did not appear at the time of sacrifice to be severely injured so far as intake of food and water, physical activity, and general appearance were concerned.

The second experiment employed 7 control and 7 treated animals. The average body weight was 175 grams and each of the 5 animals received 4.96 millicuries of K^{42}, K^{43} which, assuming uniform distribution and neglecting excretion, was 25.4 microcuries per gram and an estimated radiation dose of 1100 rep. One day before scarifice each tracer was given 1.5 microcuries of radiosodium.

In this experiment, in addition to blood counts one day before and five days after administration, counts were taken on the second day after intraperitoneal injection of radiopotassium. Here again it would appear that the difference in distribution of radiosodium was not significant; however, the radiosodium distribution is only of qualitative significance since five of the seven animals died during the five-day interval after being treated with radiopotassium. The distribution studies for the control and treated animals are given in Table III and the erythrocyte and leukocyte counts for the control and the treated animals appear in Table IV. It is of interest to note that on the second day, which was before any of the seven treated animals had succumbed, there was a marked depression of the total leukocyte count. As yet the differential counts are not available, owing to the extensive time required to do such a count in an adequate manner.

Two astatine^{211} experiments employing rats were set up in a similar manner and radiosodium was used as the tracer. In the first of the astatine^{211} studies there were 8 control and 7 treated animals averaging in weight 165 grams. In this astatine^{211} study each of the 7 treated animals received 130 microcuries of astatine^{211} or 0.76 microcuries per gram, which, neglecting excretion and assuming uniform distribution, gave a calculated dose of 135 rep. One day before the animals were sacrificed each rat in the first experiment was given 1.5 microcuries of radiosodium tracer. The animals were sacrificed at the end of the second day after administration of astatine^{211}. In Table V may be seen the distribution of sodium^{22} in the tissues, and the red cell and total leukocyte counts are in Table VI. Here again the relative distribution of radiosodium appeared to be very similar in the treated and in the control animals. The degree of leukopenia, which is indicated in Table VI, is quite marked.

The second astatine^{211} experiment employed 6 control and 6 treated animals. Each animal weighed 210 grams. The treated animals received a total of 174 microcuries of astatine^{211} or 0.83 per gram, or a calculated dose based on the previous astatine experiments, 140 rep. In this experiment the 6 control and 6 treated animals were maintained five days before sacrifice. On the fourth day they each received 1.4 microcuries of radiosodium tracer. The complete data are not available on this experiment, but it appears that there was a marked depression of the leukocyte count and that radiosodium distribution was not strikingly different in the animals treated with K^{42}, K^{43} from that in the control animals. The 6 treated rats showed evidences of what was interpreted to be radiation injury: ruffled fur, decreased dietary intake and diarrhea.

Table I

The Effect Upon the Distribution of Na^{22} in the Rat 5 Days Following Total Body Irradiation. Each Rat of the Treated Group Received 2.32 Millicuries of $K^{42, 43}$ Which Was at the Level of 12.5 Microcuries per Gram. The Radiation Dose was Estimated to be 520 rep. Both the Control and the Treated Group Received 5 Microcuries of Na^{22}. The Values are Corrected for 100 percent Recovery and are Expressed as percent of Administered Dose.

	Control			Treated		
Tissue	% per organ	% per gram	%/gm tissue %/gm plasma	% per organ	% per gram	%/gm tissue %/gm plasma
Heart	0.15	0.19	0.40	0.21	0.32	0.36
Spleen	0.06	0.13	0.27	0.06	0.27	0.30
RBC	0.30	0.06	0.12	0.63	0.12	0.13
Plasma	2.67	0.48	1.00	4.86	0.91	1.00
Liver	0.72	0.13	0.27	1.99	0.26	0.29
Kidney	0.35	0.25	0.52	0.56	0.43	0.47
Stomach	0.16	0.16	0.33	0.30	0.29	0.32
Stom. Cont.	0.07	-	-	0.52	-	-
Sm. Int.	0.98	0.24	0.50	2.40	0.42	0.46
Sm. Int. Cont.	0.40	-	-	1.64	-	-
Skeleton	6.07	0.37	0.77	11.5	0.72	0.79
Muscle	5.85	0.08	0.17	13.4	0.19	0.21
Balance	9.98	0.19	0.40	17.5	0.40	0.44
Skin	5.07	0.21	0.44	12.1	0.48	0.53
Gonads	0.02	0.14	0.29	0.03	0.24	0.26
Lym. Gland	-	0.20	0.41	-	0.37	0.41
Urine	60.8	-	-	30.0	-	-
Feces	6.41	-	-	2.59	-	-

Table II

Erythrocyte and Leukocyte Counts of 6 Rats Each of Which Received 12.5 Microcuries of K^{42} and K^{43} per Gram of Body Weight by Intraperitoneal Injection and Were Sacrificed 5 Days Later. Blood Counts were Taken One Day Prior to the Administration of Radiopotassium and at Time of Sacrifice. Similar Values are Included for the 10 Control Animals. Both the Control and Treated Groups Received 5.0 Microcuries Na^{22} 5 Days Before Sacrifice.

Rat	RBC millions per mm^3		WBC thousands per mm^3		% of lymphocytes		% of granulocytes		% of monocytes	
	-1 day	5th day	-1 day	5th day	-1 day	5th day	-1 day	5th day	-1 day	5th day
Control Group										
1	7.86	7.93	19.00	9.05	87.2	81.6	12.0	18.0	0.8	0.4
2	7.42	7.02	11.70	10.10	82.4	96.4	17.4	3.6	0.2	-
3	6.84	7.79	16.90	19.90	83.8	88.0	16.2	12.0	-	-
4	7.30	6.80	15.10	14.20	84.8	81.4	15.2	18.2	-	0.4
5	8.18	6.53	14.00	6.85	87.4	94.4	12.4	5.6	0.2	-
6	7.40	9.38	10.65	8.65	85.8	82.0	14.0	17.6	0.2	0.4
7	7.02	9.38	14.45	8.65	80.4	73.6	19.2	26.0	0.4	0.4
8	7.73	9.19	15.05	10.80	97.6	92.0	2.4	8.0	-	-
9	7.01	5.84	12.40	12.35	90.0	82.4	10.0	17.6	-	-
10	7.34	5.27	13.10	9.30	88.6	79.0	11.4	21.0	-	-
Average	7.41	7.51	14.24	10.99	86.8	85.1	13.0	14.8	0.18	0.16
Mean Std. Error	±0.13	±0.47	±0.77	±1.18	±1.51	±2.23	±1.51	±2.23		
Treated Group										
1	5.28	5.58	12.50	1.00	71.6	74.8	28.4	24.4		0.8
2	5.53	6.46	10.55	1.20	68.4	60.4	31.6	39.6		-
3	6.77	8.31	11.85	0.50	85.2	83.2	14.8	16.8		-
4	6.92	4.47	13.25	0.70	85.6	79.6	14.4	20.4		-
5	6.92	8.55	11.55	0.75	98.8	83.0	1.2	17.0		-
6	8.26	9.01	13.05	0.40	95.0	88.0	5.0	12.0		-
Average	6.61	7.06	12.13	0.76	84.1	78.2	15.9	21.7	-	-
Mean Std. Error	±0.46	±0.75	±0.39	±0.12	±4.97	±3.85	±4.97	±3.85		

Table III

The Effects Upon the Distribution of Na^{22} in the Rat 5 Days Following Total Body Irradiation. Each Rat of the Treated Group Received 4.96 Millicuries of $K^{42, 43}$ Which Was at a Level of 25.4 Microcuries per Gram. The Radiation Dose Was Estimated to be 1100 rep. Both the Control and the Treated Group Received 1.5 Microcuries of Na^{22}. The Values are Corrected for 100 percent Recovery and are Expressed as percent of Administered Dose. Only 2 Rats of the 7 Treated Rats Survived.

	Controls			Treated		
Tissue	% per organ	% per gram	%/gm tissue %/gm plasma	% per organ	% per gram	%/gm tissue %/gm plasma
Heart	0.42	0.52	0.38	0.42	0.58	0.32
Spleen	0.21	0.35	0.26	0.08	0.59	0.32
RBC	1.02	0.15	0.11	1.17	0.23	0.13
Plasma	9.16	1.36	1.00	9.41	1.84	1.00
Liver	3.27	0.36	0.26	2.82	0.44	0.24
Kidney	1.20	0.75	0.55	1.04	0.86	0.47
Stomach	0.51	0.44	0.32	1.12	0.77	0.42
Stom. Cont.	0.37	-	-	2.33	-	-
Sm. Int.	3.28	0.55	0.40	4.32	0.82	0.45
Sm. Int. Cont.	1.88	-	-	0.91	-	-
Skeleton	16.4	0.91	0.67	17.4	0.90	0.49
Muscle	21.9	0.25	0.18	17.0	0.26	0.14
Balance	14.7	-	-	24.0	-	-
Skin	16.9	0.56	0.41	14.8	0.64	0.35
Gonads	0.05	0.39	0.29	0.08	0.62	0.34
Lymph Gland	-	0.49	0.36	-	0.82	0.45
Urine	7.81	-	-	3.10	-	-
Feces	0.87	-	-	0.21	-	-

Table IV

Erythrocyte and Leukocyte Counts of 7 Rats Each of Which Received 25.4 Microcuries of K^{42} and K^{43} per Gram of Body Weight by Intraperitoneal Injection and Were Sacrificed 5 Days Later. Blood Counts Were Taken One Day Prior to and Two Days After Administration of Radiopotassium and at Time of Sacrifice. Similar Values are Included for the 7 Control Animals. Both the Control and the Treated Group Received 1.5 Microcuries Na^{22} One Day Before Sacrifice.

Control Group

Rat Number	RBC millions per mm^3			WBC thousands per mm^3		
	-1 day	2nd day	5th day	-1 day	2nd day	5th day
1	8.20	-	7.13	10.25	11.00	6.80
2	6.97	8.14	6.98	9.65	12.65	11.25
3	8.27	7.03	6.69	9.90	12.60	7.90
4	6.41	6.83	6.42	16.70	13.05	13.50
5	7.11	8.15	6.11	13.20	13.75	10.25
6	8.66	6.55	5.93	12.20	16.75	5.05
7	6.86	6.34	6.13	11.95	21.50	12.75
Average	7.50	7.17	6.48	11.97	14.47	9.64
Mean Std. Error	0.32	0.33	0.20	0.95	1.35	1.20

Treated Group

Rat Number	RBC millions per mm^3			WBC thousands per mm^3		
	-1 day	2nd day	5th day*	-1 day	2nd day	5th day
1	7.46	7.69	6.84	26.85	2.95	0.20
2	7.47	4.76	-	20.10	1.05	-
3	6.44	6.84	-	12.15	1.75	-
4	5.28	7.18	-	12.50	1.35	-
5	5.53	6.86	-	10.55	1.60	-
6	6.77	6.68	6.71	11.85	2.00	0.20
7	7.30	7.23	-	15.10	1.65	-
Average	6.61	6.75	6.77	15.59	1.76	0.20
Mean Std. Error	0.33	0.34	0.27	2.22	0.23	-

* 2 animals survived

Table V

The Effect Upon the Distribution of Na^{22} in the Rat 2 Days Following Total Body Irradiation. Each Rat of the Treated Group Received 130 Microcuries of At^{211} Which Was at the Level of 0.76 Microcuries per Gram. The Radiation Dose was Estimated to be 135 rep. Both the Control and the Treated Group Received 1.5 Microcuries of Na^{22}. The Values are Corrected for 100 percent Recovery and are Expressed as percent of Administered Dose.

	Control			Treated		
Tissue	% per organ	% per gram	%/gm tissue %/gm plasma	% per organ	% per gram	%/gm tissue %/gm plasma
Heart	0.41	0.56	0.39	0.37	0.50	0.34
Spleen	0.20	0.37	0.26	0.13	0.41	0.28
RBC	0.96	0.18	0.12	1.05	0.20	0.14
Plasma	7.56	1.45	1.00	7.50	1.46	1.00
Liver	3.00	0.52	0.36	3.21	0.45	0.31
Kidney	1.04	0.79	0.54	1.12	0.80	0.55
Stomach	0.48	0.51	0.35	0.45	0.49	0.34
Stom. Cont.	0.25	-	-	0.36	-	-
Sm. Int.	2.61	0.67	0.46	2.68	0.80	0.55
Sm. Int. Cont.	2.23	-	-	2.39	-	-
Skeleton	17.1	1.09	0.75	17.2	1.08	0.74
Muscle	21.3	0.32	0.22	17.5	0.27	0.18
Balance	18.5	-	-	25.9	-	-
Skin	16.5	0.71	0.49	13.5	0.68	0.47
Gonads	0.06	0.45	0.31	0.05	0.37	0.25
Lym. Gl.	-	0.60	0.41	-	0.42	0.29
Urine	7.21	-	-	5.31	-	-
Feces	0.54	-	-	1.17	-	-

Table VI

Erythrocyte and Leukocyte Counts of 8 Rats Each of Which Received 0.76 Microcuries of At^{211} per Gram of Body Weight by Intraperitoneal Injection and Were Sacrificed 2 Days Later. Blood Counts Were Taken One Day Prior to the Administration of At^{211} and at Time of Sacrifice. Similar Values Are Included for the 7 Control Animals. Both the Control and the Treated Group Received 1.5 Microcuries of Na^{22} One Day Before Sacrifice.

Control Group

Rat. No.	RBC total/mm^3 millions		WBC total/mm^3 thousands	
	-1 day	2nd day	-1 day	2nd day
1	6.81	Not Counted	10.45	Not Counted
2	6.44		18.0	
3	6.46		15.65	
4	7.05		11.4	
5	7.09		17.1	
6	6.13		12.35	
7	7.59		12.7	
8	5.79		13.05	
Average	6.67		13.84	
Mean Std. Error	±0.20		±0.97	

Treated Group

Rat No.	RBC total/mm^3 millions		WBC total/mm^3 thousands	
	-1 day	2nd day	-1 day	2nd day
9	7.06	6.34	6.8	1.45
10	6.74	5.48	9.85	1.10
11	4.91	5.27	13.2	0.85
12	5.93	6.09	12.25	1.55
13	5.09	5.37	9.0	0.75
14	6.25	5.03	12.35	0.85
15	5.68	5.54	10.5	2.70
Average	5.95	5.59	10.7	1.32
Mean Std. Error	±0.31	±0.17	±0.60	±0.26

Table VII

The Leukocyte Count in Monkeys Following the Intraperitoneal Administration of 0.96 Microcuries of At^{211}. 500 Leukocytes Were Counted for Each of the Control and the Injected Animals.

	Date	Days	Monkey No.	Weight Kilo.	WBC total/mm^3	Differential WBC, % on 200 Lymph	Neut.	Eos.	Baso.	Mono.
Control	2/17	0	3	2.4	17,450	79.5	19	1	0.5	-
			6	2.2	23,400	77	23	-	-	-
Expt.			2	2.4	12,000	82.5	16	-	1.5	-
			5	2.2	14,800	91	5.5	2.5	0.5	0.5
Control	2/20	3	3	-	10,950	88	11.5	0.5	-	-
			6	-	13,700	84	14	1	1	-
Expt.			2	-	1,700	65	33.5	-	1	0.5
			5	-	1,900	61	38.5	-	0.5	-
Control	2/27	10	3	2.4	13,950	89.5	10.5	-	-	-
			6	2.2	16,700	91	9	-	-	-
Expt.			2	2.4	3,500	71	29	-	-	-
			5	2.2	2,350	84	16	-	-	-
Control	3/6	17	3	-	16,400	90.5	9.5	-	-	-
			6	-	14,800	85	14	-	1	-
Expt.			2	-	4,650	89.5	10	-	0.5	-
			5	-	1,600	91	8.5	-	0.5	-
Control	3/12	24	3	-	19,700	64	34	1	-	1
			6	-	16,850	94.5	5	0.5	-	-
Expt.			2	-	6,350	95	5	-	-	-
			5	-	6,250	82.5	16.5	1	-	-
Control	3/20	31	3	2.3	18,800	87.5	12	0.5	-	-
			6	2.3	12,800	95.5	4	0.5	-	-
Expt.			2	2.3	6,250	78	20.5	1.5	-	-
			5	2.3	6,600	89.5	7.5	2	-	1
Control	3/27	38	3	-	15,100	55	42	2	1	-
			6	-	12,650	80.5	18.5	1	-	-
Expt.			2	-	8,100	69	29.5	1.5	-	-
			5	-	4,650	78.5	19	1	0.5	1
Control	4/3	45	3	-	14,850	34.5	65	-	0.5	-
			6	-	13,300	81.5	15	3.5	-	-
Expt.			2	-	7,950	78.5	17.5	2.5	-	1
			5	-	4,800	72	26	2	-	-

The observation that there was no apparent shift in the radiosodium balance in the rat proved somewhat disappointing. It may be noted that there was a high urinary excretion in the first experiment for which no adequate explanation may be given. In the treated animals the values were still high and were approximately one-half those of the control group. This, of course, made the percent per organ and per gram lower than the control and treated groups, but when expressed as percent per gram of tissue the values became almost the same for each.

A question might be raised as to the accuracy of the experiments from the point of view of recovery of the administered radiosodium from all of the tissues and organs as well as the excretion. The recovery was made with care and ranged from 95 to 100 percent of the administered dose.

Two rhesus monkeys weighing 2.2 and 2.4 kilograms respectively received by intraperitoneal injection 0.96 millicuries of astatine^{211}. The calculated dose for these two animals was 75 rep for the first monkey and 68 rep for the second animal. The weight total, leukocyte count and differential count are presented in Table VII. It may be seen that by the third day there was a profound drop in the leukocyte count and possibly a relatively greater decrease of the lymphocytes. By the tenth day the total leukocyte count had begun to rise but even at the end of 45 days there was still a marked degree of leukopenia. Apart from the leukocyte change the animals showed no outward evidences of radiation injury as far as food and water, physical activity, and general appearance were concerned.

One deduction that may be drawn from both the radiopotassium and the astatine^{211} experiments is that on a rep basis the alpha-particles from astatine^{211} are far more injurious (when calculated on a physical basis) than equivalent amounts of energy from beta-particles, such as arise from radiopotassium. Not only was this demonstrated by changes in leukocyte counts in rats and monkeys but also by the general physical appearance of the rats in the last experiment.

Two interpretations may be made. The first is that the biological effects of alpha-particles would appear to be greater than those of beta-particles. The second is that astatine^{211} localizes in the hematopoietic system to a greater degree than could be determined by the tracer studies with astatine^{211}, established earlier in this laboratory. The latter possibility seems more plausible in the light of studies done in other laboratories, in which radioelements posessing a relatively high degree of specific ionization have been used. Unfortunately the chemical properties and half-life of any suitable radioisotope of astatine render it very difficult if not impossible to carry out adequate radioautographic studies to determine selective localization within the blood-forming organs.

STUDIES ON THYROID FUNCTION AND THE DISTRIBUTION OF F^{18} IN RATS CHRONICALLY INTOXICATED WITH FLUORIDE

Patricia Wallace

Methods

Fifty-five Long-Evans femals rats approximately nine weeks of age (150 grams in weight) were divided into 11 groups of five and were placed in stock cages. The animals were maintained in two lots, 25 control animals that were given the semisynthetic ration (see previous report), and 30 experimental animals that were fed this same ration with NaF added. Each group of five rats received 75 grams of food daily or an average of 15 grams of food per rat. Tap water was given ad lib.

Sodium fluoride was added to the food of the experimental animals in sufficient quantity so that the average daily intake for each cage of five rats was 20 mg of fluoride per kilo of body weight. All the animals were weighed every 7 to 10 days at approximately the same time of day and the NaF content of the ration was adjusted for any weight changes in the experimental animals. Great care was taken to mix the fluoride supplement intimately with the food, since according to Roholm[12] this precaution reduces or prevents damage to the gastro-intestinal tract.

Twenty mg of fluoride per kilo per day was the daily fluoride supplement chosen, because it has been shown by Lamb et al[9,11] and by McClure and Mitchell[10] that a daily intake of fluoride of greater than 20 mg per kilo produces serious disturbances in the tissues of the rat other than the bones and teeth. Since the major purposes of the experiments were to determine whether or not there are any disturbances in thyroid function at this level of fluoride intake or changes in the manner in which a chronically fluorosed rat handles F^{18}, it was considered desirable to give a fairly large amount of fluoride and at the same time to maintain the animals in a state as close to physiological as possible.

The control animals were maintained on the purified diet and the experimental animals on the purified diet with the fluoride supplement for an average of 18.5 weeks. (Two control animals died in the first weeks of the experiment and one experimental animal escaped from the cage and was lost). Some of the animals were used as early as 17 weeks after the start of the experiment and others were kept for as long as 20.5 weeks. This discrepance arose because of the difficulties involved in the scheduling of cyclotron bombardments.

At the end of the feeding period some of the animals were given I^{131} to study thyroid function and the rest were given F^{18}.

(a) Ten control animals and ten experimental animals were each given 10 microcuries of I^{131} intravenously. Five experimental animals and five controls were sacrificed one day after the I^{131} injection and the other five experimental animals and five controls were sacrificed two days after the injection. At autopsy the thyroid, blood, pelt, gastro-intestinal tract, carcass and excreta were assayed for I^{131}. The blood was separated by centrifugation

and the red cells and plasma were assayed separately. The pelts were divided into four portions all of which were counted. The gamma-ray activity of these samples was measured on the same counter as was used for the measurement of F^{18}.

(b) For the studies on the distribution of F^{18} in fluorosed animals and their mature controls, the remaining animals were given F^{18} intravenously. Groups of five experimental animals were then sacrificed 1, 4, and 9 hours after the injection of F^{18} and groups of four control animals were sacrificed 1 and 4 hours after the injection and five control animals were sacrificed at 9 hours. The tissues and organs and the techniques employed in this series of six experiments were essentially the same as those described in section (1) except that the F^{18} contents of pancreas, brain, spleen and lungs were not determined and that there was no differentiation made between the extremities and bodies of the bones sampled. In addition several other measurements were made. The length of the femurs of five fluorosed and five controls were measured from the end of the head to the most prominent portion of the medial condyle. After assay for F^{18} the bone samples from 10 of the fluorosed and 12 of the control animals were ashed in a muffle furnace at 500°C for at least 24 hours and the ash contents were determined.

The four remaining fluorosed rats were sacrificed and used for histology and radioautographs.

Radioautography with F^{18}

Radioautographs were made with Eastman Kodak No-Screen X-ray film of the distal ends of the left femurs of three groups of rats one hour after the intravenous injection of F^{18}. The three groups of rats used were as follows: five young animals (150 grams) that had been fed the stock diet, five of the mature controls that had received the purified diet, and four fluorosed animals. The femur was disarticulated from the pelvis and tibia, and the patella was removed. The surrounding muscle was removed with care to avoid excessive injury to the periosteum. The bones were then cut in half with a razor blade and the proximal half was discarded. The distal end of the femur was used, because its epiphyses do not close fully until the animals reach an age of over 1000 days (Simpson et al[16]). With the posterior surface uppermost and using the intercondyloid fossa as a guide, the distal end of the femur was cut into nearly equal portions with a jeweler's saw and fine blade. A new blade was used for each bone, since it was found that bits of bone dust clung to the saw. The pieces of bone were then placed cut surface upward on a piece of sponge rubber in a light-tight box. A piece of thin mica (approximately two to five mg/cm^2) was placed between the film and the cut surface of the bone to prevent the film from sticking to the bone and to avoid chemical fogging of the film or swelling of the emulsion due to moisture seeping from the cut bone surface. A second piece of sponge rubber thick enough to to fill the box completely when it was closed was placed over the film. A weight was then placed on the lid of the box to assure close contact between the bone surface and the film. The films were exposed for from 5 to 10 minutes depending on the amount of F^{18} injected. The films were than developed for from 4 to 8 minutes in Kodak X-ray developer and fixed in hypo. The pieces of bone from which the radioautographs were made were than fixed in absolute alcohol for 48 hours and stained with silver nitrate according to the method described by McClung-Jones[8].

The right femurs were also removed from the fluorosed animals and four of their mature controls. The distal ends of the femurs were fixed for 3 days in formalin and were cut so that the sections would correspond as far as possible to the surfaces of the left femurs from which the radioautographs had been made.

(a) General findings on chronically fluorosed rats

Although there was some overlapping of the weight ranges of the two groups (the weights of the larger animals of the fluoride group were very nearly the same as the weights of the smaller animals of the control group), comparison of the mean weights of the two groups after the start of the fluoride supplement invariably gave a p value of less than 0.05, indicating that for groups of this size the differences in weight were statistically significant. (For growth curve see previous quarterly report.) It must be borne in mind that some of the animals on the fluoride diet received more fluoride than others, because the amount of fluoride added to the diet was based on the mean weight of all the experimental animals. The same holds true for the food intake, which was an average of 15 grams daily per rat for each cage of five rats.

The possibility that the slower growth rate of the fluorosed animals was due to a lower food intake than the control animals, owing either to lack of appetite for their food or difficulty in eating because of elongation of the incisors, seems to be fairly well eliminated by the following: the diet was soft and appeared to be eaten by the fluorosed rats without too much difficulty, the fluorosed animals received per cage of five rats the same amount of food as did the controls, the use of heavy pyramid-shaped porcelain feed cups reduced spillage to a minumum, and the feed cups of both groups were almost always emptied during any 24-hour feeding period.

Although most of the animals on the fluoride supplement appeared to be quite normal except for their teeth and slower growth rate, some of them showed other manifestations of intoxication such as poor coat, an untidy appearance, and lassitude.

Typical tooth symptoms, as described in the discussion of the effects of chronic fluoride intoxication on the teeth of rats, were noticeable in these animals within three to four weeks after the start of the fluoride regimen. Figure 1 shows the head of a rat after 4.5 months on a diet of 20 mg of fluoride per kilo per day and Figure 2 is the head of a normal control animal of the same age after 4.5 months on a diet containing 0.5 mg of fluoride per kilo per day. The teeth of the fluorosed rat show absence of normal pigmentation. They are opaque and almost chalky in appearance and contain dark spots. Both the maxillary and mandibular incisors are considerably thicker than those of the control animal. Note the flattening of the ends of the maxillary incisors as compared to the almost knife-edge sharpness of the corresponding teeth of the control. The spreading, elongation and backward curvature of the maxillary incisors is concurrent with the decrease in length of the mandibular incisors, which have either been broken off or worn down excessively.

All the animals on the fluoride supplement showed the above dental effects to a greater or lesser degree; however, there did not appear to be any correlation between body weight or weight gain and the severity of the tooth symptoms. The average weight gain for the fluorosed animals was 74.8 ± 3.8 grams and for the controls, 97.8 ± 4.9, comparing the two means, $p < 0.01$.

At autopsy when the femurs of the fluorosed rats were cut longitudinally to prepare them for making radioautographs, it was noticed that they seemed to be softer and more easily cut than were the control femurs. The molars of the fluorosed rats were found to be much less difficult to remove than were those of the control animals. These observations are in agreement with the findings on the ash content of the normal and fluorotic leg bones. The ash content of the leg bones of 10 fluorosed rats and 12 control rats gave the following results: control leg bones, 34.2 ± 0.5 percent ash; fluorosed, 32.5 ± 0.6 percent ash. The difference between the two means, 1.7 percent, is significant since for sets of determinations of this size, p is less than 0.05. The above figures for the ash content of the leg bones of these animals were used in the calculation of total skeletal F^{18}.

Measurement of the femur lengths of four of the fluorosed rats and four of the mature control rats gave the following results: control femur, 351 ± 6.7 mm; fluorosed femur, 328 ± 6.2 mm. A p value of less than 0.02 indicates that the difference between the two femur lengths, 23 mm, is significant.

(b) Experiments on the thryoid function of fluorosed rats

Since the late 1930's when radioactive isotopes of iodine with suitable half-lives were first produced, a great deal of work has been done on the functional state of the thyroid and the thyroidal uptake of radioactive iodine, pioneered by Hertz and his co-workers[6, 7] and by Hamilton and Soley[3, 4, 5]. More recently Scott et al[15] have devised a method whereby the ratio, red cell: plasma of I^{131} is used as a measure of thyroid function. This latter is essentially a measure of the freely diffusible iodine and of the nondiffusible, protein-bound iodine in the blood at certain time intervals after the administration of I^{131}.

In view of the controversy surrounding the subject of a possible relationship between the changes in the morphology and function of the thyroid and an elevated intake of fluoride, it was deemed advisable to make use of these two relatively new methods of measuring thyroid function in fluorosed rats.

Table I shows the I^{131} distribution in fluorosed rats and their controls 24 and 48 hours after intravenous administration. The ratios of the percent I^{131} per ml of red cells to plasma are shown at the bottom of the Table.

No differences in the sizes of the thyroids of the fluorosed and control animals were observed. The only statistically significant difference in the I^{131} distribution in fluorosed and normal rats was in the percent uptake of I^{131} by the thyroid at 24 hours, $p < 0.05$. The red cell to plasma ratios were comparable for the controls and fluorosed rats at both time intervals studied. The thyroidal uptake of I^{131} varied widely within both the fluorosed and control groups at both of the time intervals studied. This wide spread in individual values is to be expected in view of the work of other investigators who have found that the uptake of I^{131} in animals of the same age and dietary history is quite variable, Hamilton et al[2] and Taurog[18]. Consequently, results obtained using small groups of animals are to be viewed with caution, unless large differences in the thyroid uptake of I^{131} are noted between control and treated animals.

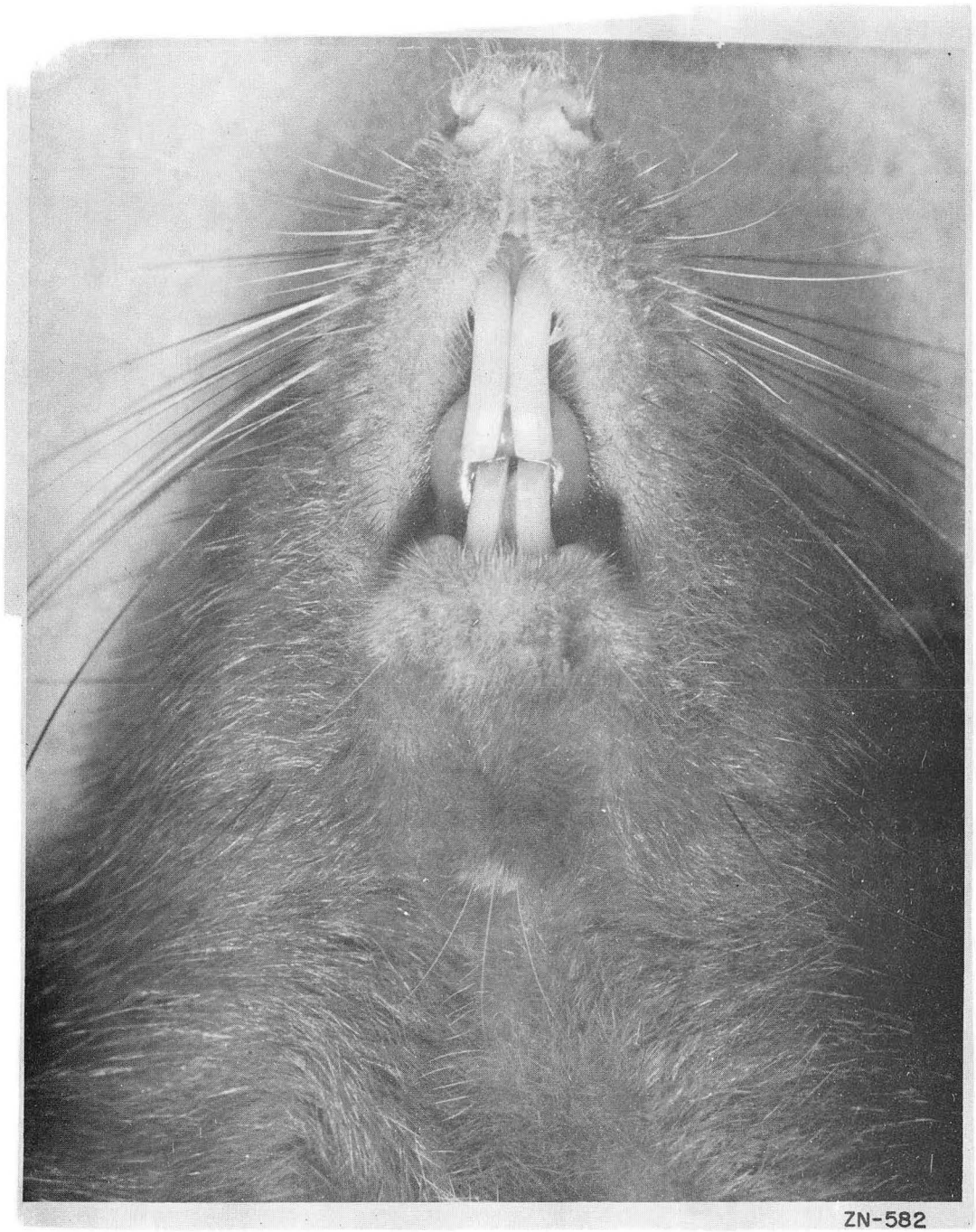

Fig. 1

The head of a female Long-Evans rat after 4.5 months on a dietary supplement of 20 mg of fluoride per kilo per day. (x2)

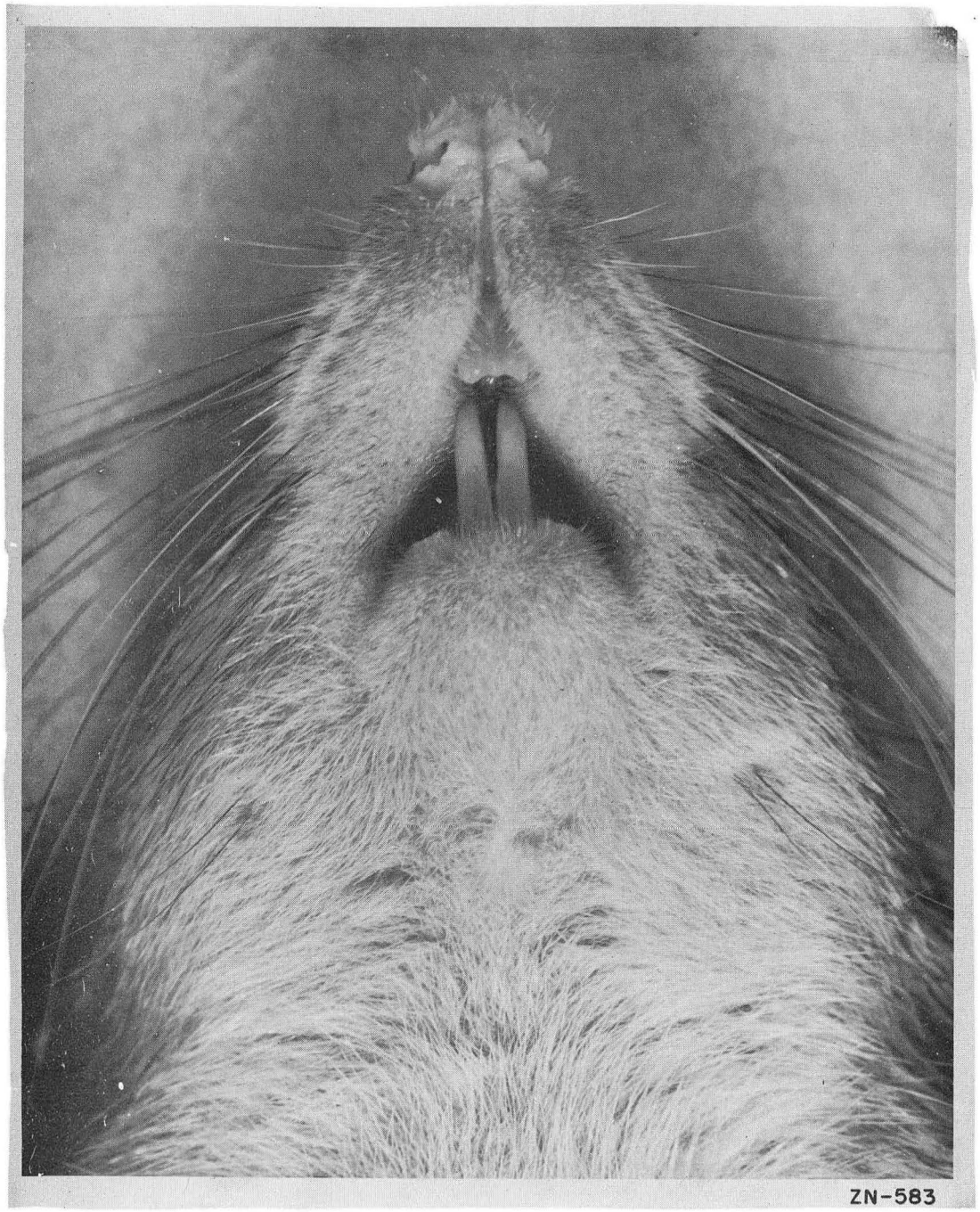

Fig. 2

The head of a normal female rat of the same age and strain as the animal shown in Figure 1. (x2)

Besides the wide fluctuations in the individual determination, the I^{131} uptakes of all the rats, both treated and controls, were lower for both of the time intervals studied than the values reported in the literature. Hamilton et al[2] found an uptake in rats on a diet comparable to "Diet 14" (see Appendix I in last quarterly report) of about 28 percent of a tracer dose of I^{131} 24 hours after intravenous administration. Taurog and Chaikoff[20] found that the thyroids of male rats of the Long-Evans strain on a similar diet accumulated 18 percent at 14 hours and 12 percent at 50 hours. There seems to be a relationship between the iodine intake in the diet and the ability of the thyorid to accumulate a tracer dose of I^{131}; the greater the daily iodine intake the lower the thyroidal uptake of I^{131} (Taurog and Chaikoff[19]). "Diet 14" contains 0.84 micrograms of added iodine per gram of food. The semisynthetic diet used in the experiments described here on fluorosed rats and their controls (see Appendix II in last quarterly report) contained 1.24 micrograms of added iodine per gram of food, or nearly 50 percent more iodine than "Diet 14", which should explain in part the low I^{131} accumulation by the thyroids of the rats that were fed the semisynthetic diet.

Taurog and Chaikoff[19] found that while the I^{131} content of the thyroid after the administration of a tracer dose was quite variable, the thryoxine content of these glands was a fairly constant proportion of the total iodine content, usually about 31 percent of the thyroidal iodine. They also found that the level of protein-bound iodine in the plasma depended on the thyroxine content of the thryoid gland. From the above observations, it would seem that the protein-bound iodine of the plasma of which the red cell: plasma ratio is a measure is actually a more reliable index of thyroid function than the thyroidal uptake of I^{131}.

Since the difference in the uptake of I^{131} by the thyroids of fluorosed and control animals was statistically significant at only one of the time intervals investigated and the red cell: plasma ratios were comparable at both time intervals, it seems safe to say that a prolonged intake of fluoride at the level employed in these experiments did not produce any untoward effects on the function of the thyroid.

(c) The metabolism of F^{18} in mature control rats and chronically fluorosed rats

The distribution of F^{18} in fluorotic rats and their normal controls 1, 4 and 9 hours after an intravenous injection of F^{18} is shown in Tables II and III. The F^{18} concentrations in the blood and soft tissues are in general slightly higher in the fluorosed animals than in the controls, a result that might be anticipated from the results obtained 15 minutes after administering F^{18} plus fluoride carrier intravenously (see Table VI).

The urinary excretion of F^{18} by the fluorosed rats was slightly more rapid than in the controls; however, since the urine was pooled for each cage of four or five animals, no conclusions can be drawn concerning quantitative differences in urinary excretion between the treated and control groups.

It was anticipated from the work of Savchuck and Armstrong[13] that the F^{18} uptake by the skeletons of the older control animals would be considerably lower than for the young adult animals discussed in Experiment (1). They found that the fluoride retention of mature rats was much lower than

Table I

The Distribution of I^{131} in Normal Mature Rats and in Rats Chronically Intoxicated with Fluoride, 24 and 48 Hours After Intravenous Administration. Values are Expressed in percent of Administered Dose and are Corrected for Deviation of Recovery from 100 percent. Each Rat Received 3.5 μc of I^{131}.

	24 hours				48 hours			
	Control		Fluorosed		Control		Fluorosed	
	% per organ	% per gram	% per organ	% per gram	% per organ	% per gram	% per organ	% per gram
Thyroid	12.0		7.50		8.62		6.65	
RBC	0.47	0.06	0.48	0.06	0.09	0.01	0.06	0.009
Plasma	0.79	0.09	0.65	0.08	0.36	0.04	0.26	0.04
Skin	5.40	0.13	3.59	0.11	2.48	0.07	2.64	0.08
G. I. Tract	5.04		3.57		1.98		0.80	
Carcass	8.31		9.32		3.40		2.33	
Urine	64.9		71.2		73.3		81.4	
Feces	3.18		3.59		9.56		5.87	
RBC: Plasma	0.61		0.70		0.26		0.25	

that of young animals. The values they obtained were 53 percent for the initial fluoride retention for young animals and 36 percent retention for older animals. The values found in the experiments reported here at the longest time interval investigated, 9 hours after injection, were 56 percent retention for the young animals and 48.5 percent retention for the older rats, indicating that very little of the fluoride deposited in the skeleton at this time would be subsequently excreted.

The F^{18} contents of the skeletons of the fluorosed rats were quite similar to the control values for the three time intervals studied. The greatest difference in skeletal F^{18} deposition, 9.4 percent, occurred 4 hours after the injection. This was also the only time interval at which there was a significant difference in the F^{18} concentrations of the leg bones of the two groups of animals. The control values were higher than the corresponding values for the fluorosed rats. Further, although the skeletal depositions and bone concentrations of the older control animals were lower than the corresponding values found for the young adults (see Tables IV and V) the pattern of uptake remained the same.

The skeletons of the older control animals accumulated F^{18} and the F^{18} concentration of the leg bones increased during the first 4 hours after the injection, until peak bone accumulation and concentration were reached. During the ensuing 5 hours both the F^{18} content of the skeleton and the F^{18} concentration of the leg bones decreased slightly; about 9.2 percent of the peak values were lost during this 5 hour interval. It will be recalled that this same pattern was seen in the young adult animals, although the peak concentration and skeletal accumulation were higher in the younger rats and the

Table II

The Distribution of F^{18} in Rats Chronically Intoxicated with Fluoride 1, 4 and 9 Hours after Intravenous Administration. Values are Expressed in percent of Administered Dose and are Corrected for Deviation of Recovery from 100 percent. The Animals Received 20 mg of Fluoride as NaF per Kilo Body Weight Daily for an Average of 4 1/2 Months.

	1 hour		4 hours		9 hours	
	% per organ	% per gram	% per organ	% per gram	% per organ	% per gram
Molars	0.13	0.94	0.17	1.39	0.16	1.15
Incisors	0.95	1.85	1.23	2.56	1.12	2.00
Skeleton	40.6	2.04	43.4	2.18	48.1	2.22
Muscle	8.30	0.08	1.06	0.01	0.61	0.006
Skin	2.91	0.08	0.45	0.01	0.60	0.02
Blood	1.43	0.09	0.25	0.015	0.12	0.008
Cartilage	-	0.28	-	0.16	-	0.15
Liver	0.89	0.10	0.12	0.01	0.04	0.005
Kidney	0.31	0.19	0.05	0.03	0.03	0.02
Stomach	0.12	0.07	0.02	0.01	0.015	0.009
Stom. Cont.	0.06	-	0.02	-	0.007	-
Sm. Int.	0.27	0.08	0.05	0.01	0.02	0.007
Sm. Int. Cont.	1.81	-	0.28	-	0.04	-
Lg. Int.	0.07	0.06	0.05	0.04	0.01	0.01
Lg. Int. Cont.	0.24	-	1.41	-	1.53	-
Cecum and Cont.	0.48	-	1.16	-	1.09	-
Sal. Gland	-	0.11	-	0.02	-	0.009
Lac. Gland	-	0.09	-	0.01	-	0.007
Lymph Node	-	0.07	-	0.01	-	0.009
Thyroid	0.002	-	<0.001	-	<0.001	-
Adrenal	0.006	-	<0.001	-	<0.001	-
Urine	38.4	-	40.4	-	42.8	-
Feces	0.30	-	1.87	-	0.85	-
Balance	2.83	-	8.04	-	3.02	-

Table III

The Distribution of F^{18} in Normal Mature Rats 1, 4 and 9 Hours After Intravenous Administration. Values are Expressed in percent of Administered Dose and are Corrected for Deviation of Recovery from 100 percent.

	1 hour		4 hours		9 hours	
	% per organ	% per gram	% per organ	% per gram	% per organ	% per gram
Molars	0.26	1.68	0.26	1.64	0.18	1.33
Incisors	1.18	2.72	1.40	3.12	1.38	3.01
Skeleton	47.2	2.32	52.8	2.74	48.5	2.46
Muscle	6.81	0.06	0.88	0.008	0.47	0.004
Skin	3.58	0.08	0.62	0.01	0.57	0.01
Blood	1.41	0.08	0.15	0.008	0.08	0.004
Cartilage	-	0.20	-	0.12	-	0.17
Liver	0.48	0.06	0.06	0.008	0.04	0.005
Kidney	0.26	0.15	0.04	0.02	0.02	0.01
Stomach	0.08	0.06	0.02	0.01	0.009	0.008
Stom. Cont.	0.12	-	0.01	-	0.003	-
Sm. Int.	0.17	0.06	0.03	0.008	0.02	0.004
Sm. Int. Cont.	3.26	-	0.22	-	0.04	-
Lg. Int.	0.06	0.04	0.02	0.01	0.02	0.01
Lg. Int. Cont.	0.19	-	0.23	-	1.65	-
Cecum and Cont.	0.42	-	0.94	-	0.95	-
Sal. Gland	-	0.08	-	0.009	-	0.003
Lac. Gland	-	0.07	-	0.01	-	0.003
Lymph Node	-	0.06	-	0.009	-	0.005
Thyroid	0.001	-	<0.001	-	<0.001	-
Adrenal	0.004	-	<0.001	-	<0.001	-
Urine	29.1	-	28.5	-	36.0	-
Feces	0.08	-	0.26	-	0.93	-
Balance	5.04	-	13.5	-	10.6	-

decline of these values in the interval from 4 to 9 hours after injection was greater. On the other hand the leg bones of the fluorosed rats did not show this same pattern, but instead continued to accumulate F^{18} slowly after the first hour so that at 9 hours the values obtained for the fluorosed animals were the same as those obtained for their controls.

Table IV gives a comparison of the F^{18} uptake of the osseous and dental samples taken from the young adult animals (Experiment 1) and from the fluorosed rats and their mature controls, Table V compares the F^{18} concentrations in these samples and Table VI compares the fresh weights of the same samples.

It is of interest to note that the mandibles of the fluorosed rats and their mature controls show no differences in F^{18} concentration or total F^{18} uptake at any of the time intervals investigated. The fresh weight of the mandibles of the flourosed rats was not significantly different from the fresh weight of the control mandibles. This is in sharp contrast to the leg bones for which there were found no differences in fresh weights, but significant differences in F^{18} content and concentration at 1 and 4 hours.

The fresh weight of the molars of the mature control animals was comparable to the molar weight of the young adult animals. It was also found that the weight of the fluorosed molars was less than that of their controls. However, if the molar weights of the fluorosed rats are compared with those of the young adult rats the difference is not statistically significant. Further, the molars of the fluorosed rats were easy to remove and were almost always removed intact, while some of the roots of the mature control molars remained embedded in the mandibles and maxillae. Therefore it is reasonable to assume that the molars of the mature controls actually weighed more than the value recorded in Table VI would indicate. It was also noted that the root structure of the fluorosed molars was not so extensive as that of their controls. Other investigators have shown that prolonged fluoride feeding does not affect either the appearance or strength of adult teeth, but that it does retard the growth and eruption of immature teeth (Roholm[12]). If the enamel of teeth that do not grow from persistent pulp is laid down during a period of fluoride feeding, it is poorly calcified and wears away with functional attrition relatively easily (Slavsgold[17]). The molars of the fluorosed animals and their controls were not fully mature when the fluoride feeding was initiated, since it has been shown that the molars of the rat do not mature until approximately 125 days of age (Schour and Massler[14]). Under the influence of the fluoride supplement the final maturation of the molars was very likely retarded and their calcification faulty, which would allow them to wear excessively. Excessive wear and retardation of root growth would account for the observed differences in molar weight.

The values obtained for the F^{18} contents and F^{18} concentrations of the molars of both groups of older animals were considerably lower than the corresponding values found for the young adult animals. This difference between older and younger animals in their ability to accumulate F^{18} seems to be a function of the decrease in pulpal activity and of the calcification of the molars with increasing age (Schour and Massler[14]).

Table IV

A Comparison of the F^{18} Content of the Molars, Incisors, Mandibles and Leg Bones of Young Adult Rats, Fluorosed Rats and Their Mature Controls 1, 4 and 9 Hours After Intravenous Administration. Values are Expressed in percent of Administered Dose and are Corrected for Deviation of Recovery from 100 percent. Standard Error and "P" Values are Calculated as Shown in Appendix III.

Tissue	Young Adult	P*	Mature Control	P**	Fluorosed
1 hour					
Molars	0.44 ± 0.04	<0.01	0.26 ± 0.02	<0.01	0.13 ± 0.02
Incisors	1.02 ± 0.07	>0.10	1.18 ± 0.08	<0.05	0.95 ± 0.04
Mandible	2.56 ± 0.04	<0.01	2.00 ± 0.11	>0.10	1.96 ± 0.05
Leg Bones	5.05 ± 0.12	<0.01	3.78 ± 0.11	<0.05	3.16 ± 0.17
4 hours					
Molars	0.41 ± 0.02	<0.01	0.26 ± 0.008	>0.10	0.17 ± 0.04
Incisors	1.13 ± 0.08	>0.10	1.40 ± 0.11	>0.10	1.23 ± 0.13
Mandible	2.97 ± 0.11	<0.01	2.04 ± 0.12	>0.10	2.11 ± 0.08
Leg Bones	5.87 ± 0.09	<0.01	4.34 ± 0.22	<0.05	3.49 ± 0.21
9 hours					
Molars	0.30 ± 0.03	<0.01	0.18 ± 0.01	>0.10	0.16 ± 0.025
Incisors	1.33 ± 0.07	>0.10	1.38 ± 0.07	>0.10	1.12 ± 0.13
Mandible	2.39 ± 0.12	<0.10	2.10 ± 0.08	>0.10	2.38 ± 0.16
Leg Bones	4.94 ± 0.14	<0.01	3.97 ± 0.13	>0.10	3.81 ± 0.15

*P compares young adults and mature controls.

**P compares mature controls and fluorosed animals.

Table V

The Concentration of F^{18} in the Molars, Incisors, Mandibles and Leg Bones of Young Adult Rats, Fluorosed Rats and Mature Controls 1, 4 and 9 Hours After Intravenous Administration. Values are Expressed in percent of Administered Dose per Gram of Wet Tissue. Standard Errors and "P" Values are Calculated as Shown in Appendix III.

Tissue	Young Adult	P*	Mature Control	P**	Fluorosed
			1 hour		
Molars	3.04 ± 0.17	<0.01	1.68 ± 0.045	<0.01	0.94 ± 0.096
Incisors	3.14 ± 0.14	>0.10	2.72 ± 0.18	<0.01	1.85 ± 0.068
Mandible	5.08 ± 0.33	<0.01	2.94 ± 0.21	>0.10	2.94 ± 0.14
Leg Bones	3.79 ± 0.12	<0.01	2.32 ± 0.19	>0.10	2.04 ± 0.16
			4 hours		
Molars	2.75 ± 0.13	<0.01	1.64 ± 0.18	>0.10	1.39 ± 0.37
Incisors	3.98 ± 0.32	<0.10	3.12 ± 0.22	>0.10	2.56 ± 0.385
Mandible	5.96 ± 0.19	<0.01	3.14 ± 0.22	>0.10	3.04 ± 0.18
Leg Bones	4.33 ± 0.12	<0.01	2.74 ± 0.225	<0.10	2.18 ± 0.13
			9 hours		
Molars	2.50 ± 0.13	<0.01	1.33 ± 0.07	>0.10	1.15 ± 0.19
Incisors	3.61 ± 0.23	<0.10	3.01 ± 0.185		
Mandible	4.61 ± 0.42	<0.05	3.22 ± 0.23	>0.10	3.54 ± 0.306
Leg Bones	3.31 ± 0.12	<0.01	2.46 ± 0.18	>0.10	2.22 ± 0.107

*P compares yound adults and mature controls.

**P compares mature controls and fluorosed animals.

Table VI

The Mean Fresh Weight of the Teeth, Mandibles and Leg Bones of Young Adult, Mature and Fluorosed Rats.

	Young Adults	P*	Mature Controls	P**	Fluorosed
Molars	0.14 ± 0.004	>0.10	0.15 ± 0.003	<0.01	0.13 ± 0.004
Incisors (Includes pulp)	0.33 ± 0.01	<0.01	0.44 ± 0.005	<0.01	0.52 ± 0.02
Mandible	0.51 ± 0.01	<0.01	0.67 ± 0.01	>0.10	0.68 ± 0.02
Leg bones (Includes marrow)	1.35 ± 0.04	<0.01	1.64 ± 0.06	>0.10	1.63 ± 0.04

*P compares young adults and mature controls.

**P compares mature controls and fluorosed animals.

The F^{18} contents and concentrations of the molars of the fluorosed rats and their controls were comparable except at the 1-hour interval. The lower F^{18} accumulation by the fluorosed molars at this time may be due in part to the inability of the enamel and exposed dentin of the rat molar to adsorb F^{18} from the saliva efficiently after a prolonged exposure to fluoride in the diet. This explanation is amplified by the findings of others. Volker et al[21] found that enamel and dentin adsorbed radiofluorine by a surface reaction according to the Freundlich adsorption isotherm equation in which case an equilibrium is set up between the material in solution and the material adsorbed on the surface. Further adsorption ceases when the surface of the crystal lattice is covered. Gol'dberg[1] found that tooth surfaces treated with NaF could still adsorb some additional fluoride but that the additional fluoride was very quickly leached.

Since the rat incisor grows from persistent pulp, it was not surprising that the incisors of the mature animals, although heavier than those of the young adults, accumulated F^{18} to the same extent as did the younger animals. The greater weight of the incisors of the mature rats would account for the differences in F^{18} concentration observed.

The F^{18} accumulated by the incisors of the fluorosed and mature rats was comparable at the 4 and 9 hour intervals. There was a significant difference noted at 1 hour after the injection, in which case the fluorosed incisors had not taken up as much F^{18} as had those of the controls. This initial lag in the ability of the fluorosed incisors to accumulate F^{18} is apparently overcome in the next 3 hours, owing perhaps to the slightly elevated level of F^{18} in the blood of these animals. The lower F^{18} content of the fluorosed incisors at 1 hour, despite the elevated blood level, could be due to the inability of the fluorosed tooth surfaces to adsorb and retain F^{18} from the saliva, as was postulated for the molars.

The greater weight of the incisors of the fluorosed rats would help to account for the differences observed in the F^{18} concentrations of the fluorosed and mature control incisors.

The results of the tracer studies described here on the distribution of F^{18} in mature adult and fluorosed rats and those discussed in the preceding sections on young adult rats have, except for the one experiment on lactating rats, been corrected for deviations of recovery of the injected F^{18} from 100 percent. The actual experimental recoveries for individual animals varied from 76 percent to 120 percent of the administered dose, owing to pooling of excretions and loss of material in the meat grinder used to prepare the carcasses. The average recoveries for each experimental group of three to five rats did not vary quite so much and were generally about 85 percent to 95 percent of the administered dose.

Radioautography With F^{18}

Figures 3ab, 4ab and 5ab show the best examples obtained of the cut silvered surfaces of the distal ends of the femurs and the corresponding F^{18} radioautographs prepared from three groups of animals that were sacrificed one hour after the intravenous injection of F^{18}. The groups employed were: (a) young adult females 70 days old, (b) mature females 200 days of age that had been maintained on a semisynthetic diet and (c) fluorosed females 200 days of age that had been fed the semisynthetic diet plus 20 mg of fluoride per kilo of body weight daily.

Unfortunately the relative darkening of the films could not be used to compare quantitatively the radioautographs obtained from the three groups of animals for several reasons: (1) The amount of F^{18} administered to each rat in any given group varied widely owing to the variations in F^{18} yields from any given bombardment, (2) There was quite a large variation in the amount of F^{18} accumulated in the bones of the individual animals in any given group and (3) The amount of F^{18} accumulated by the bones of the young animals was considerably greater than for the older animals, either fluorosed or mature controls. An attempt was made to adjust the length of exposure of the films to compensate for the above mentioned factors, however, the calculations made were quite rough and hurried to avoid excessive loss of F^{18} due to decay and to avoid autolysis of the bones themselves.

On comparison of the radioautographs no alterations in the general distributional pattern of F^{18} in the bones are to be found either as a result of advancing age or previous exposure to dietary fluoride. Several generalizations on the deposition of F^{18} in the bones of the rat can be made, however, from the inspection of the radioautographs. The area of greatest F^{18} deposition one hour after its administration was in the region of the epiphyseal cartilage, most probably in the most recently calcified bone just below the epiphyseal plate. The spongy bone of the epiphysis and of the upper end of the diaphysis, especially on the front side, were the next most heavily blackened areas. This was not quite so apparent in the radioautographs made from the young bones as it was in the mature bones. Despite the poor resolution obtainable from such relatively crude methods as were used in the preparation of these radioautographs, it can be seen that there appears to be little if any F^{18} in the marrow cavity. In the radioautograph of the fluorosed bone and to a lesser extent in that of the young adult bone, there appears to be a small amount of F^{18} in the periosteum and endosteum, but very little in the compact bone.

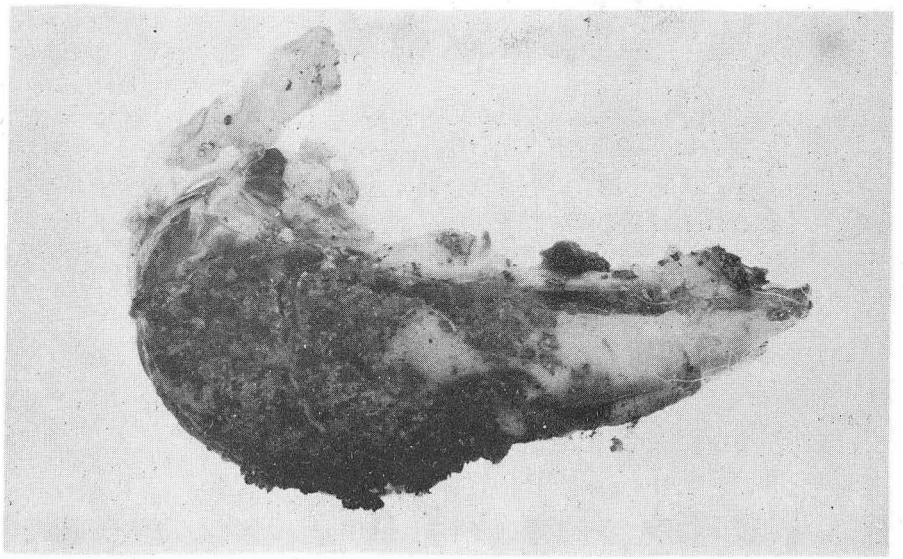

Fig. 3a

The silvered surface of the distal end of the right femur of a young adult female rat, 75 days old. (x6)

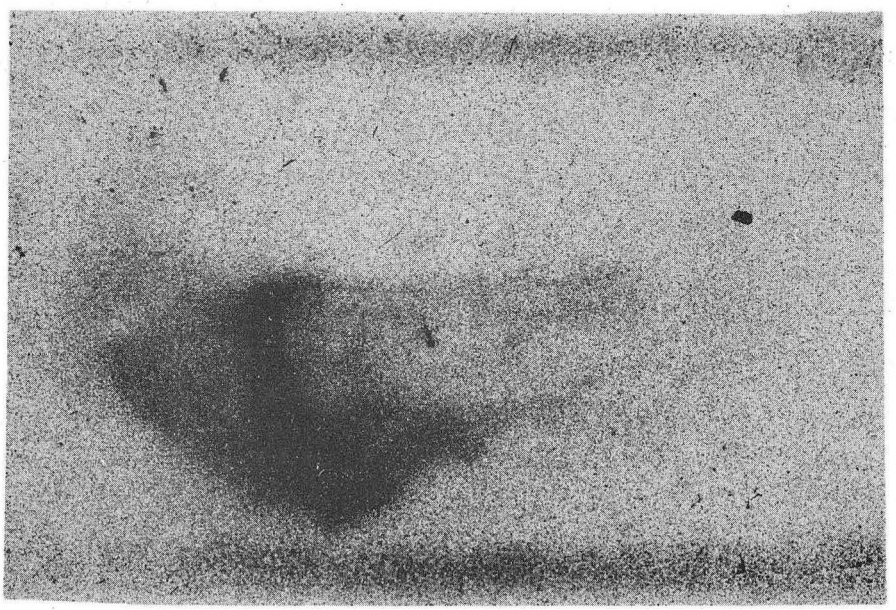

Fig. 3b

The F^{18} contact radioautograph of the bone surface shown in Fib. 3a. (x6)

ZN-586

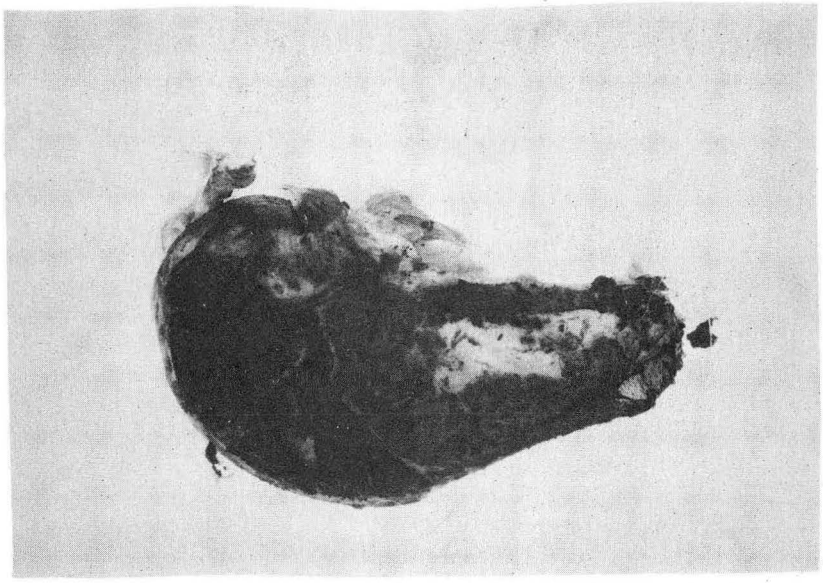

Fig. 4a

The silvered surface of the distal end of the right femur of a mature female rat, (Rat No. 50), 200 days old. (x6)

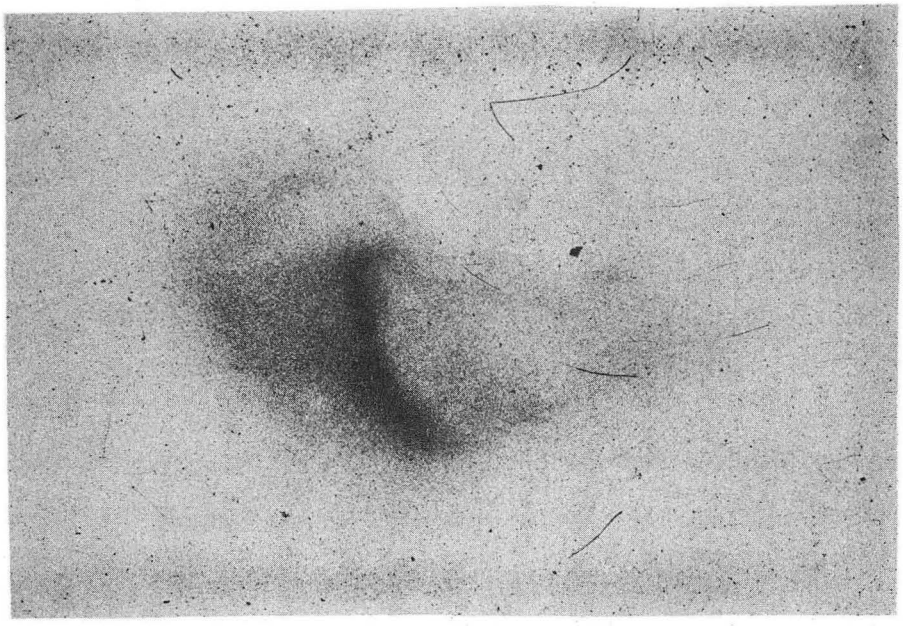

Fig. 4b

The F^{18} contact radioautograph of the bone surface shown in Fig. 4a. (x6)

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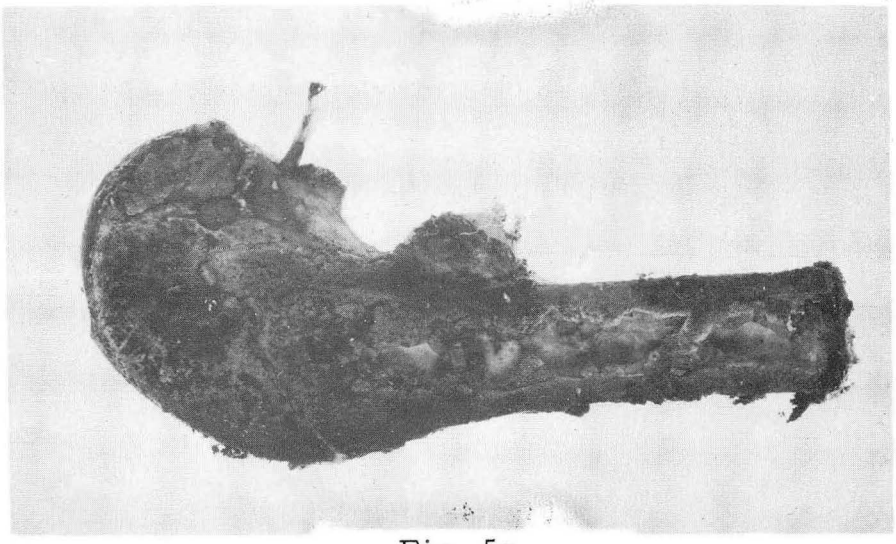

Fig. 5a

The silvered surface of the distal end of the right femur of a rat chronically intoxicated with 20 mg of fluoride per kilo per day for 4.5 months. This animal (Rat No. 30) was the same strain and age as animal No. 50. (x6)

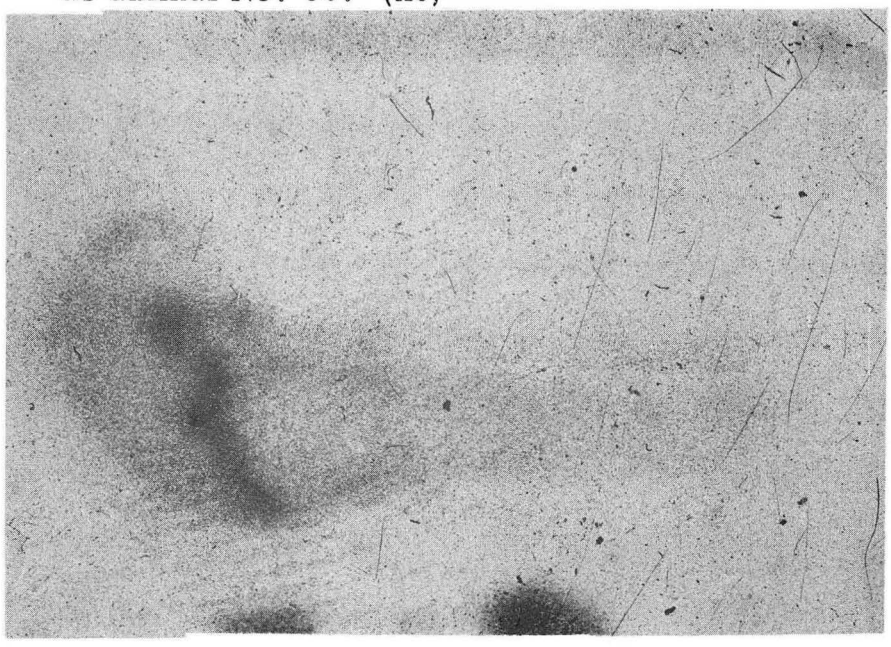

Fig. 5b

The F^{18} contact radioautograph of the bone surface shown in Fig. 5a. (x6)

ZN-584

Histological Findings on Effects of Fluoride on the Rat Femur

Sections were prepared of the distal end of the right femurs of four control rats and four rats maintained on a high fluoride diet for 4.5 months. The sections were stained routinely with hematoxylin and eosin and are shown at low-power magnification. Since such small groups of animals were used, four control and four fluorosed rats, the results obtained on the histological preparations described here can be assumed to be only semiquantitative. Before discussing the findings, it should be stated that of the four fluorosed bones studied, one appeared very nearly normal, one showed complete growth arrest, and the other two lay somewhere between the two extremes. The preparations from the latter two animals showed areas of normal bone growth and areas of growth arrest. On the other hand the control bones showed quite a bit of variation so far as the quality of the marrow was concerned. The marrow of one animal was almost 90 percent cellular and of another only about one-half cellular, again with the others lying somewhere in between the two extremes. The control animals also showed variation in the number of layers of enlarged, degenerating chrondrocytes. However, when the slides were randomized with the labels face down, it was still possible to align them into two distinct groups, with the position of only one control and one treated animal in doubt.

Measurement of the width of the epiphyseal plate taking the average of four separate determinations on each section gave the following results: control, 182 ± 6.9 microns, and fluorosed, 145.4 ± 5.8 microns, with a difference of 37.4 microns between the two groups and a p value of less than 0.01, a surprisingly significent result for the small groups employed. These findings are consistent with the observation mentioned earlier that the femurs of the fluorosed rats were shorter than those of the controls by some 23 mm.

The slides chosen for presentation here are those that were considered approximately the midpoints of each group, both on the basis of histological evidence and on the basis of the width of the epiphyseal plate and femur length.

Figure 6, the control, shows short columns of proliferating chondrocytes with one to two layers of slightly vacuolated enlarged chondrocytes. Marrow tufts are abundant and there are quite a few delicate, short primary trabeculae. Secondary trabeculae are less abundant and are located mainly on the front side of the bone. The marrow appears to be about two-thirds cellular and about one-third adipose. There are no signs of growth in the cortical bone. The general impression is that of slow growth and is approximately normal for rats of this strain and age.

Figure 7, the fluorosed animal, shows a few columns of proliferating chondrocytes. However, some chondrocytes are arranged in clumps and in coniform clusters. From the front side of the bone to the center there are some vacuolated cartilage cells, there is some erosion, and some formation of a few primary trabeculae. From the center to the back side of the bone there is no evidence of bone formation and no erosion. At this point the marrow lies inactive against the cartilage, although there is no bone seal as was seen in another of the animals. There are no sites of really abundant bone formation at any point. There are a few secondary trabeculae on the front side, however. The marrow is predominantly adipose. The general impression is one of almost complete growth arrest resembling advanced age or hypophysectomy.

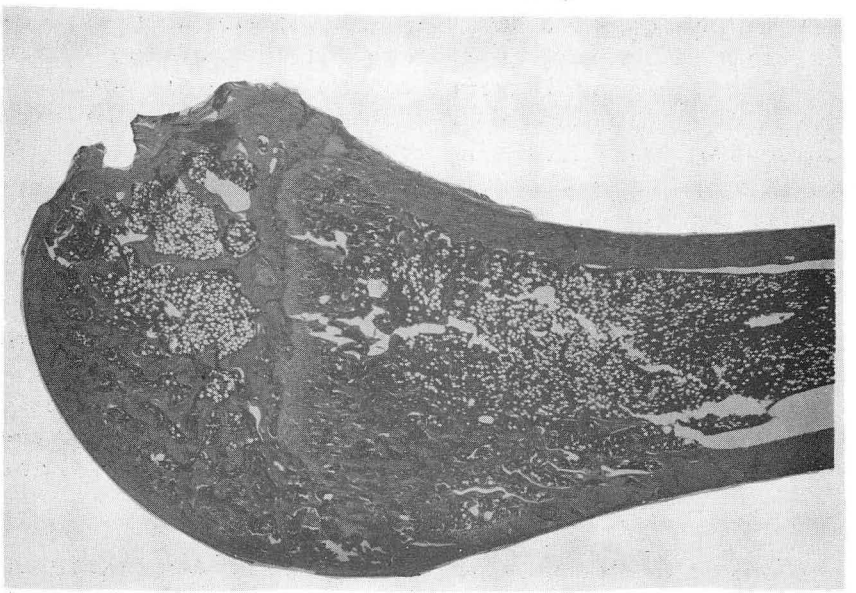

Fig. 6

Histological section of the distal end of the left femur of mature control rat No. 50, stained with hematoxylin and eosin. See text for description. (x12)

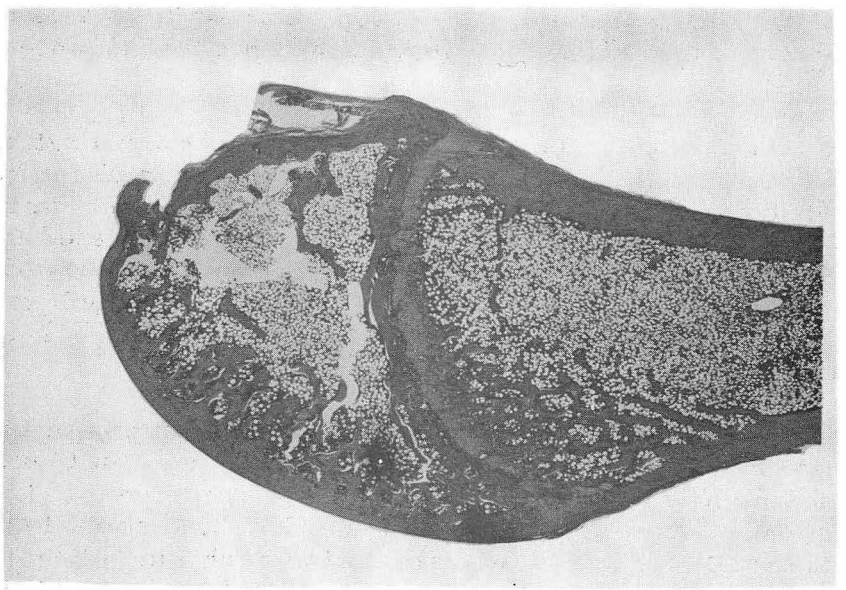

Fig. 7

Histological section of the distal end of the left femur of a fluorosed rat, No. 30, stained with hematoxylin and eosin. See text for description. (x12)

ZN-587

Unfortunately, there was no real correlation obtained among any of the following factors: weight gain, severity of tooth symptoms, femur length and extent of marrow effect.

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HISTOLOGICAL STUDY OF A MONKEY FOLLOWING ALPHA-IRRADIATION

C. W. Asling and J. G. Hamilton

This is to supplement data given in earlier reports concerning a monkey which received At^{211} and was sacrificed two years later.

Gross Appearance of Viscera

No major differences were noted grossly in the viscera of the control or of the astatine-treated monkey with the exception of marked fibrous replacement of thyroid tissue in the latter.

Histology

Lungs. The control shows mild pleural fibrosis and thickening. About half of the alveoli contain an exudate which, however, contains very few cells (chiefly polymorphonuclear elements). There is a spotty distribution of golden-brown pigment in the alveolar walls and occasionally in free macrophages. (This is not found in the aerated portions of the lungs.) The bronchioles show some desquamation and a peribronchial mononuclear cell infiltration. The impression is one of a subacute pneumonitis.

The astatine-treated animal shows an essentially normal lung, except for a scanty alveolar exudate. The pleural fibrosis, pigmentation, round cell infiltration, and bronchiolar desquamation seen in the control are not found here.

Heart. All layers of the heart of the astatine-treated monkey are essentially normal.

Aorta. The aorta of the astatine-treated monkey is essentially normal. In particular no signs of degenerative changes in the intima could be found.

Liver. The organization of the liver of the control is intact. However, the parenchyma shows cloudy swelling and some vacuolar degeneration.

The liver of the astatine-treated monkey shows cloudy swelling, but no vacuolar degeneration. It appears normal otherwise.

Kidney. The kidney of the control is essentially normal. Though some convoluted tubules have a reduced lumen, no marked cloudy swelling is seen.

With the rare exception of a shrunken, fibrosed glomerulus the kidney of the experimental monkey appears normal.

Pancreas. The acini, ducts and islet tissue of the astatine-treated animal appear normal.

Stomach. In the control there is a marked surface autolysis, with breakdown and desquamation of mucous neck cells. Otherwise the stomach appears normal. Parietal cells are less frequently found than is usual in some other species (for example, man, dog) but show their customary position and ersinophilia.

In the astatine-treated animal the epithelium is intact and all elements are structurally normal. Parietal cells are more easily found than in the control. In a section through another level (for which no control is available) the pyloric glands appear normal.

Small Intestine. The surface and villus tips are broken down in the control, but the intact portions appear normal. The lumen is filled with much cellular debris, among which round cells predominate. In addition to much amorphous mucus, there also appear patches of degenerating mucus epithelium (representing some of the shed gastric mucosa).

In the injected monkey the mucosa is intact and normal. A Peyer's patch found in the section appears normal. At another level, the duodenum (including Brunner's glands and an occasional solitary lymphatic nodule) are of normal appearance.

Salivary Gland. The salivary gland of the control is normal with an exception noted below. It is predominantly serous, with some mucous acini.

The greatest portion of the salivary gland of the injected animal is normal and consists of a chiefly serous gland with scattered mucous acini. However, there are sharply circumscribed small areas (the largest being 1/3 the diameter of a low-power field, the total number being about a dozen in a section approximately 1.5 cm^2 in area) whose structure appears abnormal (Fig. 1). (Two similar regions were found in a control section almost double this area.) The cells are markedly basophilic and their nuclei pyknotic. They lie in cords or clusters suggesting an acinar arrangement. All cells are markedly compressed so that the structure appears dense. In two such areas, which lie at a distance from ducts, a central mass of slightly lighter staining reaction suggests a former duct structure with lumen now occupied by cells. The remainder of these cellular aggregates are adjacent to functioning ducts. None show more than slight fibrosis or round cell infiltration.

Lacrimal Gland. The structure of this gland in the control appears normal.

In the astatine-injected monkey only slight differences from the control are seen. The less prominent walls of acinar cells may be related to fixation of the tissue. There is slightly more fibrous tissue in the stroma. The chief difference is a spotty, but minor, interstitial edema.

Spleen. The spleen of the control contains a high proportion of white pulp (and Malpighian corpuscles) to red pulp. The sinusoids of the red pulp are depleted of erythrocytes.

In the injected animal the proportion of red and white pulp is like that in the control. The sinusoids are abundantly filled with erythrocytes.

Lymph Nodes. The cervical nodes of the control are normal. Lymphopoiesis is active. The mesenteric lymph nodes of the control are active. Germinal centers are much more frequent in the cortical nodules of the mesenteric nodes than in the cervical lymph nodes.

The lymph nodes, both cervical and mesenteric, of the injected monkey are within normal limits. Compared to the control, their cortices show less active lymphopoiesis, and their medullary sinusoids are more widely dilated.

Suprarenal. No control sections of the adrenal being available, judgment of any adrenal pathology is being reserved. It may be said that conventional histologic sections showed no alteration in structure or organization of cortex or medulla which suggest radiation damage. Fat stains demonstrated much less cortical lipid than is usually seen in the normal adrenals in other species, a finding which would be consistent with the reduced adrenocortical function in hypothyroidism.

Testis. This organ is of special interest since the possibility of the thyroid deficiency effected by astatine might be reflected in testicular structure. The sections of the control and the experimental animal are not entirely comparable, the former having been embedded in paraffin (with resultant shrinkage of seminiferous tubules and slight disruption of the stroma) while the latter was embedded in nitrocellulose to give maximal visulization of cytologic detail. The differences disclosed in the two might have been more clearly shown had technical conditions been more nearly identical. It is also to be remembered that the control was just entering adolescence, and that the gland, therefore, is not likely to show all the signs of activity to be seen in mature males.

The cross section area seen in the control is two to three times that in the experimental animal, though the section planes are comparable. The majority of seminiferous tubules show at least beginning canalization (Fig. 2), but only a few show active mitosis and spermatogenesis (Fig. 3). In even fewer has this progressed to the development of spermatids. No spermatozoa are seen. In the interstitial tissue Leydig cells are sparse but identifiable. They are large, pale cells with a somewhat foamy cytoplasm and a round deep-staining nucleus whose chromatin is diffusely distributed in coarse granules. Other cells (besides fibrous tissue elements) are seen with a less abundant cytoplasm, somewhat fusiform and not foamy. These may be undifferentiated or immature Leydig cells.

In the epididymis the duct epithelium is proliferating to a slight extent. The cells are of medium height and often appear stratified. Stereocilia are present. The lumen contains some amorphous eosinophilic material, but only very rarely can elements be found whose form suggests that of a spermatozoan.

The impression gained is that of an essentially normal testis, just beginning spermatogenesis and function as a hormone-producing organ, with an epididymis beginning to respond to the hormone.

In the astatine-treated monkey the seminiferous tubules are not canalized (Fig. 4). There are at most two layers of nuclei, and mitotic figures

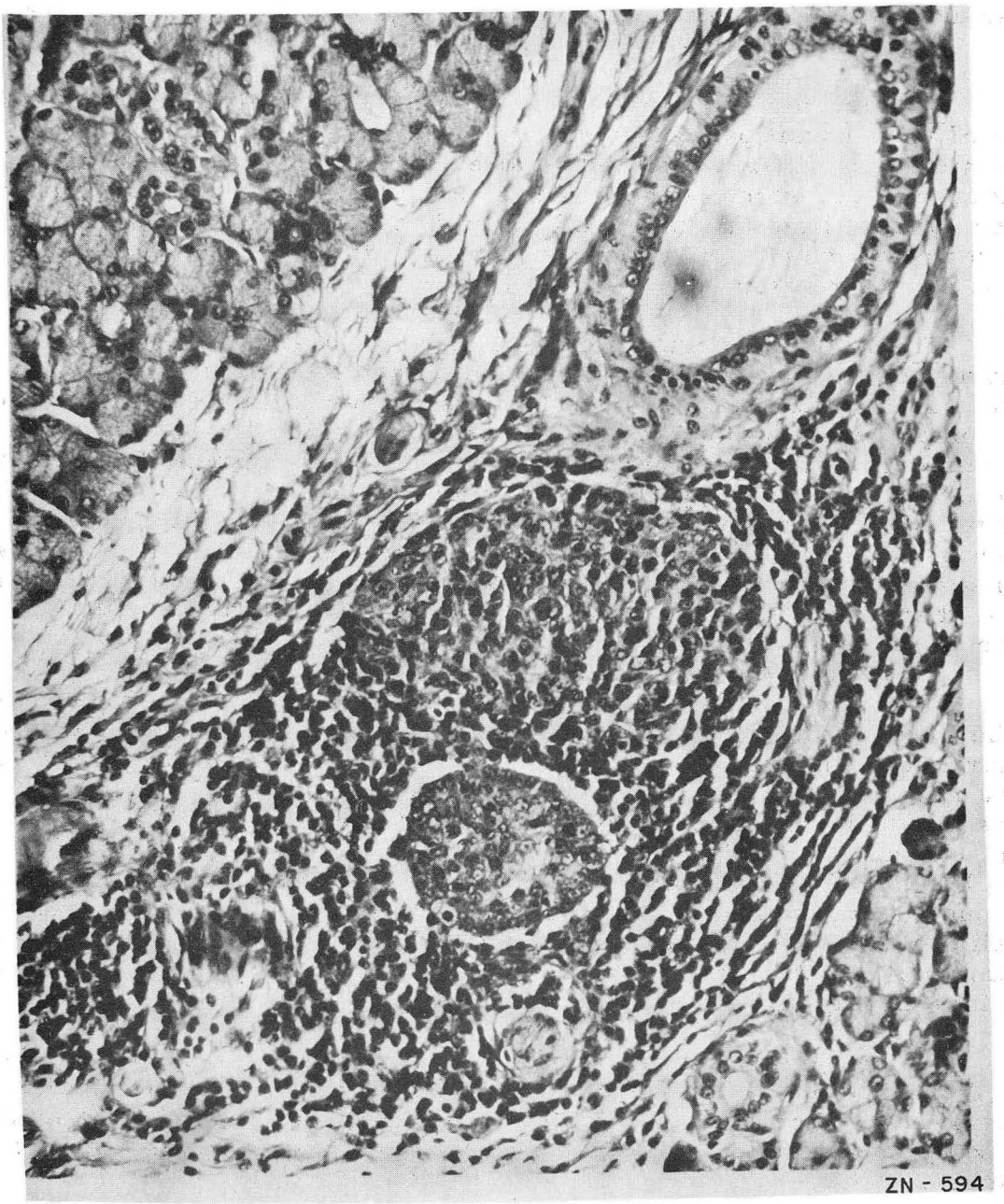

Fig. 1

Salivary gland of astatine-treated monkey. Magn. x 220. Normal tissue at upper left and lower right. Note nodule composed of cords and clusters of shrunken cells in center and lower half of field.

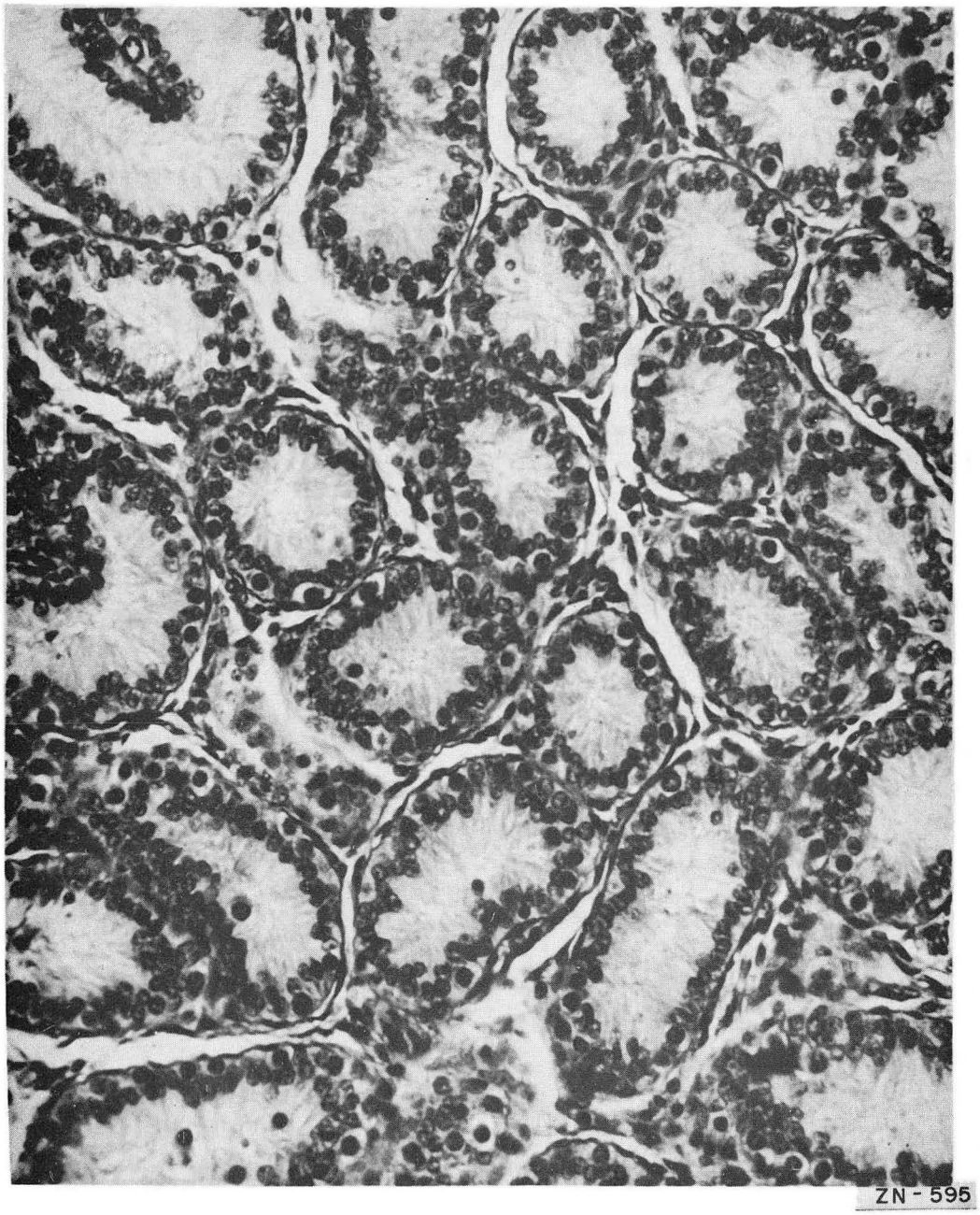

Fig. 2

Testis of control monkey. Magn. x 240. The majority of the tubules have established lumens.

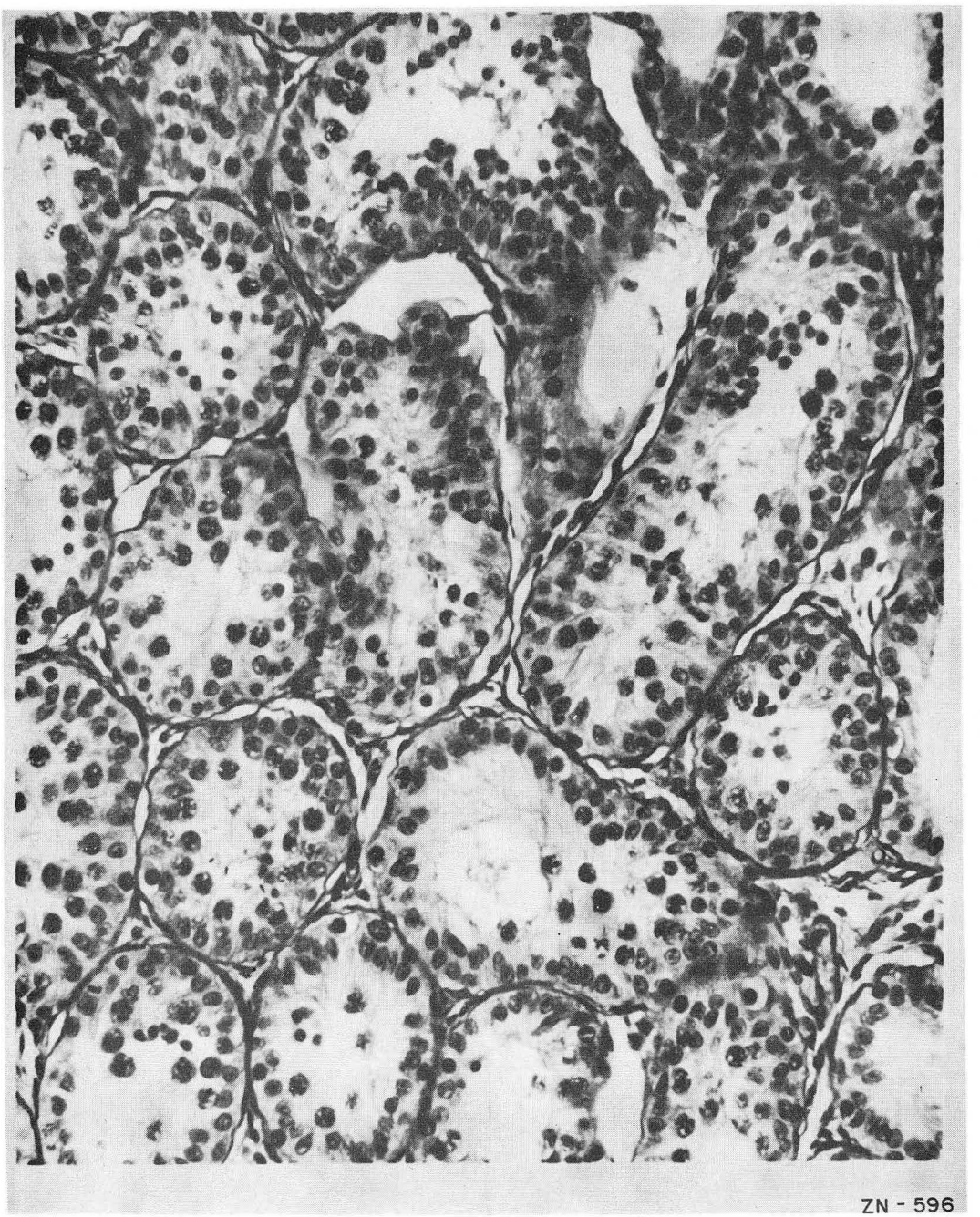

Fig. 3

Another field of testis of control. Magn. x 240. Spermatogenesis is beginning.

are found only after prolonged search. Rare interstitial cells are found, which are even more rarely foamy and large. The majority are somewhat fusiform with fairly homogeneous cytoplasm.

In the epididymis the tubular epithelium is not prolifereating, and is clearly a simple columnar type. The cells possess stereocilia. They are, however, definitely taller than in the control. (This may result from the different embedding techniques, or may be a real difference.) The lumens contain a scanty amorphous eosinophilic material.

The impression gained is that of a small testis as yet not functioning by production of spermatozoa or hormone.

Pituitary. The pituitary of the control monkey cannot necessarily be considered normal, in view of the demonstration previously made that the thyroid of this animal was hyperplastic. However, there were few deviations from expected histology and cytology. Figure 5 is representative of the distribution of anterior lobe acidophils, basophils and chromophobe cells. (Color filters were used in photography to render eosinophils very dark and basophils medium gray). As expected, acidophils predominated amongst the granule-containing cells. Some regions showed virtually all the cells to be acidophilic.

The astatine-treated monkey had a pituitary cytology which deviated markedly from normal and corresponded in all essentials to that resulting from thyroidectomy (Figures 6, 7). Even in the densest areas normal acidophils were sparse, but degranulated eosinophils were common. A few of the characteristic "thyroidectomy basophils" were found -- large colloid-filled cells with sparse cytoplasm and nucleus compressed to one side. Other basophils were found to be degenerating with irregular (even stellate) cell outline and pyknotic nuclei. Finally a new crop of basophils was appearing, such cells being small but with a regular nucleus and light basophilic cytoplasms.

The posterior lobes appeared normal in both animals.

Muscle. No significant changes in the striated skeletal musculature are observed in the treated animal.

Bone and Cartilage. A small segment of the costochondral junction of the control was available for study. The bone and cartilage, per se, appear normal. Some endochondral osteogenesis was in progress, as shown by cartilage proliferation and erosion, with replacement by delicate bony trabeculae. The region is not illustrated, the section plane not being comparable to that in the experimental animal.

In the astatine-treated monkey a marked difference is seen in that endochondral osteogenesis is almost completely arrested (Fig. 8). Few columns of proliferating chondrocytes are seen. There is very little enlargement and vacuolation of cartilage cells near the marrow junction. Virtually no capillary erosion is occurring and no bony trabeculae are being formed. Instead, the cartilage is sealed from the marrow by a transverse lamina of bone. This lamina is identical with that described by Erdheim (Beitr. path. Anat. 62:302, 1916) in the costochondral junction of a human being suffering a growth arrest due to pituitary hypofunction. It has since been described histologically in skeletal growth arrest from several causes, and probably corresponds to the

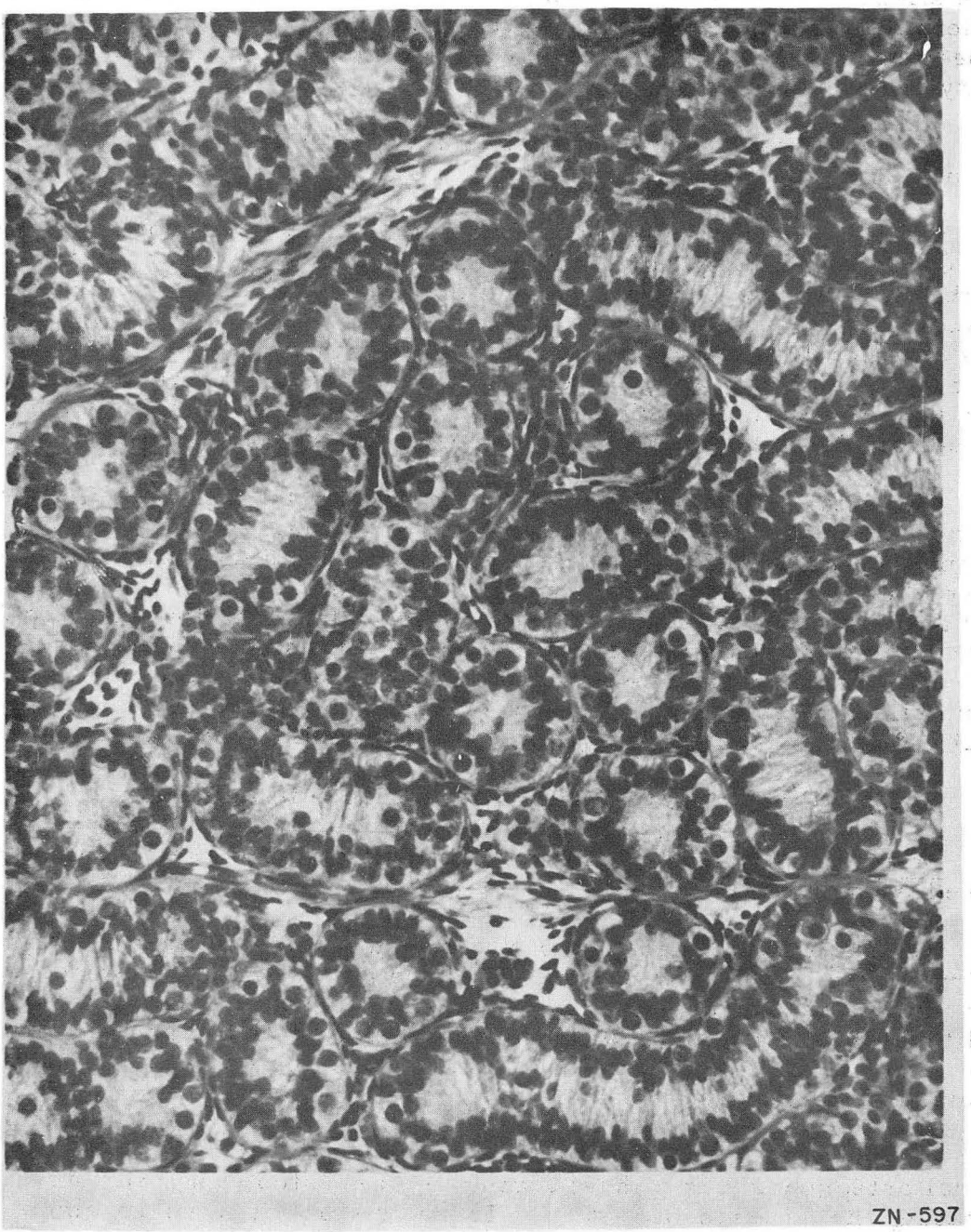

Fig. 4

Testis of astatine-treated monkey. Magn. x 240.
No canalization of tubules, no spermatogenesis.

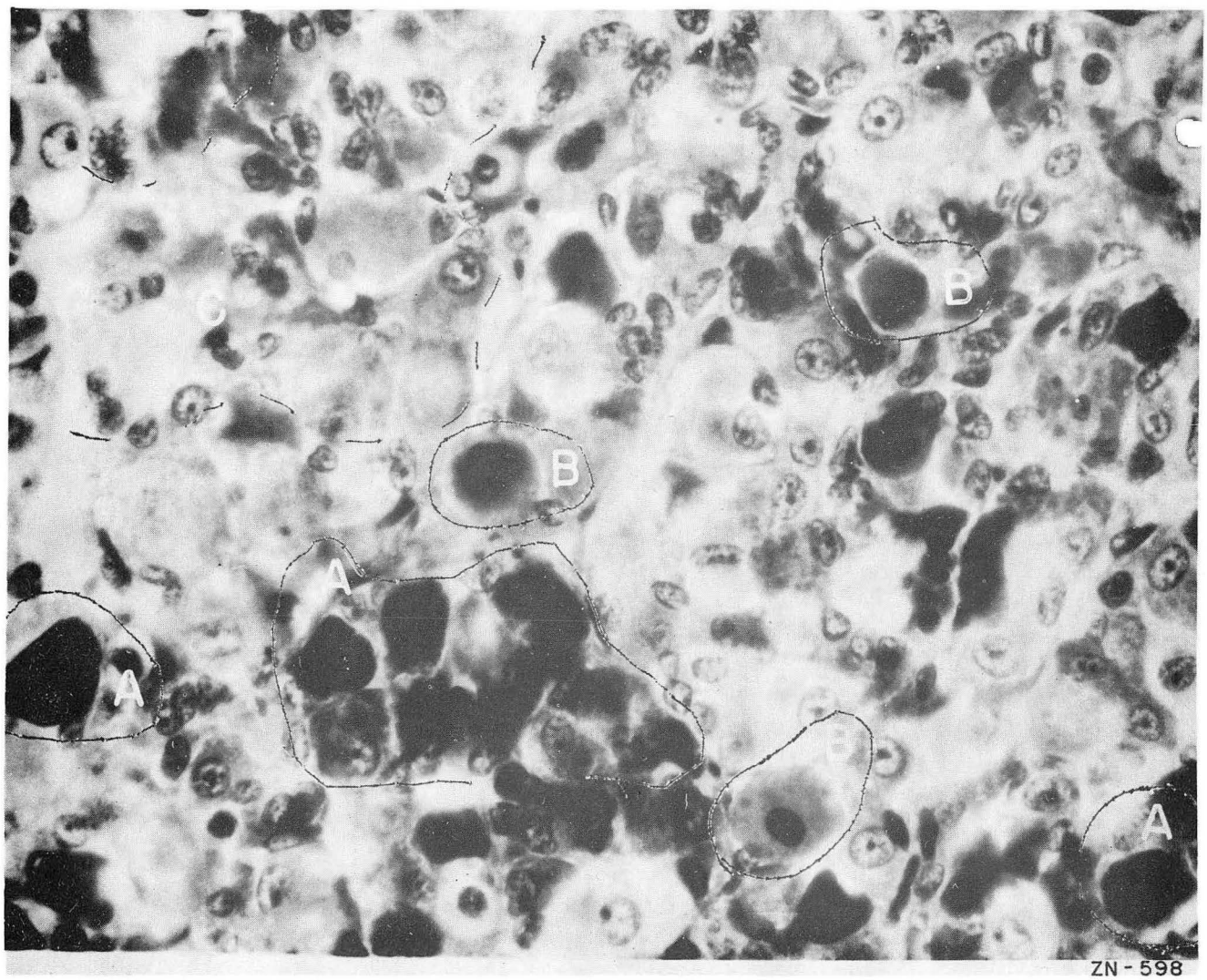

Fig. 5

Pituitary (anterior lobe) of control monkey. Magn. x 480.
A, A, A = acidophils (rendered very dark in photograph).
B, B, B = basophils (medium gray cytoplasm, dense nucleus).
C = chromophobes, which represent majority of cells seen here.

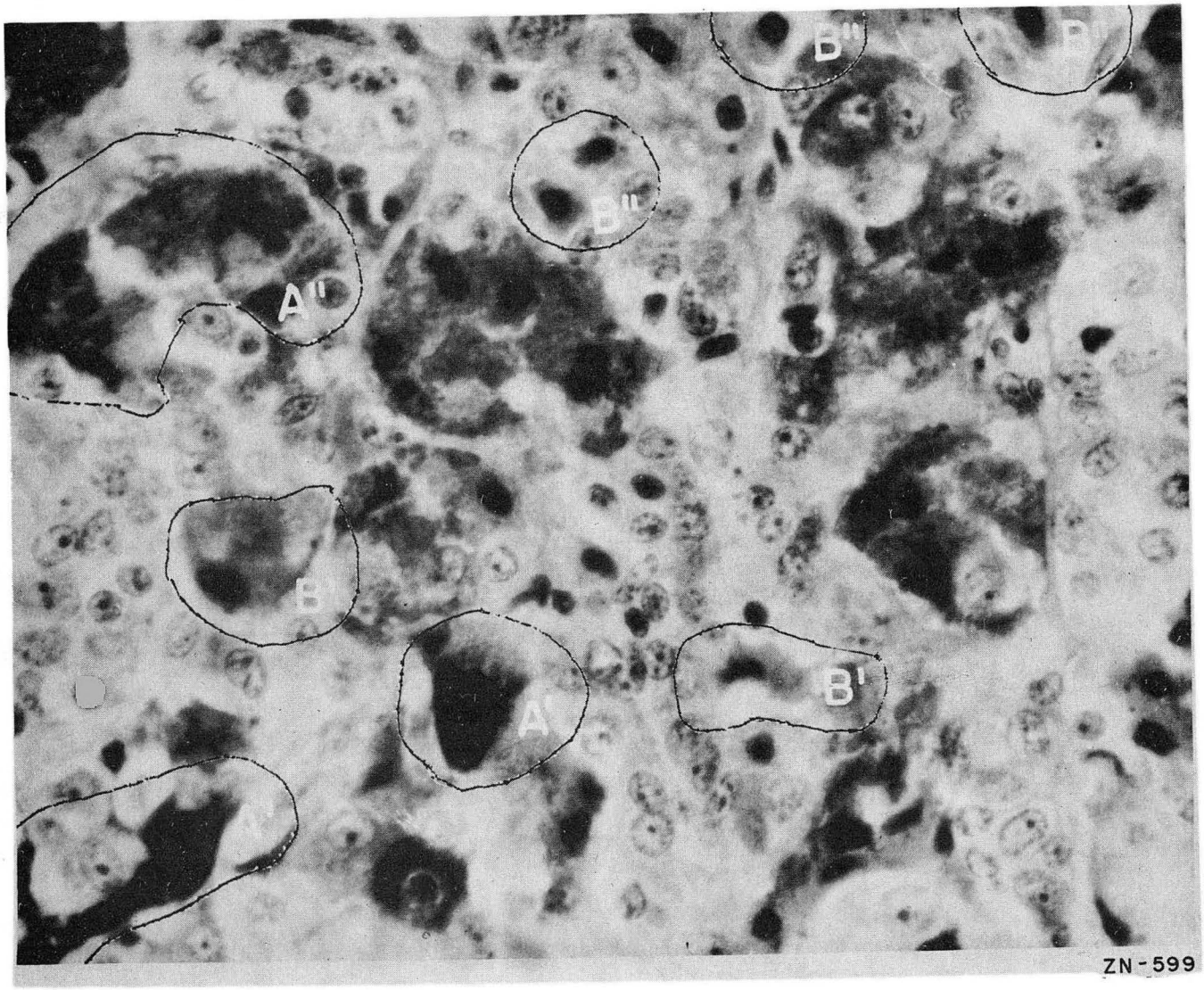

Fig. 6

Pituitary of astatine-treated monkey. Magn. x 480. A'A' = acidophils of structure within normal limits; even in this field, where they were most numerous, they are less common than in control. A'' = degranulating acidophils, common in this field. B'B' = degenerating basophils - irregular contour, pyknotic nuclei. B''B''B'' = a few of the considerable crop of small new basophils.

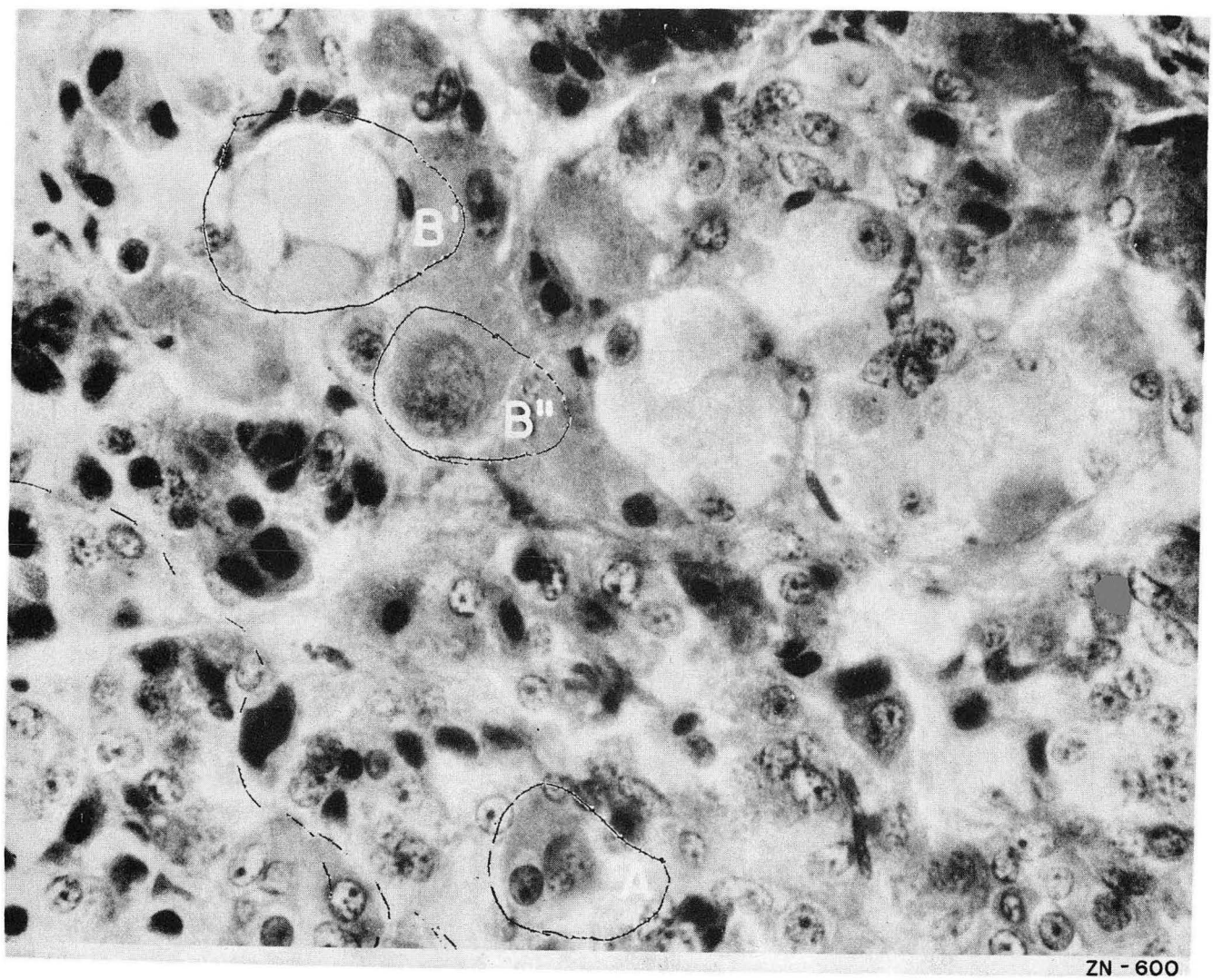

Fig. 7

Another field of pituitary of astatine-treated monkey. A = degranulated acidophil. B' = "thyroidectomy basophil", note large multiocular colloid and nucleus compressed to one side. B'' = a very active basophil, as evidenced by the large Golgi apparatus (seen in negative image as a delicate light net above nucleus). C = chromophobes.

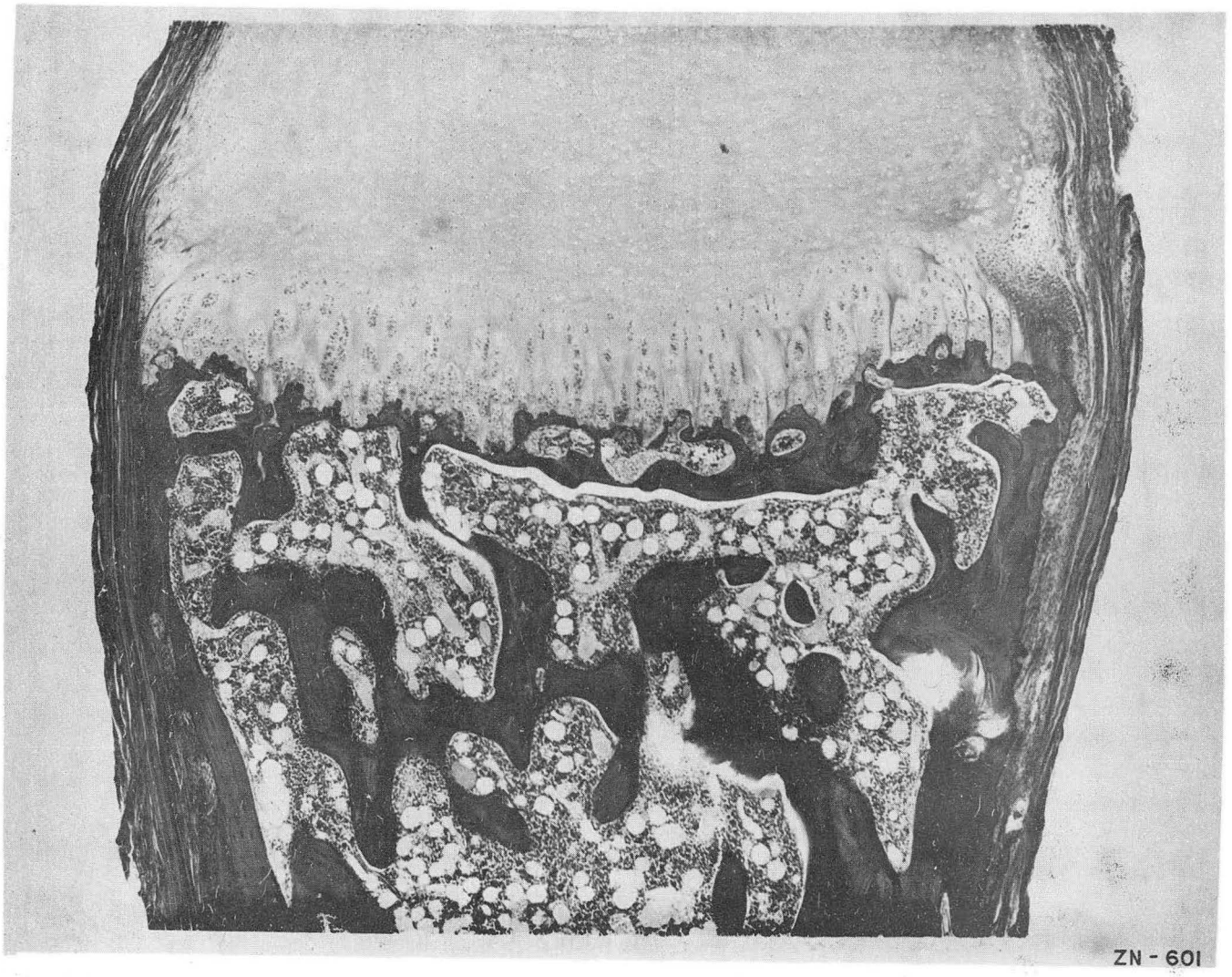

Fig. 8

Costochondral junction of astatine-treated monkey. Magn. x 32.6. Cartilage proliferation is virtually arrested. Marrow is restrained from contact with cartilage by a transverse bony lamina. No primary spongiosa indicative of growth is seen. Note fatty marrow.

"lines of growth arrest" described by radiologists under certain clinical conditions. By one of the present authors (C. W. A.) it has been domonstrated (in rats) that it is related not only to growth arrest but to arrest of maturation (i.e., retarded bone age).

The proximal tibial epiphysis, sectioned histologically, also shows evidences of arrested growth and maturation. The epiphyseal ossification center is incompletely expanded in the cartilage at the end of the bone (Fig. 9). The cartilage plate shows virtually no proliferation (Fig. 10) and, like the costochondral junction, is sealed from the marrow by a layer of bone, rather than showing the active erosion and osterogenesis characteristic of skeletal growth.

The sternum, though sectioned primarily with the intention of observing bone marrow, also showed arrested development. It still consisted of individual sternebrae separated by bars of cartilage, rather than being fused into a single bone (Fig. 11). The processes of cartilage obliteration and bony fusion were in abeyance, as shown by the characteristic transverse bony laminae at chondromedullary junctions like those described above.

In connection with the retarded maturation it should be reported that in the present experiment X-rays were prepared of representative growth centers (shoulder, elbow, wrist and paw, knee and ankle). Though similar ones had not been made of the control, it has been possible, through the courtesy of Dr. Gertrude van Wagenen, of Yale University School of Medicine, to have the X-ray status of these epiphyses compared with those of normal monkeys of a series of known chronologic ages. It can thus be said that the bone age of the astatine-treated monkey appears to be substantially less than the estimated chronologic age, namely two and one-half years. This is based on incomplete fusion of the distal humeral epiphysis, lack of beginning fusion of the olecranon epiphysis, absence of one of the secondary centers in the proximal end of the tibia, and absence of the tarsal sesamoid bone. Roentgenograms of representative osseous centers are seen in Figs. 12-16. In the X-rays of the paws in particular the shadow of the bony lines of arrested growth can be seen.

Bone Marrow. In the control only a small portion of marrow was visible in histologic section in a portion of a rib. As seen in Fig. 17 it is densely cellular.

All marrow examined in the astatine-treated monkey was considerably aplastic (see low magnifications in Figs. 8-11 and high magnification in Fig. 18 in region corresponding to that of Fig. 17). It was predominantly adipose in all regions.

Thymus. No control section being available, it can only be stated that the thymus of the experimental monkey shows an abundance of thymocytes and no substantial fibrosis. Thymic corpuscles are as yet absent.

Skin. Sections of skin of the astatine-treated monkey were taken from chest and leg. The epidermis is thin (Fig. 19), the total thickness not exceeding four to five cell layers, and virtually no proliferation is seen in the stratum germinativum. Granules are very scanty in the granulosum. In lucidum and corneum there are very few flattened cells. The surface bears

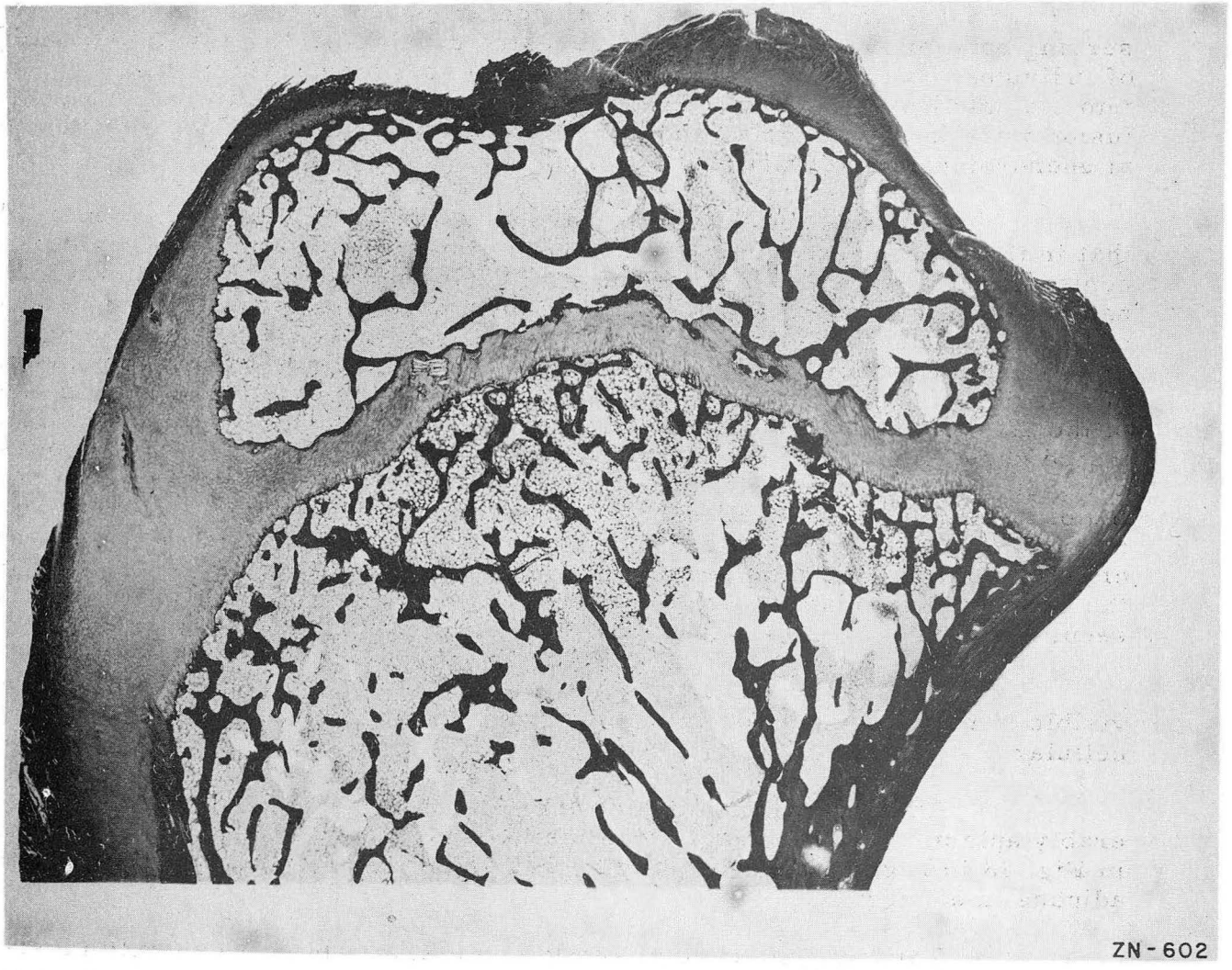

Fig. 9

Proximal end of tibia of astatine-treated monkey. Magn. x 6.7. Note that the epiphyseal ossification center has not expanded fully into the cartilage and that the epiphyseal cartilage plate is invested by bone on both sides, indicating arrested growth and maturation. Note fatty marrow.

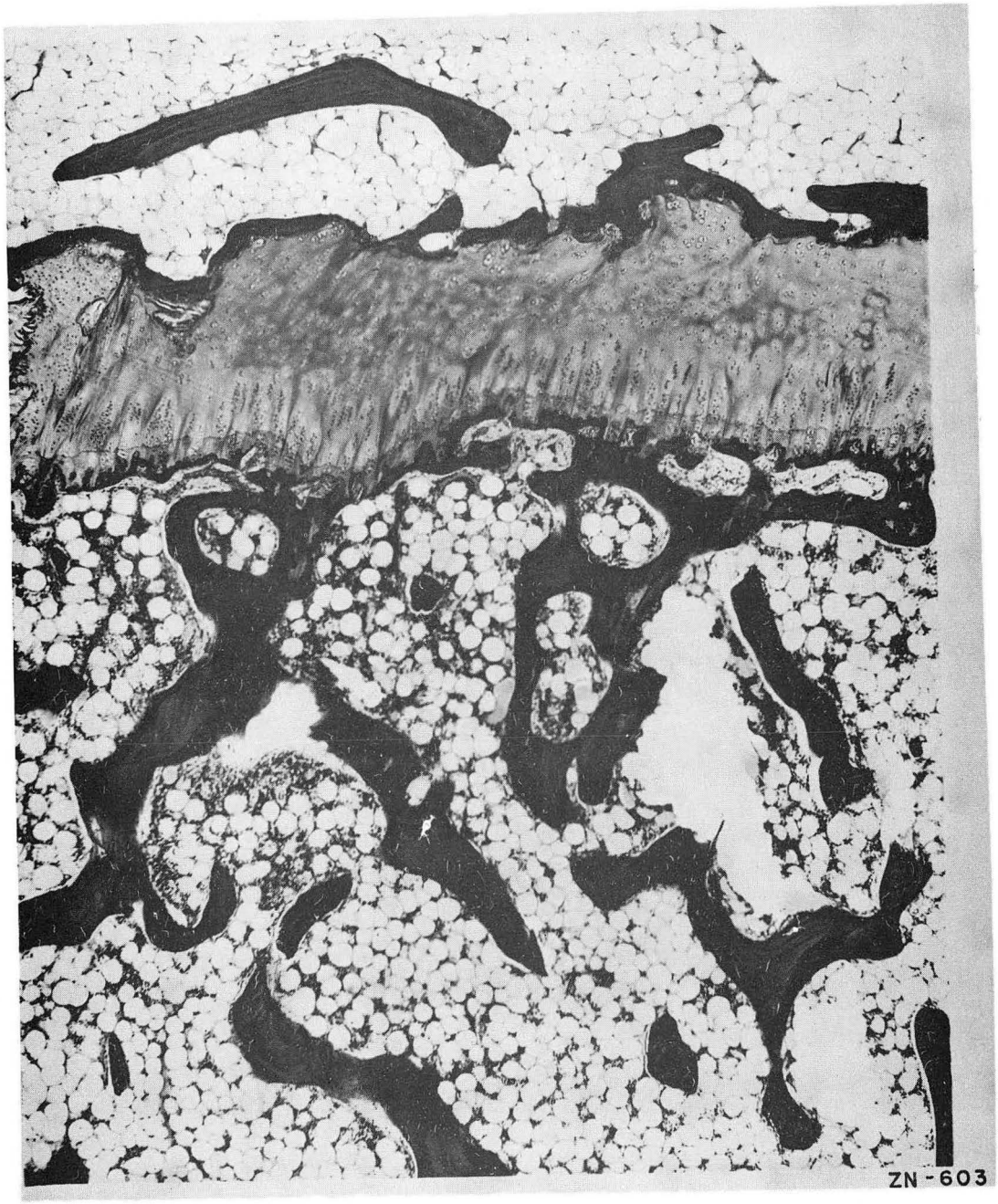

Fig. 10

Higher magnification (x 32.6) of tibial epiphysial cartilage plate. The lesion corresponds essentially to that described for the costochondral junction (compare Fig. 8).

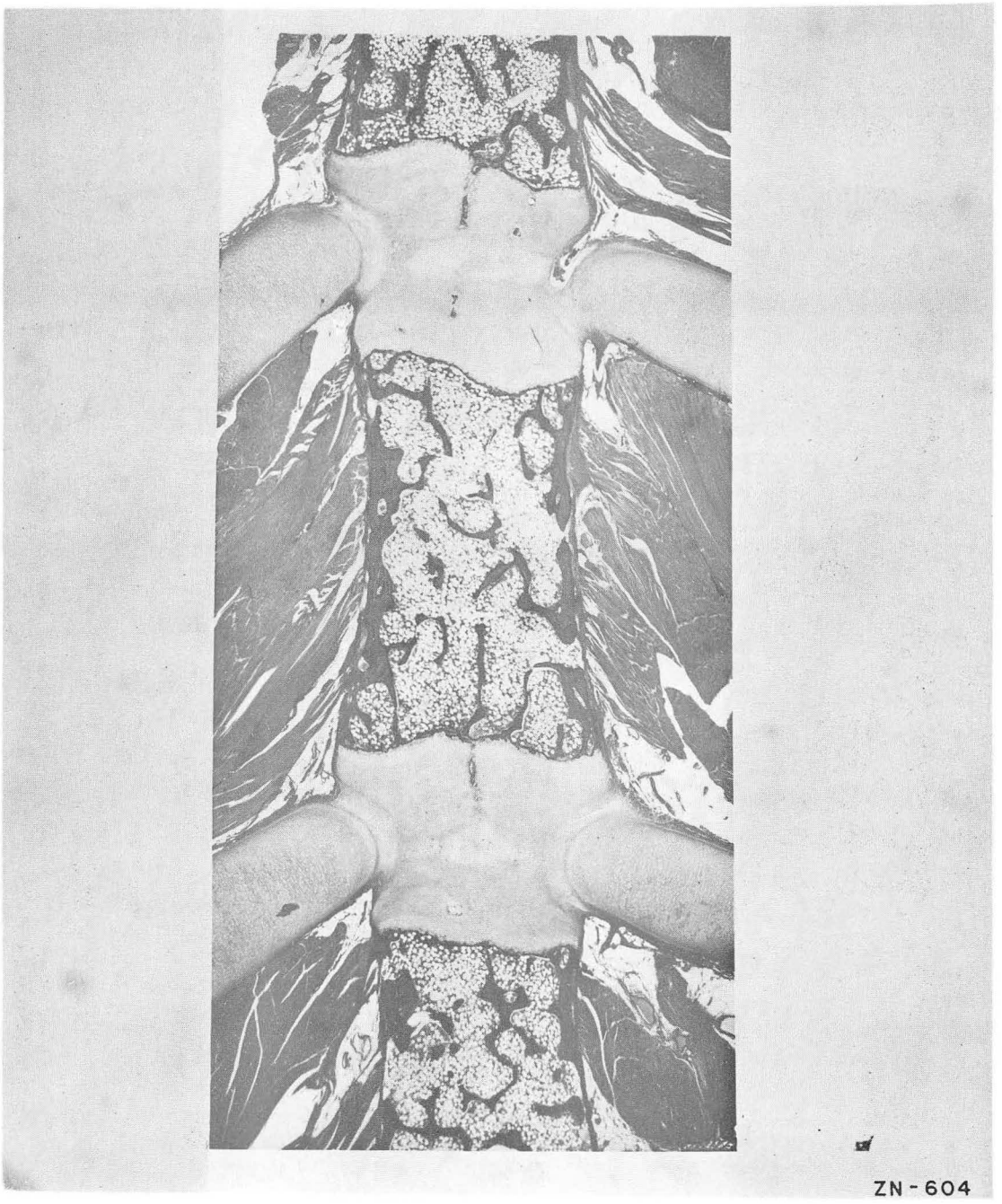

Fig. 11

Sternum of astatine-treated monkey. Magn. x 6.7. Note separate sternebrae and arrested endochondral osteogenesis. Note also the fatty marrow.

a moderate amount of shredded keratin. Hair shafts are slender and the root structure shows only slight proliferative activity. The dermis is of very loose structure, but lacking comparable control material it cannot be established whether this is related to a subcutaneous myxedema.

DISCUSSION

The histologic studies of the control are consistent with the picture of a male monkey in early adolescence suffering from a subacute inflammatory disorder, possibly primarily pulmonary. There is a suggestion of slight liver damage and gastroenteritis. As described in an earlier report the thyroid gland appeared hyperplastic. To this extent the control cannot be considered to have been normal.

The morphologic alterations in the astatine-treated monkey fall into three chief groupings:

(a) Those resulting from irradiation. The thyroid is the chief example of this. The changes in the bone marrow may also have been related to this, as also the less marked changes in lymphatic organs.

(b) Those resulting from impaired thyroid function. The chief of these are the pituitary cytologic changes, namely degranulation of acidophils and degeneration of basophils. It is particularly remarkable that these changes were demonstrated successfully in view of the fact that during part of the experiment thyroid hormone was administered. This hormone is known to correct completely the pituitary cytologic alterations which follow thyroidectomy.

The delayed bone maturation (diagnosed roentgenographically) and the associated histologic changes are probably directly referable to hypothyroidism.

The skin atrophy also falls in this category, as may the reduced hematopoiesis in part.

(c) Those resulting from impaired pituitary function (and therefore secondarily from hypothyroidism). In this category are impaired growth (and impaired osteogenesis), delayed sexual maturation, and reduced adrenal cortical activity. The rates of all these are under pituitary hormonal control.

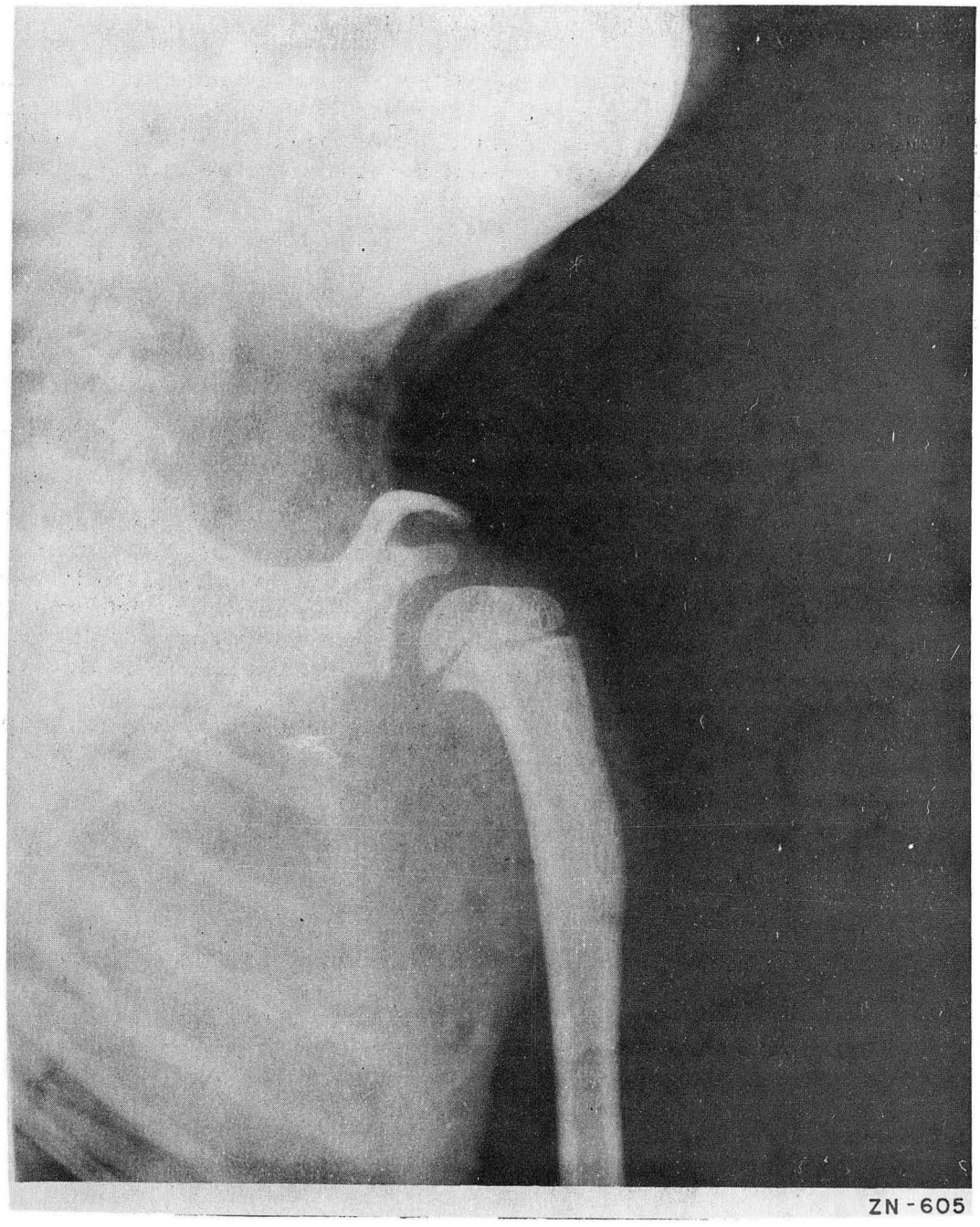

Fig. 12

Roentgenogram of occiput and shoulder of astatine-treated monkey. Actual size. The posterior fontanelle is widely open, suggesting immaturity.

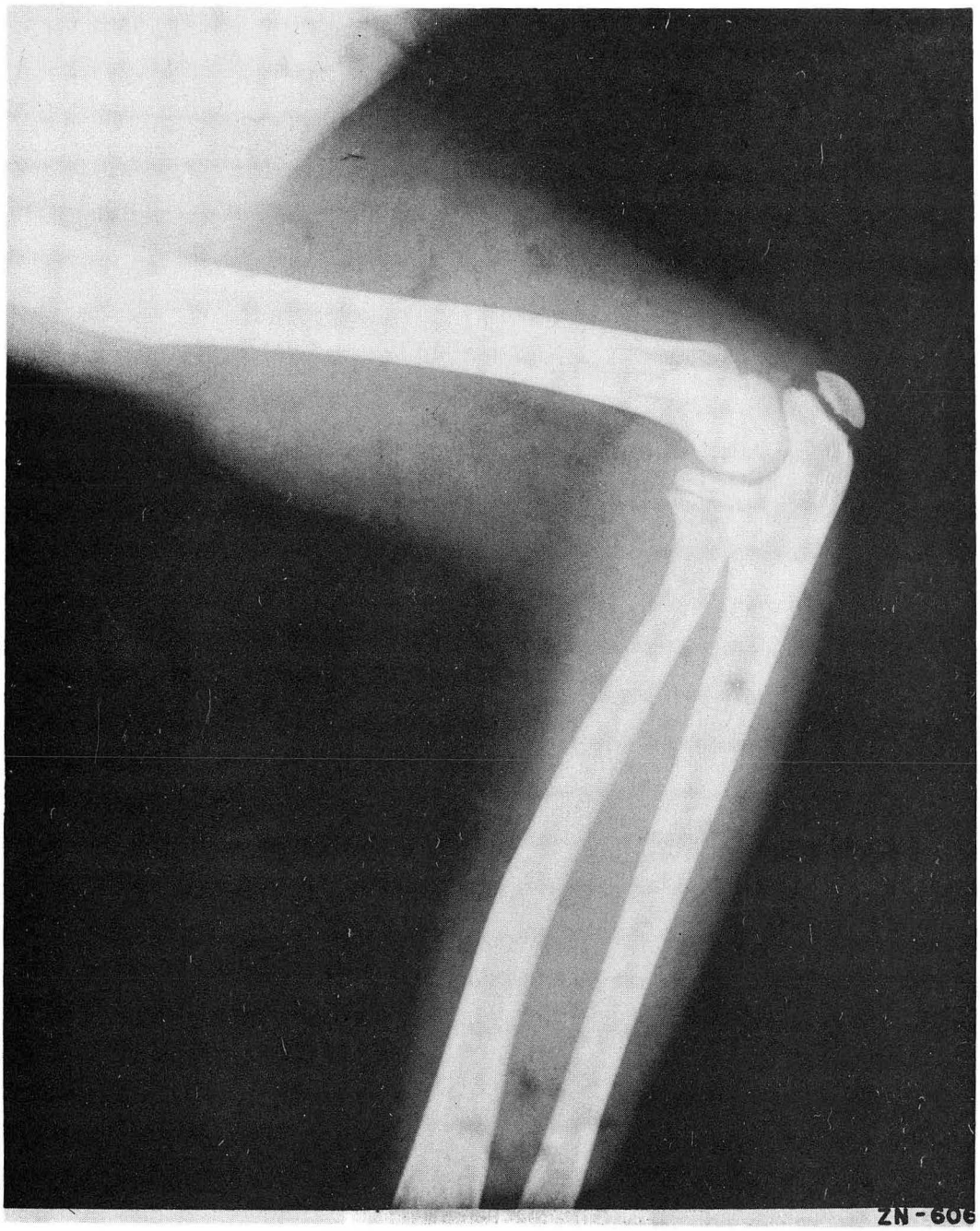

Fig. 13

Elbow, conditions as in Fig. 12. Distal humeral epiphysis is incompletely fused and olecranon fusion to ulna has not commenced. Both represent retardation in a monkey of this age.

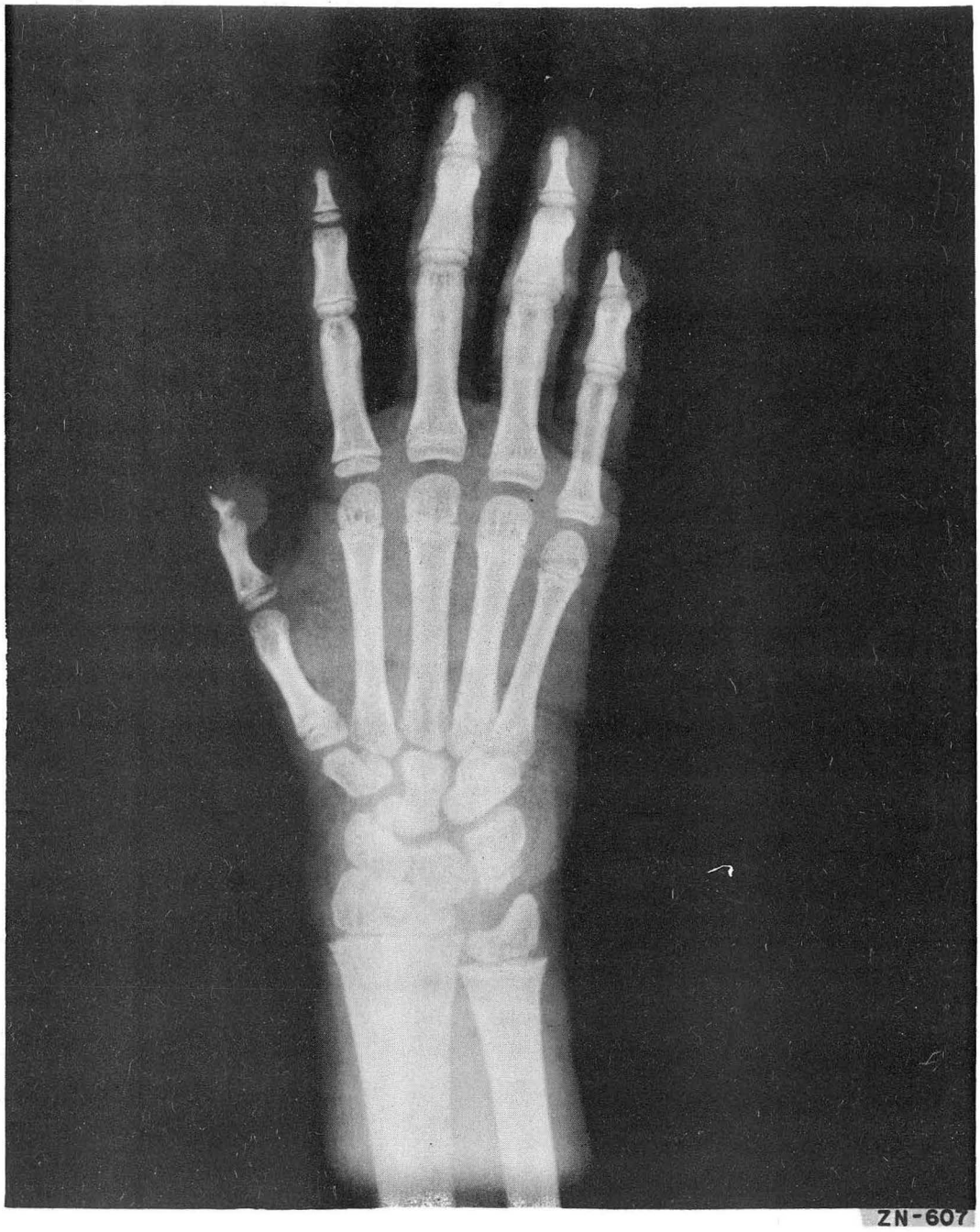

Fig. 14

Forepaw, conditions as in Fig. 12. The sharp white lines at all epiphyseal plates correspond to lines of arrested growth (as shown histologically by bony laminae in Figs. 8-11).

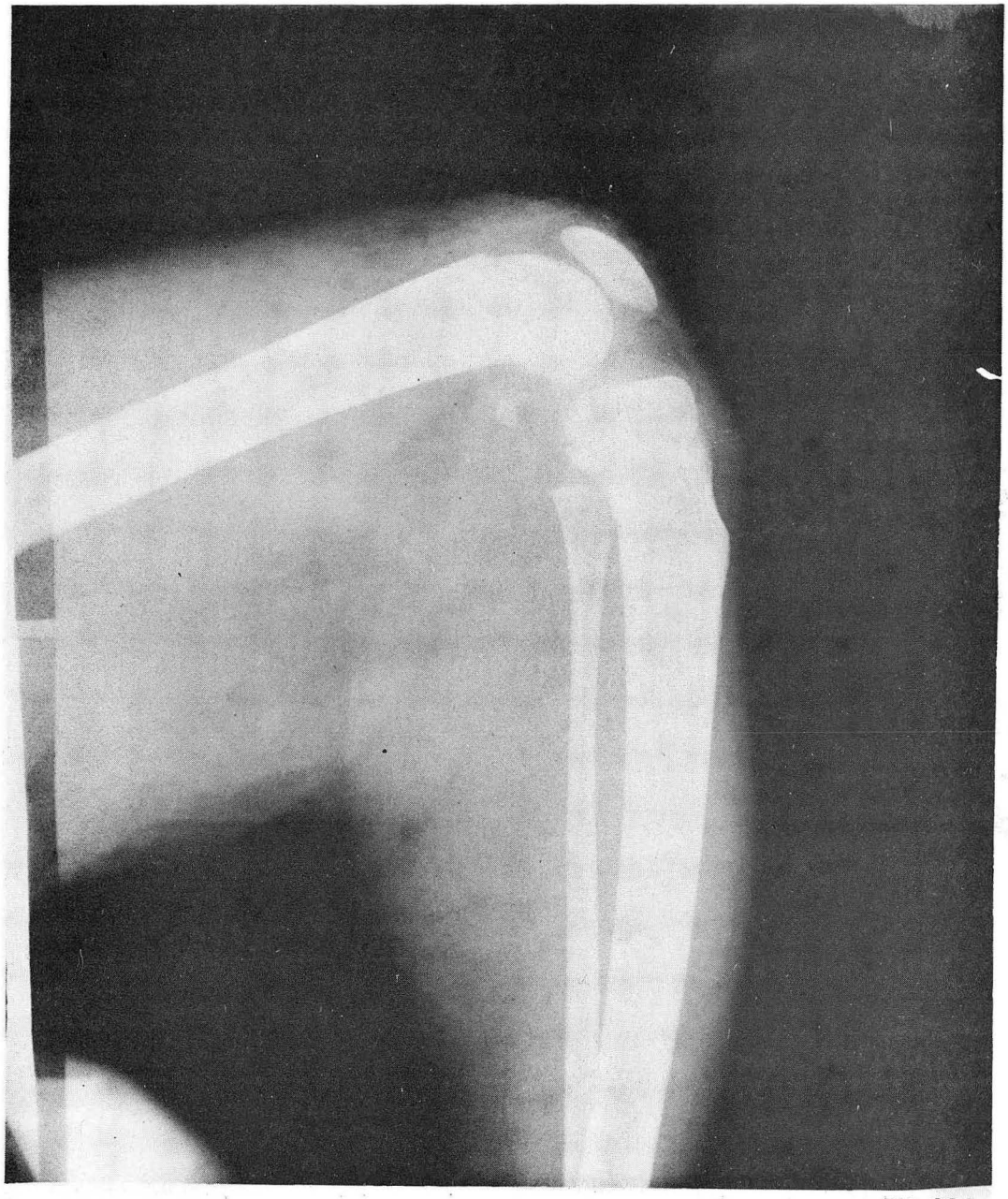

Fig. 15

Knee, conditions as in Fig. 12. A secondary tibial ossification center, due to appear at this age, is delayed.

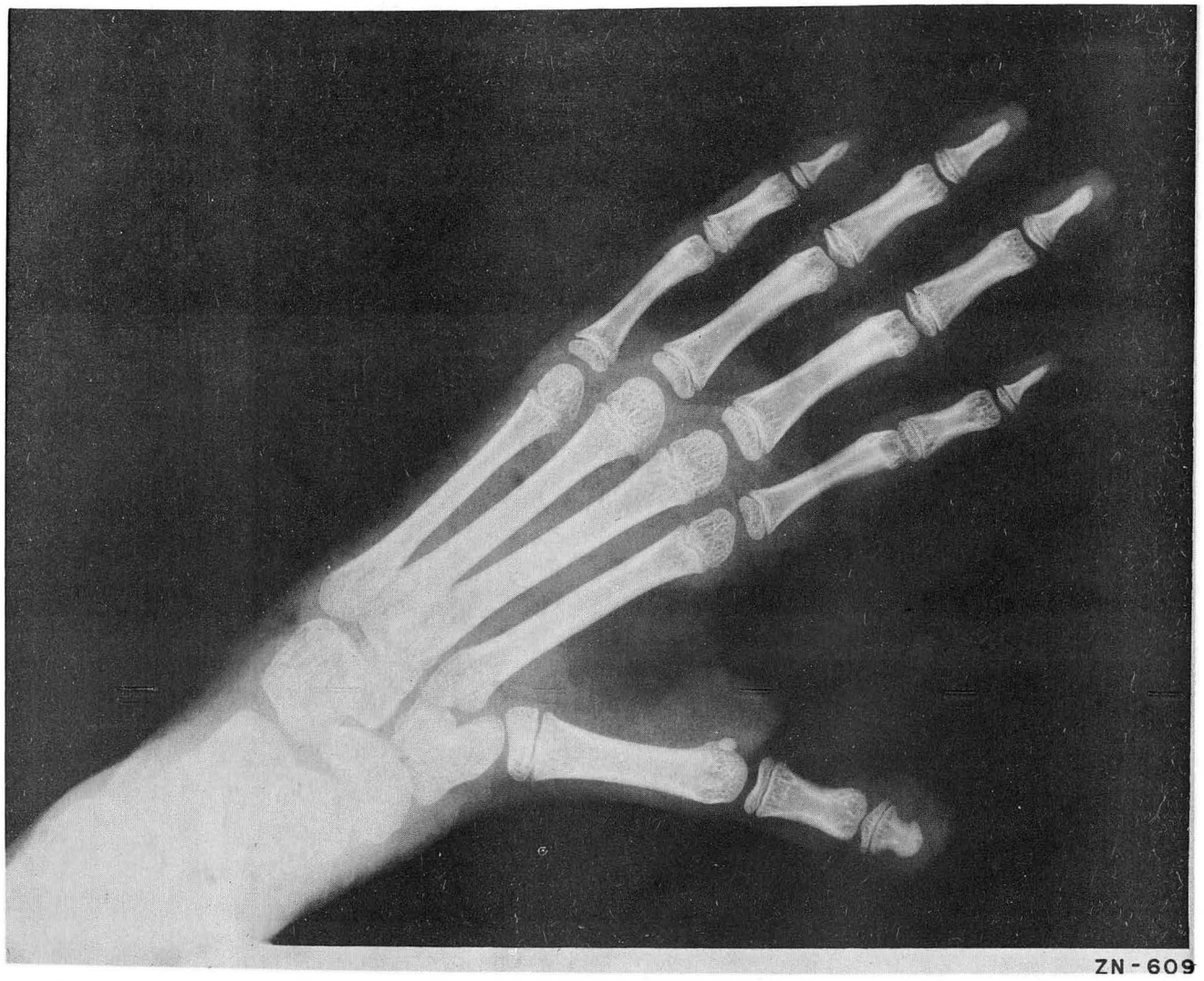

Fig. 16

Hind paw, conditions as in Fig. 12. A tarsal sesamoid bone is lacking, although that of the great toe is present. Compare also with Fig. 14.

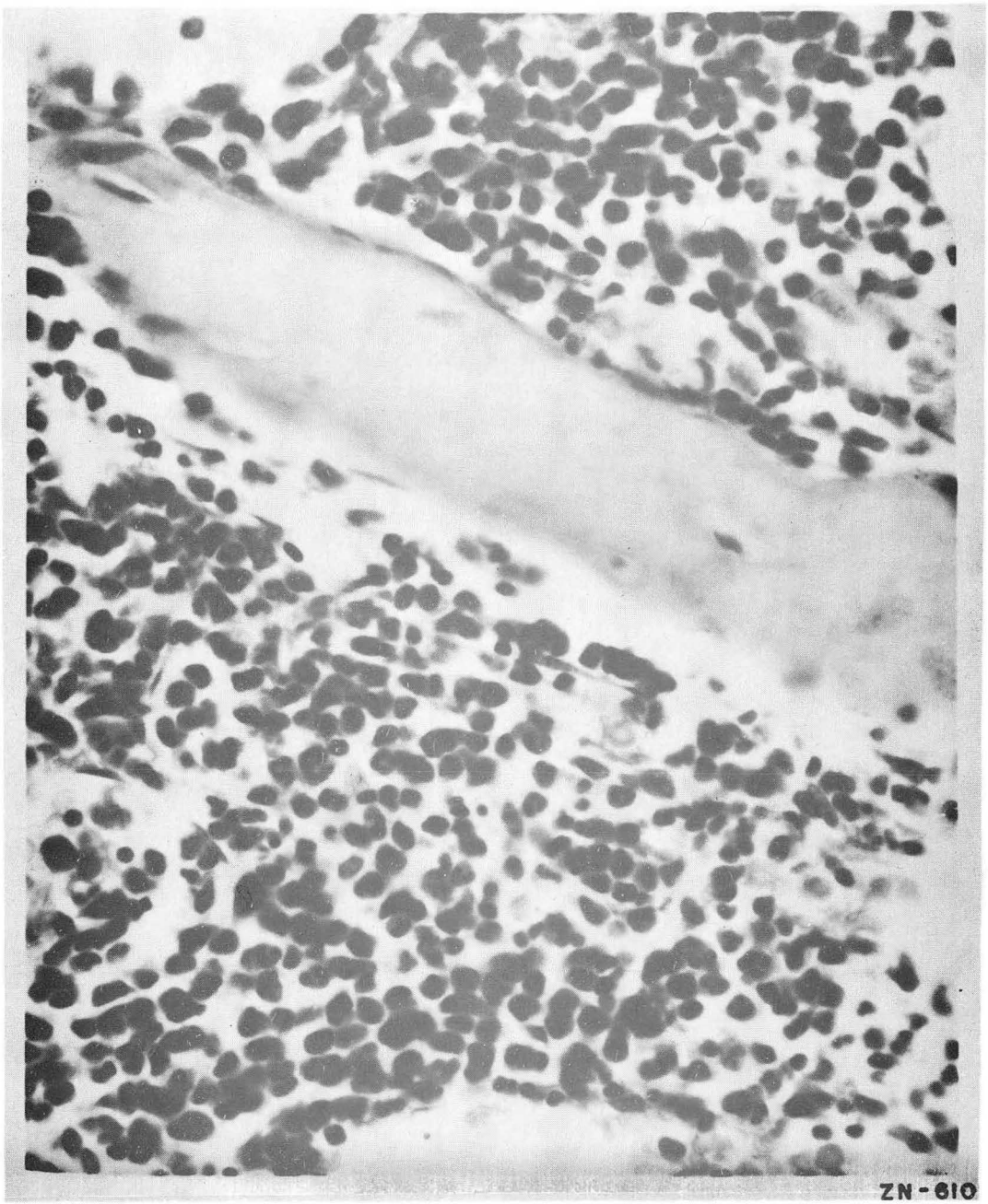

Fig. 17

Bone marrow, from costochondral junction of control. Magn. x 480. The densely cellular character is seen. (A bony spicule crosses the field.)

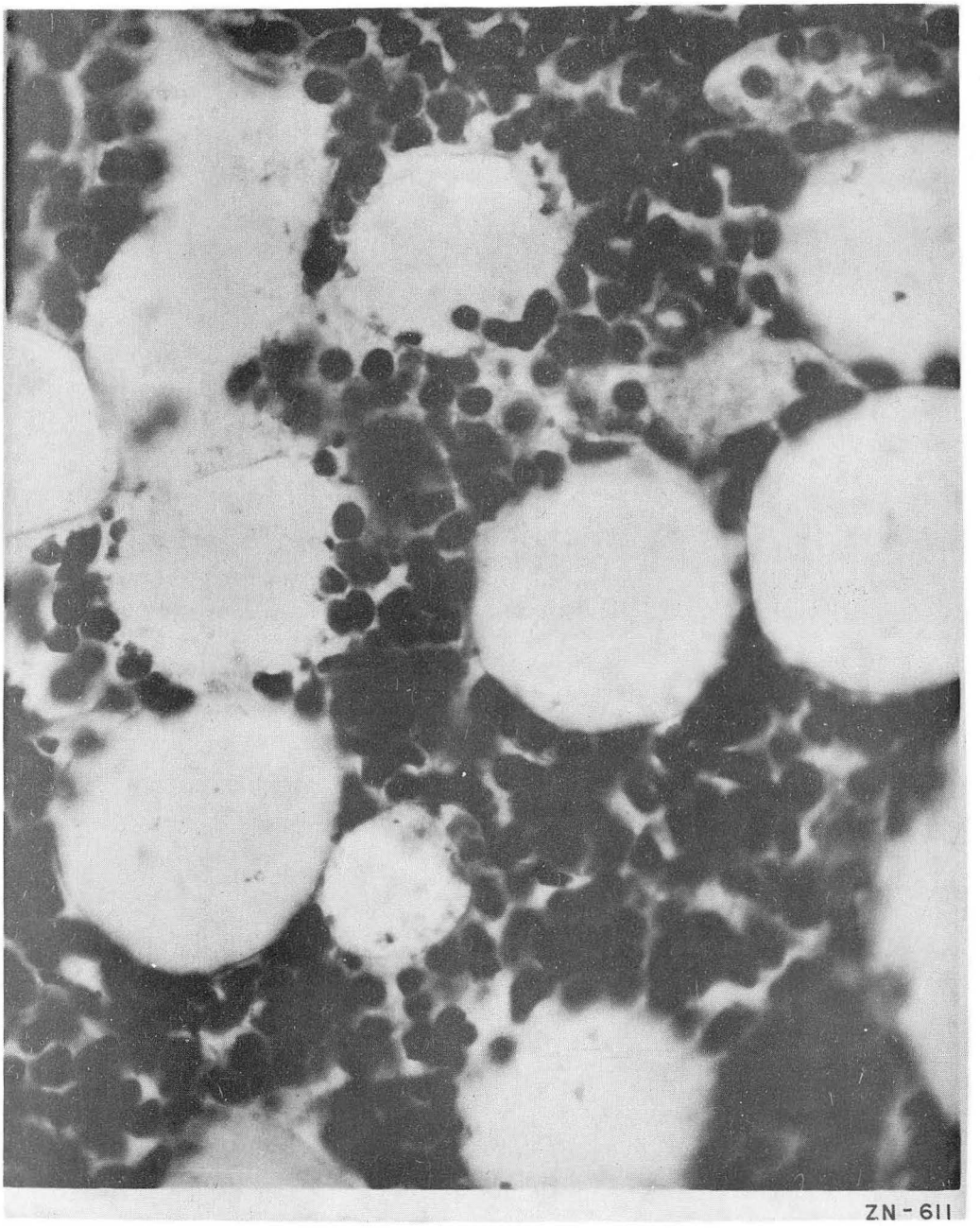

Fig. 18

Bone marrow, from costochondral junction of astatine-treated animal. Magn. x 480. The majority of the field is occupied by fat cells.

Fig. 19

Skin of astatine-treated animal. Magn. x 110. The epidermis is very thin, but shows extensive flaking of keratinized surface. The dermis occupies the rest of the field and is of very loose structure.

RADIATION CHEMISTRY

Warren M. Garrison

During the last quarter, the work of the radiation chemistry group involved primarily the study of radiation-induced changes in aquo-organic systems. In studies of dilute solutions in which the H and OH radicals are produced in the primary radiation chemical act, it is becoming increasingly apparent that the relative probability for subsequent reactions of these radicals with different types of functional groups in the organic solute molecule may be quantitatively evaluated. Because of the importance of this type of information in the elucidation of radiobiological phenomena, it has been felt that a detailed kinetic study of radiation-induced chemical processes in aqueous solutions of simple organic molecules would be of practical and theoretical interest. In previous quarterly reports and in other publications[1-3] we have presented kinetic studies of radiation-induced reactions in aqueous solutions of carbonic acid, formic acid, acetic acid and glycine. The work discussed in this report is a continuation and extension of these studies.

Acetic Acid Studies

Warren M. Garrison, Boyd M. Weeks, and Herman R. Haymond

Irradiation of aqueous acetic acid solutions with high-energy helium ions results in the formation of succinic acid as the principal nonvolatile product at radiation doses of less than 1×10^{21} ev/ml. Hydrogen peroxide, hydrogen, carbon dioxide, and methane are also produced. At higher radiation doses the reactions became considerably more complex because of subsequent reactions of succinic acid to form tricarballylic, malic, citric, and several other acids, as yet unidentified. The production of succinic acid has been explained[2] in terms of a reaction mechanism involving the CH_2COOH radical as an intermediate, i.e.:

$$CH_3COOH + OH \rightarrow CH_2COOH + H_2O \qquad (1)$$

$$CH_3COOH + H \rightarrow CH_2COOH + H_2 \qquad (2)$$

$$2CH_2COOH \qquad (COOH) - CH_2 - CH_2 - COOH \qquad (3)$$

The relative importance of ractions (1) and (2) in the production of the CH_2COOH radical is being determined from radiation yield studies of HD formation in irradiated CD_3COOH solutions.

The radiation yield of succinic acid (G_s) in both evacuated and oxygen-saturated solutions increases with acetic acid concentrations and

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1 W. M. Garrison and G. K. Rollefson, Discussions Farad. Soc., 12, 155 (1953).

2 W. M. Garrison, H. R. Haymond, D. C. Morrison, and B. M. Weeks, UCRL-1863.

3 W. M. Garrison, D. C. Morrison, H. R. Haymond, and J. G. Hamilton, J. Am. Chem. Soc., 74, 4216, (1952).

approaches a limiting value. A detailed study has been made of the effect of acetic acid concentration on the radiation yield of succinic acid, hydrogen peroxide, hydrogen, carbon dioxide and methane in evacuated and oxygen-saturated solutions. The experimental procedures which were employed have been previously described[2, 4]. The effect of acetic acid concentration on product yield in evacuated and oxygen-saturated systems is shown in Figs. 1 and 2 respectively. The corresponding target data are given in Tables I and II. It is seen that the radiation yield of succinic acid, hydrogen, and hydrogen peroxide approaches a limiting value in both the evacuated and oxygen-saturated solutions. The methane yield increases linearly with the acetic acid concentration, while carbon dioxide shows a fairly sharp rise above 0.25 M acetic acid.

If we assume that two primary radiation processes[5] occur when dilute aqueous solutions are irradiated,

$$H_2O \rightarrow H + OH \qquad (4)$$

$$2H_2O \rightarrow H_2 + H_2O_2 \qquad (5)$$

reaction (5) represents the combination of like radicals in regions of high ionization density along the track of the ionizing particle and (4) represents the production of radicals which escape combination and are available for reaction with solute molecules. In the absence of added solute or at low solute concentration the radicals produced in (4) react with H_2O_2 and H_2 (5) giving rise to a radiation-induced back reaction, i.e.:

$$H + H_2O_2 \rightarrow H_2O + OH \qquad (6)$$

$$OH + H_2 \rightarrow H_2O + H \qquad (7)$$

In the presence of acetic acid we have the following possible competing reactions involving possibly both H and OH

$$OH + CH_3COOH \rightarrow CH_2COOH + H_2O \qquad (1)$$

$$H + CH_3COOH \rightarrow CH_2COOH + H_2 \qquad (2)$$

which result in the production of succinic acid via (3) above. The radicals which react with acetic acid via (1) and (2) are not available for reactions (6) and (7). The radiation yield of succinic acid, hydrogen and hydrogen peroxide would therefore be expected to increase with acetic acid concentration as seen in Fig. 1. In a previous report, from a kinetic consideration of the competing reactions, it was shown that a value for the yield of reaction (4) could be estimated in terms of the limiting yield for succinic acid at the higher acetic concentrations. If it is assumed that all of the OH or all of the H produced in (4) reacts via (1) or (2), then the following relationship can be derived[2]

$$\frac{1}{G_s} = \frac{1}{2k_4}\left[1 + \frac{C}{(A)}\right]$$

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4 Quarterly Report, Oct., Nov., Dec., 1952.
5 A. O. Allen, J. Am. Chem. Soc., 56, 575 (1952).

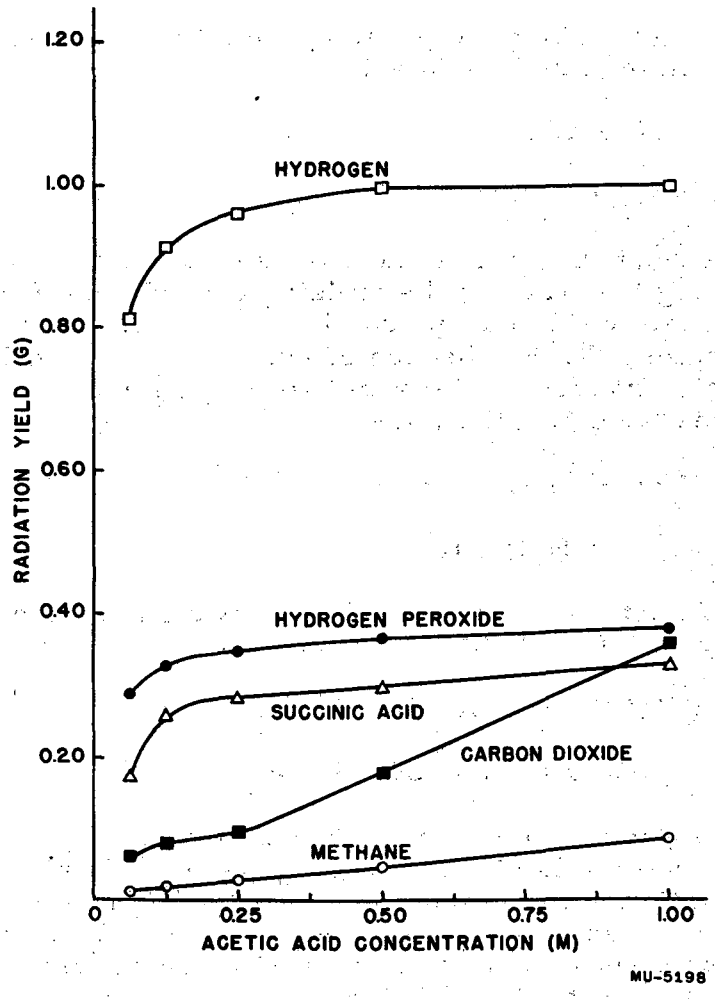
1.20
1.00
0.80
0.60
0.40
0.20
RADIATION YIELD (G)
HYDROGEN
HYDROGEN PEROXIDE
SUCCINIC ACID
CARBON DIOXIDE
METHANE
0
0.25
0.50
0.75
1.00
ACETIC ACID CONCENTRATION (M)
MU-5198

Fig. 1

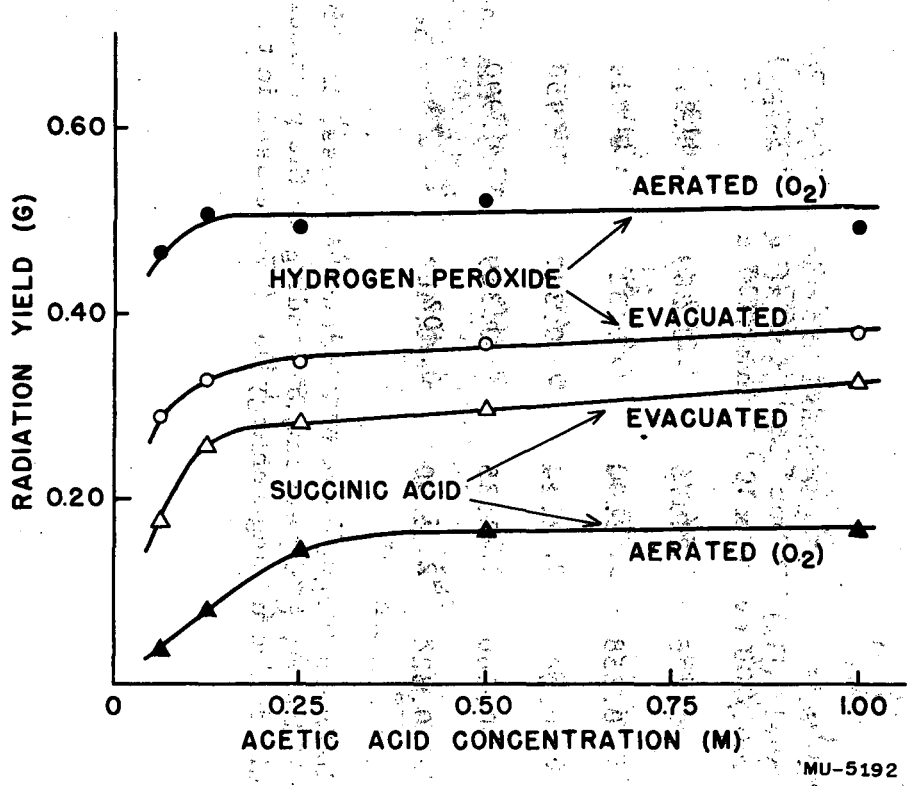
0.60
0.40
0.20
RADIATION YIELD (G)
AERATED (O_2)
HYDROGEN PEROXIDE
EVACUATED
EVACUATED
SUCCINIC ACID
AERATED (O_2)
0
0.25
0.50
0.75
1.00
ACETIC ACID CONCENTRATION (M)
MU-5192

Fig. 2

Table I

Radiation Yields in Evacuated Solutions of Acetic Acid.
Target Vol., 100 ml; Beam Intensity, 0.200 μa; Bombardment, 0.100 μa hr.

Acetic Acid conc. M	ev/ml* x 10^{-20}	Succinic Acid conc. M x 10^3	Succinic Acid yield (G_s) mol/100 ev	Hydrogen Peroxide conc. M x 10^3	Hydrogen Peroxide yield (G_s) mol/100 ev	Hydrogen (G_{H_2})	Carbon Dioxide (G_{CO_2})	Methane (G_{CH_4})
0.0625	3.69	1.07	0.175	1.76	0.289	0.813	0.0588	0.011
0.125	3.68	1.57	0.258	2.00	0.329	0.914	0.0791	0.0167
0.25	3.73	1.73	0.281	2.14	0.347	0.950	0.096	0.0268
0.50	3.69	1.84	0.300	2.25	0.369	0.990	0.176	0.0463
1.00	3.62	1.94	0.324	2.40	0.402	0.994	0.284	0.0795

* Because of small differences between target cells in window thickness, the energy of helium ions absorbed in the solution varied slightly (< 3 percent) depending on the particular cell window used. The corrected energies, calculated from range energy data, were used in the calculation of G.

Table II

Radiation Yields in Oxygen-Saturated Solutions of Acetic Acid. Target Vol., 75 ml; Beam Intensity, 0.200 μa; Bombardment, 0.100 μa hr.

Acetic Acid conc. M	ev/ml x 10^{-20}	Succinic Acid conc. M x 10^3	Succinic Acid yield (G_s) mol/100 ev	Hydrogen Peroxide conc. M x 10^3	Hydrogen Peroxide yield (G_{H_2O}) mol/100 ev
0.0625	3.91	0.31	0.040	4.04	0.469
0.1250	3.91	0.69	0.087	4.40	0.512
0.250	3.91	1.25	0.145	4.20	0.484
0.500	3.91	1.44	0.180	4.48	0.521

where G_s is the experimentally observed radiation yield for succinic acid, C is a constant involving the concentration of H_2, H_2O_2 or an intermediate produced, (A) is the acetic acid concentration and k_4 is the yield of reaction (4). A plot of $1/G_s$ as a function of 1/(A) for the data given in Figs. 1 and 2 is shown in Fig. 3. It is seen that the same extrapolated value for $1/G_s$ is obtained at a beam intensity of 0.2 μa both in the evacuated system (curve I) and the oxygen saturated system (curve III). Curve II is a similar plot of previously reported data which were obtained at a beam intensity of 1 μa. The intensity effect seen at the lower acetic acid concentration in the oxygen-saturated solution (compare curves II and III) is attributed to the removal of radicals by the reaction

$$H + O_2 \rightarrow HO_2$$

$$HO_2 + OH \rightarrow O_2 + H_2O$$

which becomes less important as a competing mechanism for radical removal at the higher acetic values.

A plot of $1/G_{H_2O_2}$ as a function of 1/(A) for the data obtained at 0.2 μa is shown in Fig. 4. It is readily seen that the extrapolated values for $G_{H_2O_2}$ in the evacuated and oxygen-saturated systems do not coincide. Since the extrapolated values for G_s are the same for these two cases it would appear that hydrogen peroxide is formed in the oxygen-saturated system at the higher acetic acid concentrations by a reaction of the type

$$H + HO_2 \rightarrow H_2O_2$$

or possibly

$$HO_2 + CH_3COOH \rightarrow H_2O_2 + CH_2COOH$$

The effect of added hydrogen peroxide on the product yield in 0.25 M acetic acid is shown in Fig. 5. The corresponding target data are given in Table III.

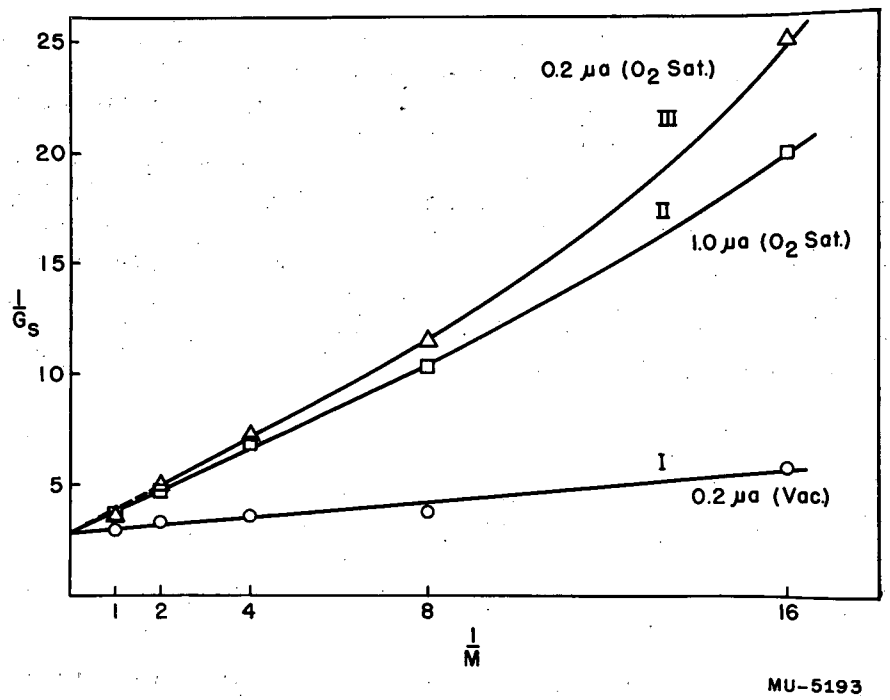
25
20
15
10
5
$\frac{1}{G_s}$
0.2 μa (O_2 Sat.)
III
II
1.0 μa (O_2 Sat.)
I
0.2 μa (Vac.)
1
2
4
8
16
$\frac{1}{M}$
MU-5193

Fig. 3

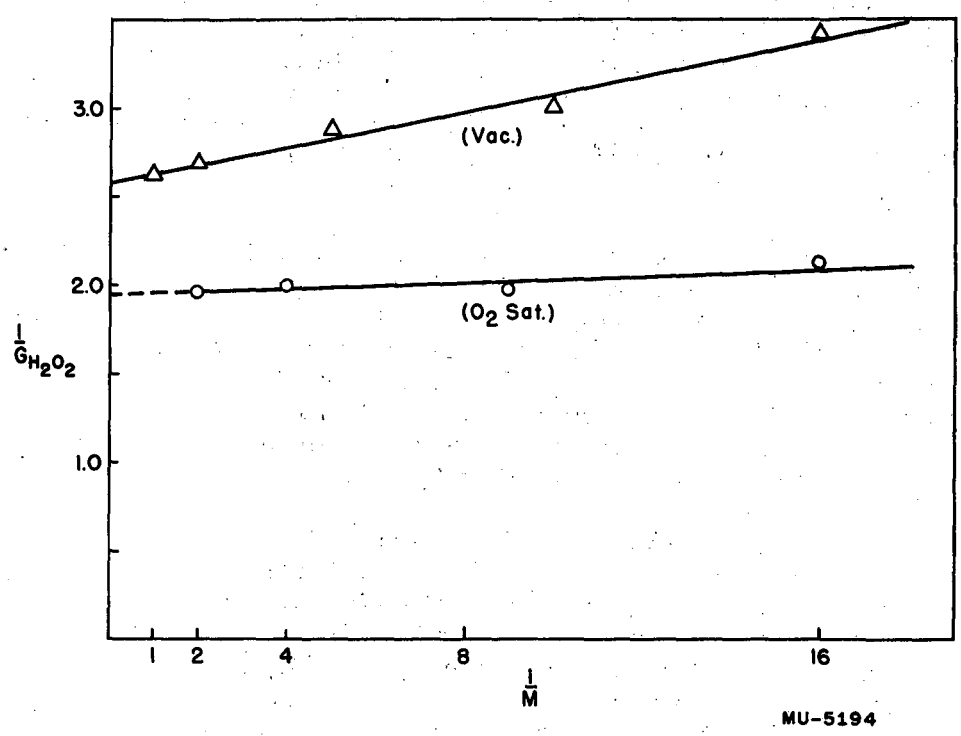

Fig. 4

The value of G_{H_2} at the higher peroxide concentrations presumably represents the molecular yield via reaction (5). At the present time it is not possible to state whether the decrease of G_s and increase of G_{CO_2} with increasing initial peroxide are caused by further oxidation of the CH_2COOH radical or whether independent processes are involved. The data of Fig. 5 will be discussed in greater detail in a subsequent report.

Consideration has been given to the possibility that organic products other than succinic acid are produced in the irradiation of acetic acid at the lower dose levels. In previous reports it was shown by methods of partition chromatography on silicic acid columns that succinic acid is the principal non-volatile acid product at radiation doses below 5×10^{20} ev/ml. More recently it has been found by an independent analytical procedure that succinic acid accounts for over 95 percent of all nonvolatile products. A solution of 0.25 M acetic acid containing $CH_3C^{14}OOH$ was bombarded with 35 Mev helium ion to give a radiation dose of 3×10^{20} ev/ml. A sample of the nonvolatile fraction was chromatographed[6] on filter paper with butanol-acetic acid-water and phenol-water as the developing solvents. A radioautogram of the two-dimensional chromatogram is shown in Fig. 6. It is seen that the principal activity ($C^{14}OOH$) - CH_2 - CH_2 - $C^{14}OOH$ accounts for over 95 percent of the nonvolatile activity.

Preliminary work has also been done toward the development of methods for separation and identification by chromatographic techniques of the volatile nonacid products resulting from irradiation of acetic and formic acids. (See section II). Studies were made of the properties of the 2,4-dinitrophenylhydrazones of acetone, acetaldehyde, formaldehyde, glyoxal and diacetyl on columns of silicic acid when eluted with mixtures of high boiling petroleum ether and ether or with mixtures of petroleum ether and chloroform. Marvel et al[7] have reported a method of this type.

The dry silicic acid used as the adsorbent column was prepared as has been previously described.[2] The silicic acid was suspended in the first solvent mixture to be used, poured into the chromatographic tube and allowed to settle against a glass-wool plug. After the excess solvent had been drained off a chloroform solution of the ketone or aldehyde derivatives to be studied was put on top of the column and drained into the column. The brightly colored band thus formed at the top of the column could be followed easily during its development with various eluting solvents. The ratio of the volume of solvent used to volume of column through which a band had moved was recorded as a measure of the eluting power of a particular solvent for a particular 2,4-dinitrophenylhydrazone. This ratio varied considerably with different batches of silicic acid, and a quantitative method such as that used for the nonvolatile acid products has not yet been worked out. From the data already obtained, however, it appears that a method can be developed by utilizing mixtures of ether and petroleum ether as eluting solvents.

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6 A. A. Benson et al, J. A. Chem. Soc. 72, 710 (1950).
7 C. S. Marvel, C. Bluestein, W. M. Schilling and P. G. Sheth, J. Org. Chem., 16, 854 (1951).

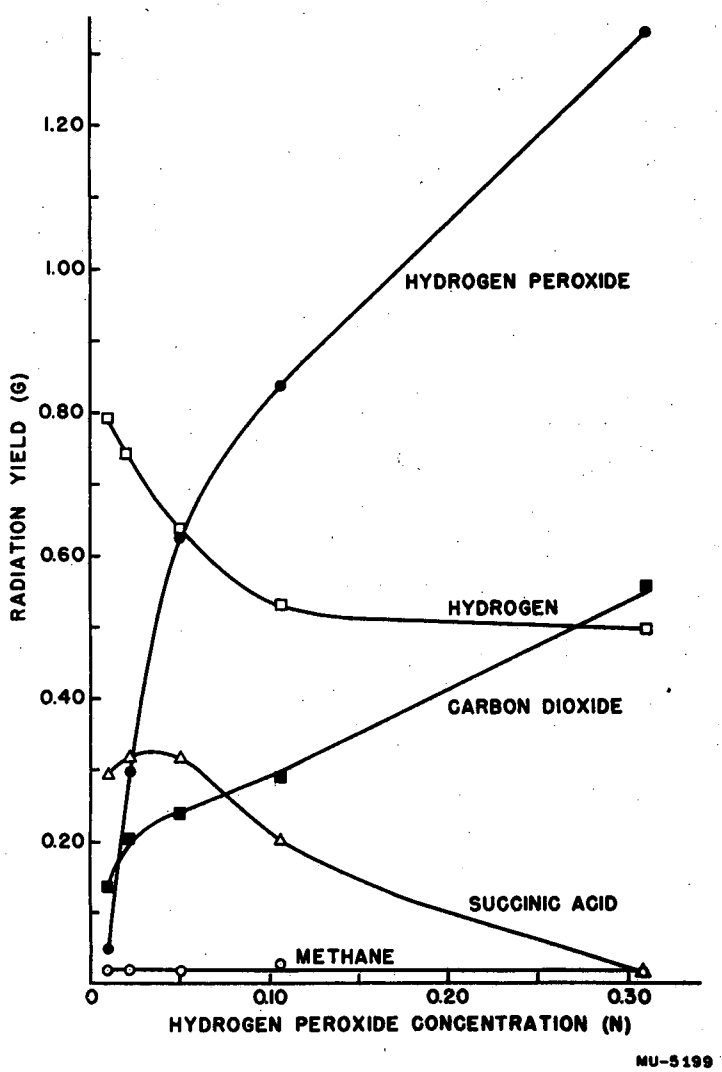
1.20
1.00
0.80
0.60
0.40
0.20
RADIATION YIELD (G)
HYDROGEN PEROXIDE
HYDROGEN
CARBON DIOXIDE
SUCCINIC ACID
METHANE
0
0.10
0.20
0.30
HYDROGEN PEROXIDE CONCENTRATION (N)
MU-5199

Fig. 5

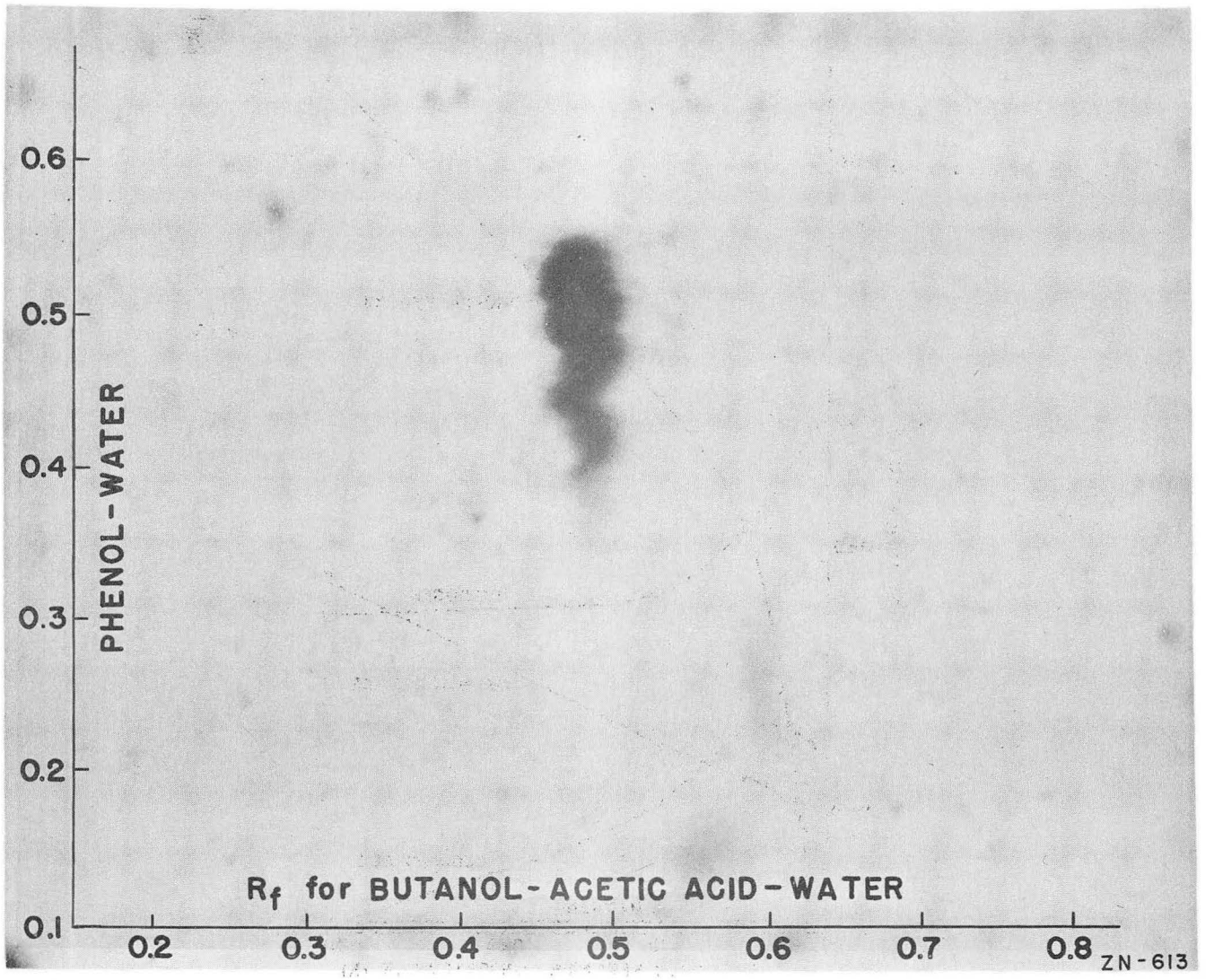
0.6
0.5
0.4
0.3
0.2
0.1
PHENOL-WATER
R_f for BUTANOL-ACETIC ACID-WATER
0.2
0.3
0.4
0.5
0.6
0.7
0.8
ZN-613

Fig. 6

Table III

Radiation Yields in Solutions of Acetic Acid Containing Added Hydrogen Peroxide
Target Vol., 100 ml; Beam Intensity, 0.200 μa; Bombardment, 0.100 μa hr;
Acetic Acid Conc., 0.25 M.

ev/ml x 10^{-20}	Hydrogen Peroxide init. conc. M	Hydrogen Peroxide final conc. M	Hydrogen Peroxide -yield ($-G_{H_2O_2}$) mol/100 ev	Succinic Acid conc. M x 10^3	Succinic Acid Yield (G_s) mol/100 ev	Hydrogen (G_{H_2})	Carbon Dioxide (G_{CO_2})	Methane (G_{CH_4})
3.93	0.0042	0.0039	0.0493	1.90	0.293	0.794	0.136	0.023
3.87	0.0103	0.0084	0.298	2.05	0.322	0.742	0.204	0.024
3.87	0.025	0.0214	0.626	1.72	0.269	0.637	0.240	0.024
3.91	0.053	0.047	0.837	1.32	0.204	0.533	0.290	0.032
3.88	0.15	0.146	1.40	0.12	< 0.02	0.492	0.569	0.016

Formic Acid

Warren M. Garrison, Herman R. Haymond, and Boyd M. Weeks

In the previous quarterly report it was shown that the principal nonvolatile acid product formed in aqueous formic acid by helium ion irradiation is not oxalic acid. Although this product has not been positively identified, it has been found to correspond closely to tartronic acid in partition chromatographic separation. Figs. 7, 8 and 9 show elution curves obtained by the method of Bulen[8] with samples of the nonvolatile fraction corresponding to radiation doses of 4×10^{20} ev/ml, 1×10^{21} ev/ml and 1.5×10^{21} ev/ml respectively. Peak III has a peak effluent volume which agrees closely with that of tartronic acid. Further data are being obtained with C^{14}-labeled formic acid solutions.

Glycine

Boyd Weeks

In the last quarterly report (Oct., Nov., Dec., 1952) the details of a proposed study of the radiation chemistry of aqueous solutions of glycine were laid out and the initial steps in this program described. The present report will therefore be confined to work carried out in the past quarter (Jan., Feb., March, 1953).

I. Preparation of Target Solutions

One hundred ml of purified 0.25M aqueous glycine solution was prepared for irradiation. A small amount of crude glycine (Nutritional Biochemicals Corp.) was first added to a cation exchange column and eluted with HCl. The glycine hydrochloride thus obtained was passed through the same cation exchange column using NH_4OH as eluent. The free glycine was then dried *in vacuo* to remove NH_3 and the 0.25M target solution was prepared from the perfectly white crystals so obtained. As a test for NH_3 a little of the glycine used for the target solution was dissolved in water and again distilled *in vacuo*. Phenolphthalein gave no pink color to this distillate. As a test for Cl, $AgNO_3$ added to a solution of the crystals gave no precipitate. All H_2O used for target solutions as well as in the purification procedures of the glycine is doubly distilled; first in an ordinary Barnsted still and secondly in an all-glass system from alkaline permanganate. Elution of the column with NH_4OH was continued after the recovery of the free glycine and occasional ninhydrin tests of the effluent were made. In addition to the glycine peak which was at about 1150 ml on the NH_4OH elution curve there was an unidentified peak at about 2000 ml. This was a white crystalline material which gave a deep blue color with ninhydrine (glycine gave a more magenta color) and formed a slightly yellowish precipitate with $AgNO_3$ which dissolved in dil. HNO_3 without evolution of gas. The amount of this material did not exceed 2 percent of the glycine.

II. Bombardment

One hundred ml of 0.25M glycine in H_2O was irradiated in a sealed, evacuated glass cell* with agitation during irradiation. The dose given was

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8 W. A. Bulen *et al*, Anal. Chem., 24, 187 (1952).

* This cell is described in the quarterly report for Oct., Nov., and Dec., 1952 in connection with the acetic acid work.

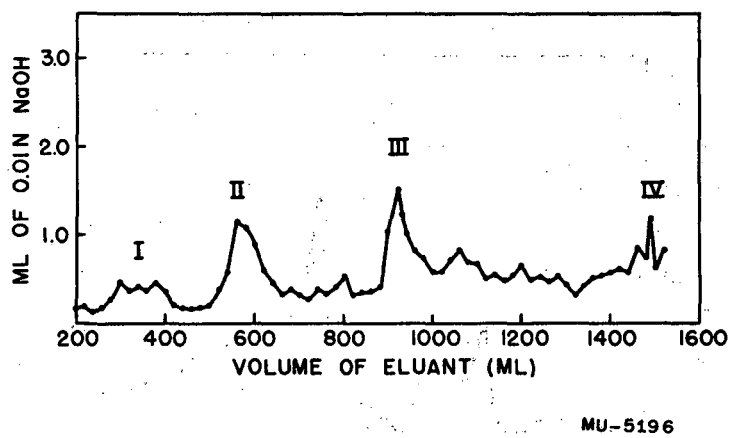
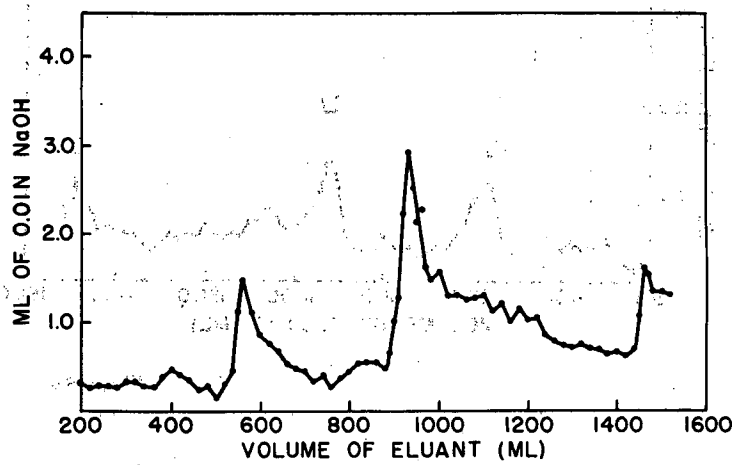

ML OF 0.01N NaOH
3.0
2.0
1.0
I
II
III
IV
200
400
600
800
1000
1200
1400
1600
VOLUME OF ELUANT (ML)
MU-5196

Fig. 7


4.0
3.0
2.0
1.0
ML OF 0.01N NaOH
200
400
600
800
1000
1200
1400
1600
VOLUME OF ELUANT (ML)

MU-5197

Fig. 8

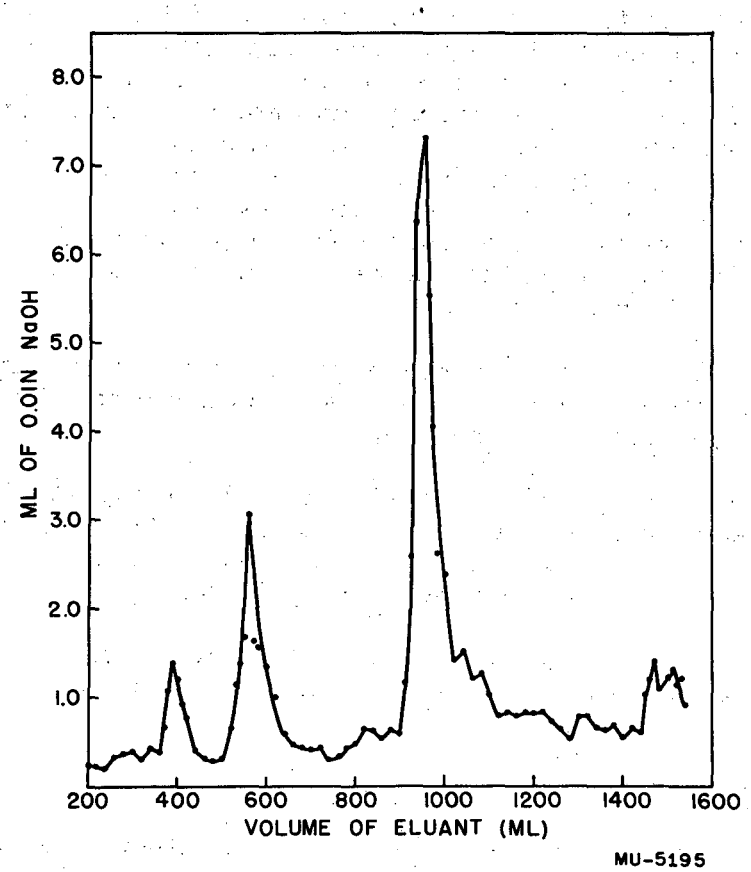

8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
ML OF 0.01N NaOH
200
400
600
800
1000
1200
1400
1600
VOLUME OF ELUANT (ML)
MU-5195

Fig. 9

3.9×10^{20} ev/ml, which is fourfold reduction in dose from the proposed "standard" dose in the preliminary glycine studies. The lower dose should reduce the complexity of the product pattern and permit a clearer interpretation of the primary reactions.

III. Determination of Products

No attempt was made to fractionate either the cationic products (including the parent glycine) on a cation exchange column or the acidic products on a silica column. Portions of an earlier C^{14}-labeled target were chromatographed by these two methods and several distinct products are indicated in both fractions. None of these indicated products has been identified yet. Work is now in progress to prepare purified glycine labeled in both 1 and 2 positions with C^{14} in order to identify and determine these two groups of products. By the methods outlined in the last quarterly report the following products were determined: H_2 CO_2, volatile base, volatile acid, H_2O_2. The values for these products remain to be established by repeated experiments. A positive test for carbonyl as the 2,4 dinitrophenylhydrazone derivative was obtained in the nonvolatile fraction but not in the volatile fraction of a portion of the target solution. CH_4 was absent.

Purification of the target material remains the most urgent problem. Although the method outlined has been used, new methods of purification and other criteria of purity are being sought.

The chromatographic analyses in the above studies were made by Mrs. Sibyl Cole and James T. Post. The cyclotron irradiations were made possible by the cooperation of G. Bernard Rossi, William B. Jones and the crew of the Crocker Laboratory 60-inch cyclotron.

We are indebted to Dr. Amos Newton for the mass spectrometric analysis of the gaseous products and for helpful discussions on product identification.

(1) Publications which have appeared:

Warren M. Garrison and G. K. Rollefson: Radiation Chemistry of Aqueous Solutions Containing Both Ferrous Ion and Carbon Dioxide. The University Press, Great Britain, Aberdeen, Faraday Society Discussion, 1952, No. 12.

J. G. Hamilton, C. W. Asling, W. M. Garrison, and K. G. Scott: The Metabolism and Biological Effects of Astatine in Rats and Monkeys. University of California Publications in Parmacology, 2, 283-344.

Joseph G. Hamilton, Thomas M. Putnam, and Martin L. Ehrmann: Effect of Heavy Charged Particle and Fast Neutron Irradiation on Diamonds. American Mineralogist, 37, 941-949 (1952).

(2) Submitted for Publication:

Warren M. Garrison, H. R. Haymond, H. Powell, C. Corum, and J. G. Hamilton: A Centrifugal-Pump Target Assembly for the Cyclotron Bombardment of Liquids.

Joseph G. Hamilton and Kenneth G. Scott: The Effect of the Calcium Salt of Versene Upon the Metabolism of Plutonium in the Rat.

Warren M. Garrison, H. R. Haymond, D. C. Morrison, B. M. Weeks, and J. G. Melchert: High Energy Helium-Ion Irradiation of Aqueous Acetic Acid Solutions.

BIOLOGICAL STUDIES OF RADIATION EFFECTS

John H. Lawrence, M.D. - in charge

Project 48A-1

IRRADIATION OF THE PITUITARY OF THE RAT WITH HIGH-ENERGY DEUTERONS[+]

C. A. Tobias, D. C. Van Dyke, M. E. Simpson, H. O. Anger
R. L. Huff and A. A. Koneff[++]

This paper describes the use of deuteron particles to partially or completely destroy the pituitary of rats. For three decades attempts have been made to study the effects of radiation on the pituitary, not only to further our understanding of its function but also with a view to human therapy. The pituitary has been studied after irradiation by a variety of methods ranging from total body X-irradiation (guinea pigs, Strauss, 1920[1]) to focused high-speed electron irradiation of the pituitary (rats, Mateyko, et al[2,3] 1952). The early investigators were handicapped because the available radiation, X-rays, scattered widely in tissue so that it was impossible to confine the irradiation to the pituitary alone. It must be considered that even under the most favorable circumstances a substantial part of the head was irradiated. Several investigators have reported evidence of brain damage associated with attempts to irradiate the pituitary (Selle[4], 1935 and Cicardo[5], et al, 1951). In such cases injury to the hypothalamus would complicate interpretation of specific effects on the pituitary. In investigations where the method used amounted to external head X-irradiation the dose internally was limited by the radiation tolerance of the skin. In some reported work the dose remains uncertain in terms of modern units; however, externally applied doses probably seldom exceeded those of Lawrence, et al[6], who gave 3,000 r to the rat head and produced marked retardation in the growth of the animal, similar to that seen following hypophysectomy. At such doses many investigators reported changes in function of the pituitary target organs. Both retardation and stimulation of pituitary function have been reported. Rahm[7] (1922) noted a greater weight gain in rabbits and Epifanio and Cola[8] (1932) reported accelerated body growth and increased adrenal size of rabbits after head irradiation. Martinalli[9] (1929) found an increase in the interstitial tissue of the rabbit ovary but a decrease in the number of follicles following exposure of the head. Many have reported partial or complete destruction of the anterior lobe with atrophy of all the target organs after high doses of radiation, and some describe more inhibition of one function

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[+] From the Donner Laboratory of Medical Physics (supported in part by the United States Atomic Energy Commission) and the Institute of Experimental Biology (supported in part by United States Public Health Service Grant GG 409 (C-3)), University of California, Berkeley.

[++] We are indebted to Joseph Sayeg, G. Welch, and V. Burns for their participation and to Mr. J. Vale and the cyclotron crew for their collaboration and help in the course of these experiments.

of the pituitary than of others but without agreement as to which function is more easily influenced by radiation. The same lack of agreement exists concerning the histologic appearance of the cells of the anterior pituitary after irradiation. Ghilarducci[10] (1922) and Epifanio[8] (1932) using rabbits, and Lawrence, et al[6], (1937) using rats found a decrease in the number of acidophiles and in dwarfism, whereas Podljaschuk[11] (1927) found an increase in acidophiles of rabbit pituitaries following irradiation. Brunner[12], using cats and dogs (1920), and Podljaschuk[11], using rabbits (1927), found a reduction in the number of basophiles in the anterior lobe after irradiation. As pituitary stains were not in common use at the time many of these papers were published, it is probable that not too much emphasis should be placed on the contradictory claims of specific cell changes.

Truly localized irradiation of the pituitary was first accomplished by Lacassagne and Nyka[13,14,15,16] (1934), who surgically implanted a capsule of radon in the center of the pituitary of rabbits. All anterior lobe cells were equally damaged. Recently Mateyko, et al[2,3] (1952) used high-speed electrons from a Van der Graaf machine to study the immediate postirradiation effects on the pituitaries of rats. The electrons can be brought to a focus within the head. The changes in pituitary hormone content in the first 24 hours after irradiation were studied.

Accelerated high-energy nuclei (protons, deuterons and alpha particles) have definite advantages for localized radiation of small volumes of tissue within the body. 190-Mev deuterons penetrate approximately 15 cm. into tissue and they travel in approximately a straight line. The scatter is considerably less than that of any other radiation used at present in clinical radiology. Collimated beams of these particles penetrate tissue like a straight pencil and the edge of the radiation field produces sharp lines of demarcation of radiation effects, somewhat like a surgical knife. The radiological properties of these particles have recently been described[17].

The present study was designed to demonstrate deuteron-radiation destruction of a tissue lying deep within the body. The pituitary was chosen because of its central position within the head, its well defined volume and the multiple criteria by which the functional state of the pituitary can be estimated in the living animal during the postirradiation period as well as at autopsy. Because the pituitary can be removed surgically in the rat, satisfactory controls could be provided. It was also hoped that these experiments would shed some light on the radiation sensitivity of the pituitary and perhaps show a specificity in reaction of certain cell types.

Material and Methods

The position of the pituitary within the skull of the rat is such that if the beam is directed laterally through the pituitary, little or no brain tissue is irradiated. If the beam is limited by an aperture of proper size, it passes through the tympanic bulla and the third, fifth and sixth cranial nerves. Fig. 1 shows the path of the deuteron beam superimposed on a frontal section through the pituitary to illustrate the structures through which it passes. The median sagittal section of the pituitary of a rat of the age used (28 days) is a triangle of base 2.0 mm and height 1.0 mm (Fig. 1). The deuteron beam was collimated to conform to the shape and dimensions of the pituitary by being passed through a brass aperture of triangular shape slightly larger than the pituitary to allow

for slight errors in placing the beam (0.4 mm greater in each direction). Fig. 2 is a roentgenogram showing the relation of the pituitary to the base of the skull. The constant position of the pituitary over the occipitosphenoid synchondrosis makes it possible to direct the deuteron beam correctly by relating it to this landmark in a roentgenogram.

To hold the rat's head in position during the irradiation, a head holder was built which was slightly modified from the type described by Asling[18]. The rats were anaesthetized with nembutal during the procedure. The animal's head was held rigidly in the horizontal plane, anteriorly by a pin between the incisor teeth and posteriorly by tapered plugs which fit into the angle between the mandible and the tympanic bulla (Fig. 3). Screw adjustments were provided for moving the animal horizontally or vertically relative to the base of the holder to an accuracy of 0.1 mm. Correct alignment of the head in proper position in the path of the deuteron beam was accomplished with the aid of roentgenograms. For this purpose two film holders were attached to the base of the apparatus, one 5 cm. and one 12.4 cm. behind the rat's head (Fig. 3). A set of crossed wires was also mounted to the base so that its image would be superimposed on the roentgenographic image of the rat's skull. The steps in the procedure of the alignment of the pituitary in the path of the deuteron beam are explained in four parts in Fig. 4. The crossed wires were adjusted in such a way that the deuteron beam passed through the intersection when the holder was placed in proper position in the radiation chamber. Fig. 4, a and b, show the deuteron beam superimposed on the shadow of the crossed wires on both the near and distant roentgenograms. These films were developed after exposure to both X-rays and deuterons. The superimposed images on near and distant plates eliminate error through parallax. After a rat was placed in the holder a roentgenogram was taken to show the position of the skull in relation to the crossed wires. On the basis of the film obtained the animal was moved with the adjusting screws until the occipitosphenoid synchondrosis was in the desired relation to the crossed wires (Fig. 4c). A new film was then exposed to X-rays and the animal holder was moved to the deuteron radiation bench. By short exposure the deuteron beam was superimposed on the X-ray image of the skull and crossed wires (Fig. 4d). This picture shows the alignment of the skull to the crossed wires and the deuteron beam. Once the alignment procedure was found to be satisfactory each new animal had only to be aligned to the crossed wires in the roentgenogram.

Dose Measurements

The average dose delivered by the deuteron particles was recorded and measured by a parallel-plate ionization chamber. This ion chamber was placed in front of the beam-limiting aperture and the measured dose in roentgen equivalent physical (1 rep = 93 ergs/g) units represented the average dose before the particles were scattered by the walls of the aperture, the air molecules and the animal tissues. Owing to the scattering phenomena the edge of the beam becomes somewhat blurred. This is seen schematically in Fig. 1.

To obtain information on the actual dose received by the pituitary gland and the surrounding tissues, two types of measurements were performed. First, the dose distribution was measured in a lucite phantom substituted for the head of the animal. A small cadmium sulphide crystal detector* was used

*Made available through the courtesy of the General Electric X-Ray Corporation.

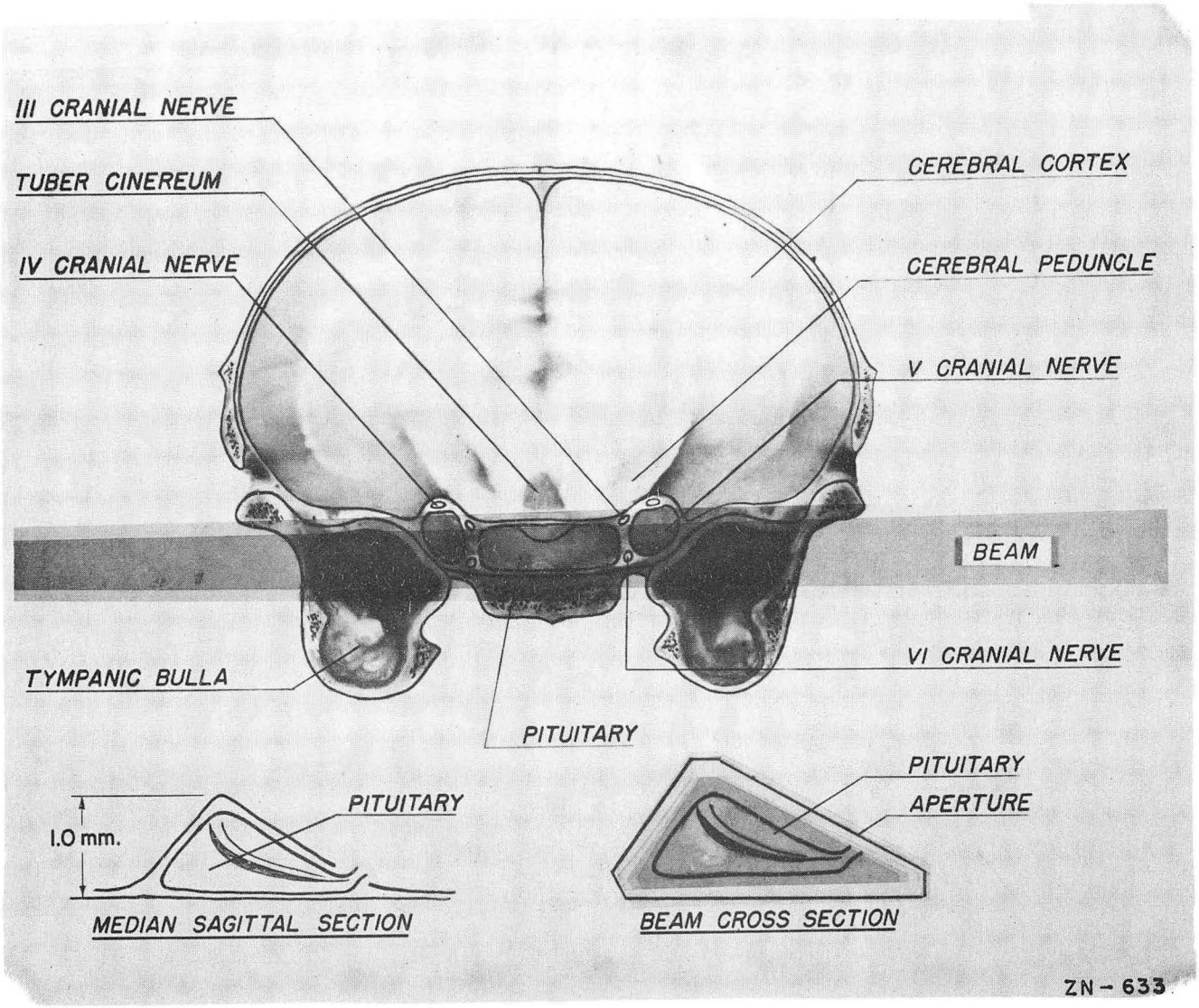

Fig. 1

The path of the deuteron beam superimposed on a frontal section through the pituitary of a 28-day-old rat to illustrate the structures through which it passes. Lower left shows median saggital section of pituitary. Lower right shows the cross section of the beam superimposed on the saggital section of pituitary. It represents the size of the aperture in relation to the pituitary and also represents the intensity and scatter of the beam.

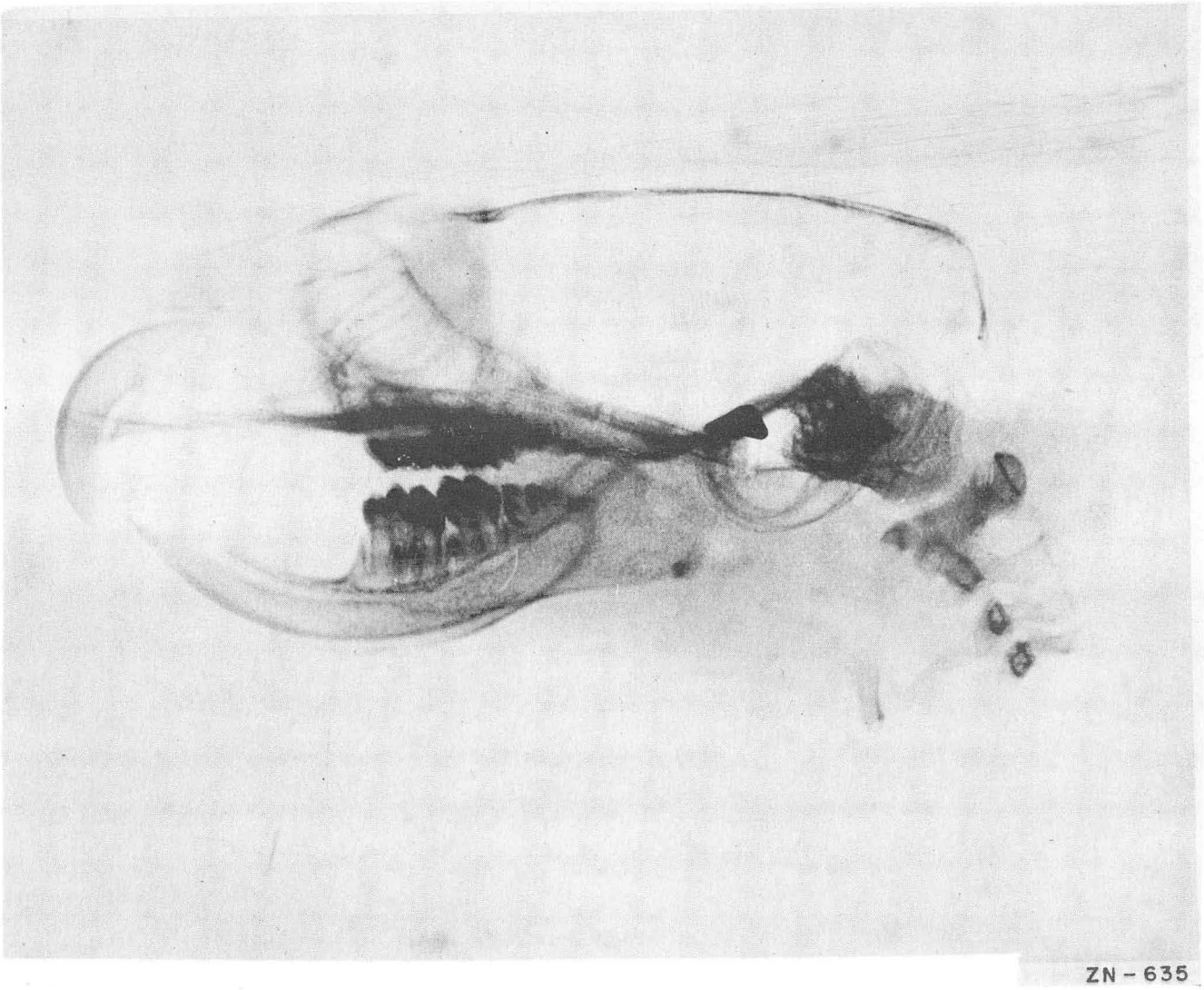

Fig. 2

Roentgenogram showing the relation of the pituitary to the base of the skull. The block triangle represents a lead block of the exact size and shape of the pituitary gland, which was placed in the position the pituitary usually occupies. The pituitary consistently lies 3/4 anterior and 1/4 posterior to the occipitosphenoid synchondrosis.

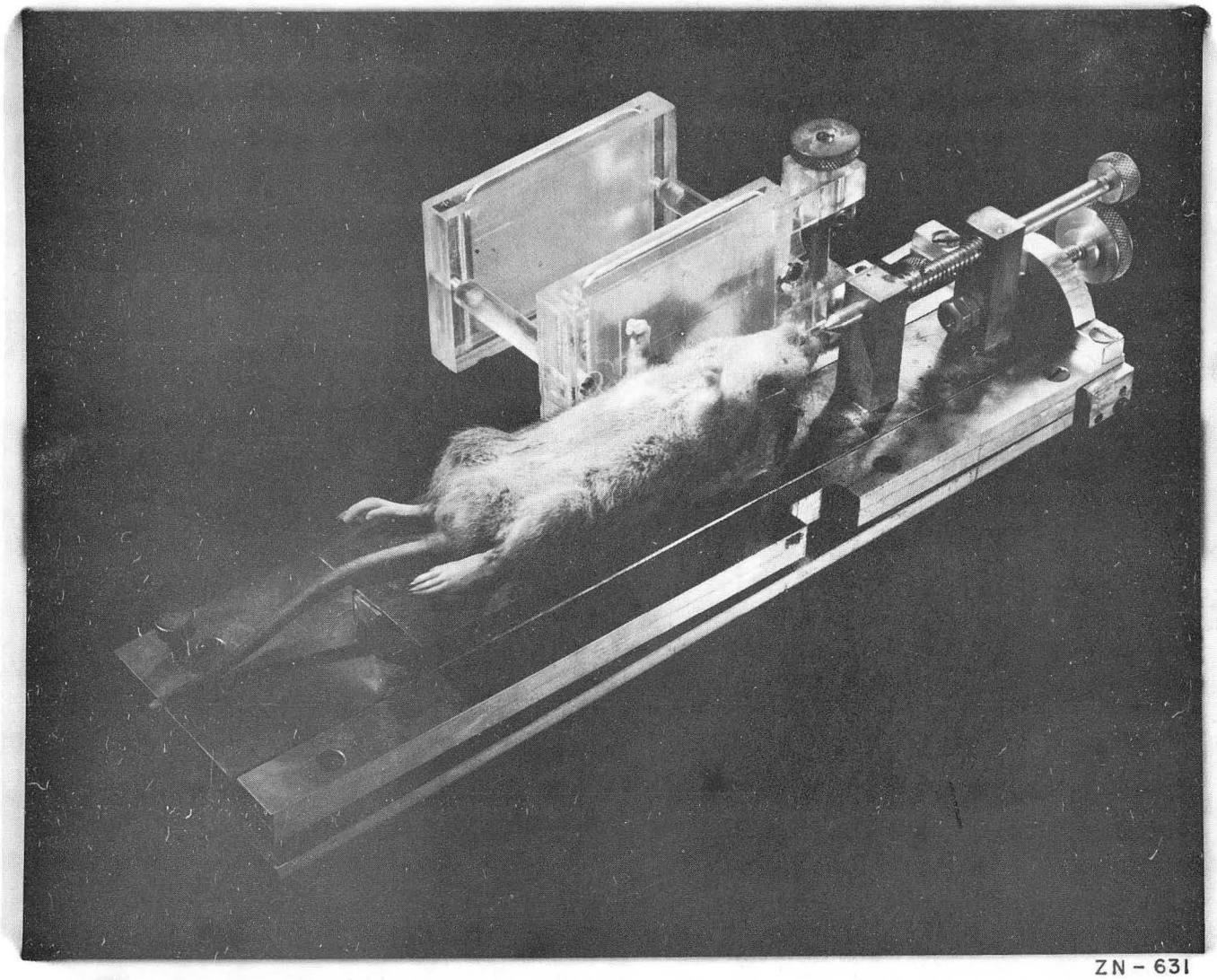

Fig. 3

Rat mounted in the head holder used during the irradiations. The figure illustrates the three-point suspension of the head, the screw adjustments, and the two film holders used for correct alignment of the head. Note the adjusting screws for horizontal (H) and vertical (V) movement of the animal, and of the crossed wires (C).

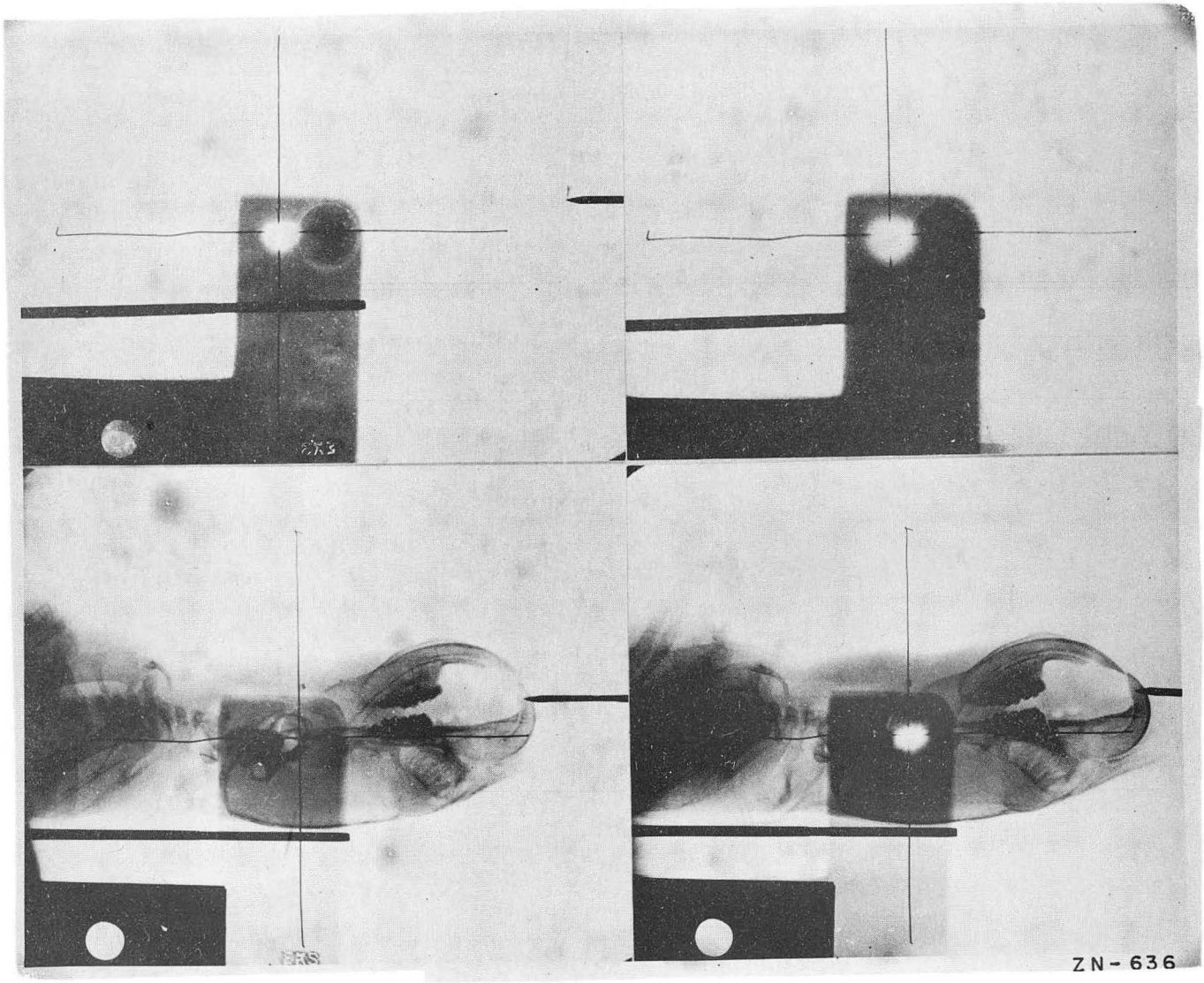

Fig. 4

The four steps in the procedure of aligning the pituitary in the path of the deuteron beam: (a) shows the deuteron beam superimposed on the shadow of the crossed wires on the near roentgenogram; (b) shows the deuteron beam superimposed on the shadow of the crossed wires on the distant roentgenogram; (c) shows the crossed wires in proper position to the occipito-sphenoidal synchondrosis; (d) shows the alignment of the skull to the crossed wires and the deuteron beam.

to scan across the beam[19]. The electric impedance of the crystal decreases as radiation impinges on it. The dose distribution in the horizontal plane obtained by this method is plotted in Fig. 5. The figure shows that the dose is not uniform for the entire area irradiated. It falls off near the edge of the pituitary and some irradiation reaches the immediately surrounding area. It is well known from surgical hypophysectomy that a very small fragment of gland will produce sufficient hormone to partially maintain the target organs (Smith[20], 1932). Therefore, after damage to the pituitary by irradiation any remaining biological activity is determined by that part of the pituitary which received the least dose. The average doses stated in this paper therefore do not represent the actual minimum doses that are able to destroy individual anterior pituitary cells but rather the doses which must be given to allow for the inaccuracies of the method.

Because of the scattering and the lack of perfect alignment of the beam one expects fluctuations in the actual amount of radiation received by the pituitaries of the individual animals. To obtain an approximate figure for these variations the overall dose received by the pituitary has been measured by assaying the radioactivity induced in the gland itself[21,22]. The deuteron particles induce activity in the atomic nuclei of the tissue. With no limiting aperture in the path of the beam (whole-head irradiation), there is no problem of positioning, and scattering can be neglected, so that the dose recorded in the monitoring ionization chamber is the dose delivered to the pituitary. Immediately after exposure of several animal heads to 10,000 rep doses of deuterons with no limiting aperture in the path of the beam the pituitary was removed, weighed, and then counted in a scintillation counter. A typical postirradiation decay curve for the pituitary is shown in Fig. 6. This curve illustrates three components with different half lives. The C^{11} activity is induced chiefly from the irradiation of C^{12} isotope by deuterons and has a 20.5-minute half life.

With a second group of rats the usual aperture for pituitary irradiation was inserted and the animals were carefully aligned by the technique described above. The same dose was given and the pituitary was again removed immediately and counted. Table I shows that the pituitaries irradiated with the limiting aperture in place have an average of only 63 percent of the activity induced in the pituitaries after whole-head irradiation (standard deviation of 14 percent). No doubt the lesser activity induced and the greater fluctuations when the beam was limited to the pituitary are due to lack of perfect alignment and to scattering of the beam at the edge of the aperture. From the activation analysis it is assumed that the probable dose received by the pituitaries is 63 percent of the measured dose. In this paper only the probable dose will be given.

The fact that only 63 percent of the radiation is intercepted by the pituitary implies that the immediately surrounding tissues receive approximately 37 percent. It is obvious from Fig. 5 that the dose falls off rapidly immediately outside the pituitary.

Biological Technique

Male rats of the Long-Evans strain were subjected to pituitary irradiation at 28 days of age. Doses of 3,150 to 25,200 rep were given to the pituitaries. The controls consisted of normal rats which received no irradiation,

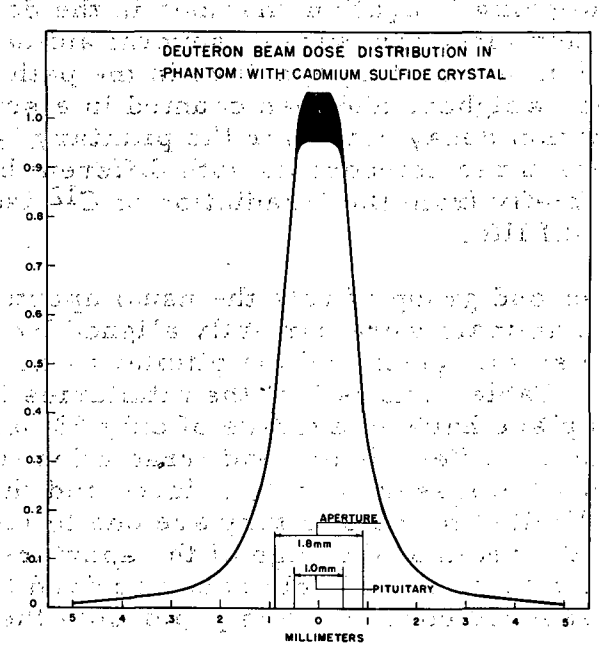

Fig. 5

Beam intensity distribution in lucite phantom measured in the position of the rat pituitary. The distribution is plotted in relative units. The measurements were carried out with a cadmium sulphide crystal.

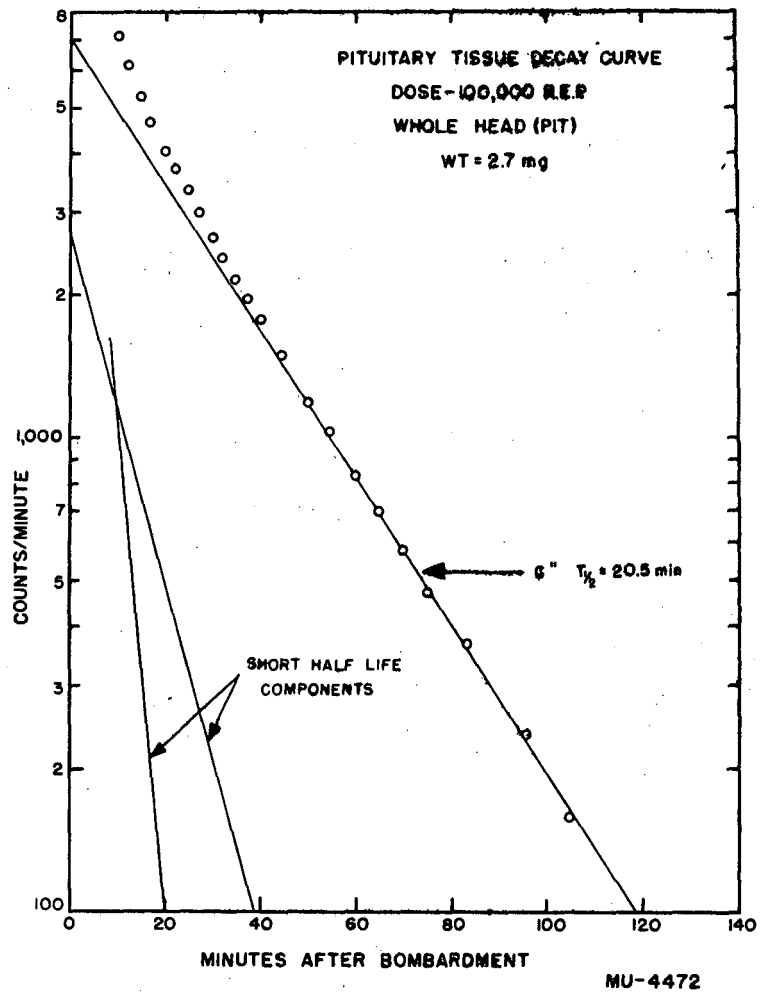

Fig. 6

The decay curve of a single pituitary irradiated with 10,000 rep deuterons to the whole head. Immediately after irradiation the pituitary was removed, weighed and counted directly in a scintillation counter.

Table I

Induced C^{11} Activity of the Rat Pituitary After Exposure to 10,000 rep Measured Dose Through Large and Small Beam Apertures.

Animal No.	Type Exposure	Exposure Time (Sec.)	Measured Dose rep	Pituitary Wt. mg.	Counts per minute, mg. and measured rep	% induced radioactivity in pituitary
1	Whole Head	169.5	10,000	3.3	0.038	100
2	"	235	"	2.9	0.038	100
3	Small Aperture	249.5	"	3.4	0.018	48
4	"	199	"	3.3	0.021	56
5	"	212	"	3.2	0.025	64
6	"	145.5	"	2.5	0.032	85

Average amount received by pituitary with small aperture 63 ± 14%

rats which received the same background irradiation as the experimental animals (1/2 or less of the dose delivered to the pituitary) and surgically hypophysectomized rats. Approximately 1,000 rats were used in this study. All animals were fed a complete diet*. Periodically the general appearance, weights and tail lengths were recorded. At autopsy the testes, seminal vesicles, prostrate, adrenals, thyroid, thymus and pituitary were dissected, weighed and fixed for histologic study. The testis and thyroid were fixed in Bouin's fluid and imbedded in paraffin. The adrenals were fixed in neutral formalin and sectioned by the frozen method to be stained for lipid.

In one group of rats given 12,600 rep to the pituitary the circulating red cell volumes were determined in order to demonstrate whether the anemia which characteristically follows surgical hypophysectomy[24] could be shown to develop after injury of the pituitary by irradiation. The red cell volumes were determined by the Fe^{59} labeled red cell dilution method[25].

Results

By the fifth day after irradiation there were small ulcerations on each side of the head where the beam penetrated (Fig. 7). These ulcerations healed after three weeks and in no instance was a permanent ulceration observed. The new hair in these areas was white. These patches of white hair are shown in Fig. 7 and they demonstrate the direct path of the beam. The animals receiving pituitary irradiation showed none of the effects of total body irradiation. No diarrhea, postirradiation hypothermia or transient loss of weight were observed.

Changes in production of growth hormone by the pituitaries of the irradiated rats were estimated by measurements of gain in weight and tail length. The average gains in weight and increase in tail length of groups of rats given doses of 3,150, 9,450 and 18,900 rep to the pituitary are plotted in Figs. 8 and 9. These figures show that at each dose level (from 3,150 to 18,900 rep) the weight gain and tail length attained are below normal, the retardation being progressively more at higher doses. The weight curve was modified by radiation in two distinct ways. Within a few days after irradiation there was an abrupt dose-dependent decrease in the daily rate of weight gain followed by a more gradual decrease in growth rate until it approached that of surgically hypophysectomized controls (Fig. 10). There is no evidence of recovery in growth in any of the experiments, even with the lowest doses. Some of the rats which survived the highest doses showed as complete failure to gain in weight and length as did the surgically hypophysectomized controls. Fig. 11 shows one such animal 5 months after irradiation (18,900 rep), flanked by normal and surgically hypophysectomized controls of the same age. Such irradiated animals were comparable to surgically hypophysectomized rats, with small well formed bodies without obesity and with soft infantile fur.

The pituitary target organs were studied in groups of rats given from 3,150 to 31,500 rep to the pituitary. Groups of animals were autopsied

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*Diet I consists of: Ground whole wheat, 67.5 percent; casein technical, 15 percent; skim milk powder, 7.5 percent; sodium chloride (iodized), 0.75 percent; calcium carbonate, 1.5 percent; melted fat, 6.75 percent; fish oil (vitamin A and D concentrate), 1.0 percent.

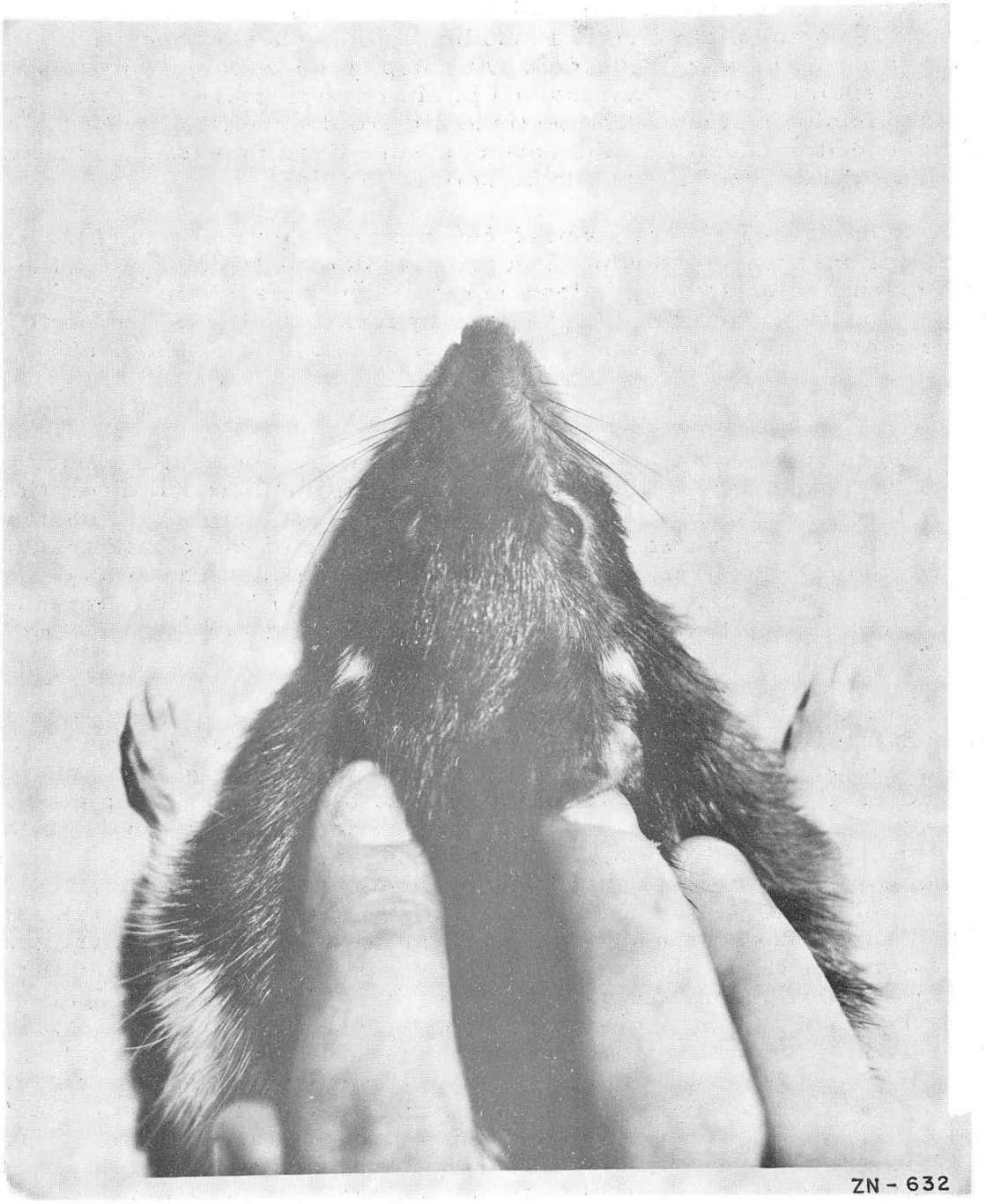

Fig. 7

Areas of white hair at the point of entry (left) and exit (right) of the deuteron beam. These patches of white hair appeared after the small ulcerations which formed at the same sites had healed.

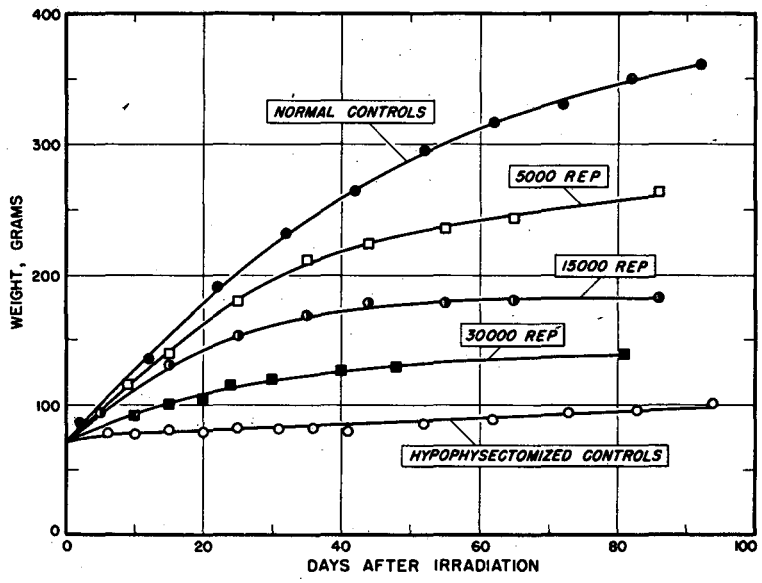

Fig. 8

Average gain in weight of groups of rats given various doses to the pituitary as compared to normal and to surgically hypophysectomized controls of the same age.

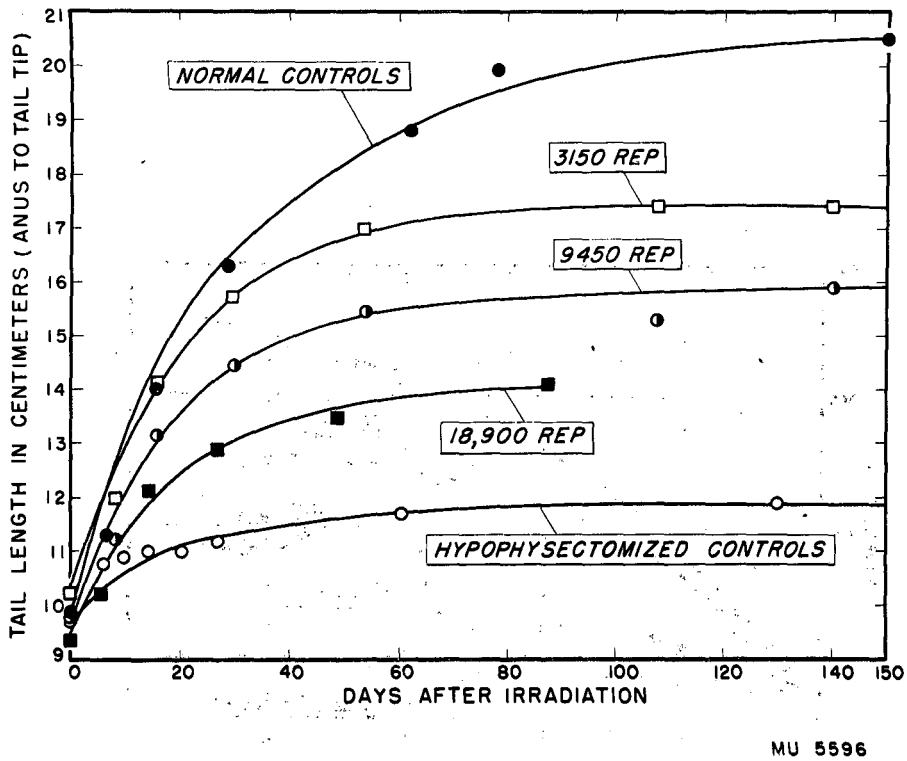

Fig. 9

Average gain in tail length of groups of rats given various doses of irradiation to the pituitary as compared to normal and to surgically hypophysectomized controls of the same age. These are the same groups as illustrated in Fig. 8.

at intervals from 4 to 270 days after irradiation. From these autopsies it was found that only at very high doses (25,200 rep or more) did the irradiation cause cessation of pituitary function within a few days. At lower doses (12,600 rep or less) it was found that complete atrophy of the pituitary target organs occurred only after the lapse of several months. Fig. 12 shows the average weights of some of the endocrine organs and the pituitaries at various intervals after irradiation of the pituitary with 12,600 rep. Up to 40 days postirradiation there was evidence of only moderate damage to pituitary function, but after that time the weights of the pituitary and pituitary target organs rapidly declined. Examination of the figure indicates that there was an immediate effect on growth of the thyroid, whereas the testes and dependent organs showed no great difference in weight from the weight of the testes of normal rats of comparable age during the first 45 days. At a later time all of the target organs became atrophic, following atrophy of the pituitary itself. By 130 days there was almost complete atrophy of all pituitary target organs. The testes descended into the scrotum, enlarged and differentiated normally during the early period, subsequently atrophied and returned to the abdominal cavity. The speed with which atrophy occurred varied with dose. In one half of the animals, testes had atrophied and left the scrotum three months after irradiation of the pituitary with 12,600 rep. After 6,300 rep testes of only one half of the animals had withdrawn from the scrotum 10 months postirradiation. Thus the different doses resulted in similar final degrees of pituitary destruction, but required different postirradiation intervals to become manifest. The only outstanding difference in the effect of pituitary irradiation on the different pituitary functions was that body growth and growth of the thyroid were affected almost immediately at all doses, whereas the testes were the last of the target organs to atrophy.

Forty days after treatment the microscopic appearance of the pituitary target organs of animals given large doses of irradiation to the pituitary (18,900 and 25,200 rep) was that which is characteristic after surgical hypophysectomy. The thyroid epithelium became flattened and the colloid compact, the adrenal cortex became thin and a characteristic subglomerular lipid-free zone was present, and the testis showed atrophy of interstitial tissue as well as failure of spermatogenesis in the seminiferour tubules. For representatives organ weights, see Table II.

In animals given smaller doses (12,600 rep), the microscopic examination of the target organs revealed the same sequence of development of atrophy, paralleling the change in organ weights. In the early postirradiation period the histology of the thyroid, adrenal and testis was not different from that of normal controls. At later periods these organs became more atrophic. By 4 months or more the microscopic appearance of the target organs of the animals receiving the lower doses of irradiation approached that of the completely hypophysectomized rat.

At 6,300 rep to the pituitary and a postirradiation period of 9 months, the pituitary target organs had undergone almost complete atrophy. The thyroid epithelium was flattened, being only slightly higher than after surgical hypophysectomy. The adrenal cortex was depleted of lipid almost to the same degree as after hypophysectomy, showing the coarse lipid granules and subglomerular lipid-free area. The interstitial tissue of the testis was deficient and the tubules were atrophied to two cell layers with only a few desquamating primary spermatocytes.

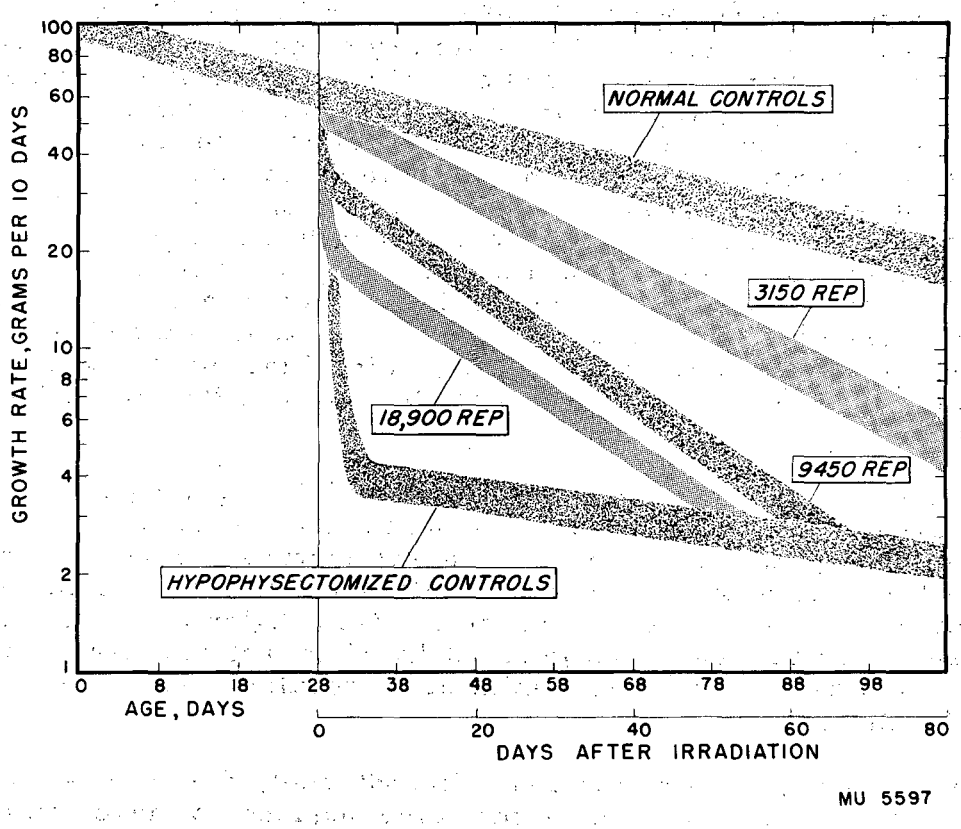

Fig. 10

Average growth rates for rats reconstructed from weight data. Because of the great natural fluctuation in animal weights, this chart is very inaccurate. Nevertheless it shows a striking difference in growth rates of normal and of surgically hypophysectomized rats, also the drop in growth rate immediately after irradiation with increasing doses. The initial drop in growth rate increases with dose. At a later time the growth rate decreases faster than normal, indicating progressive decrease in function of the pituitary.

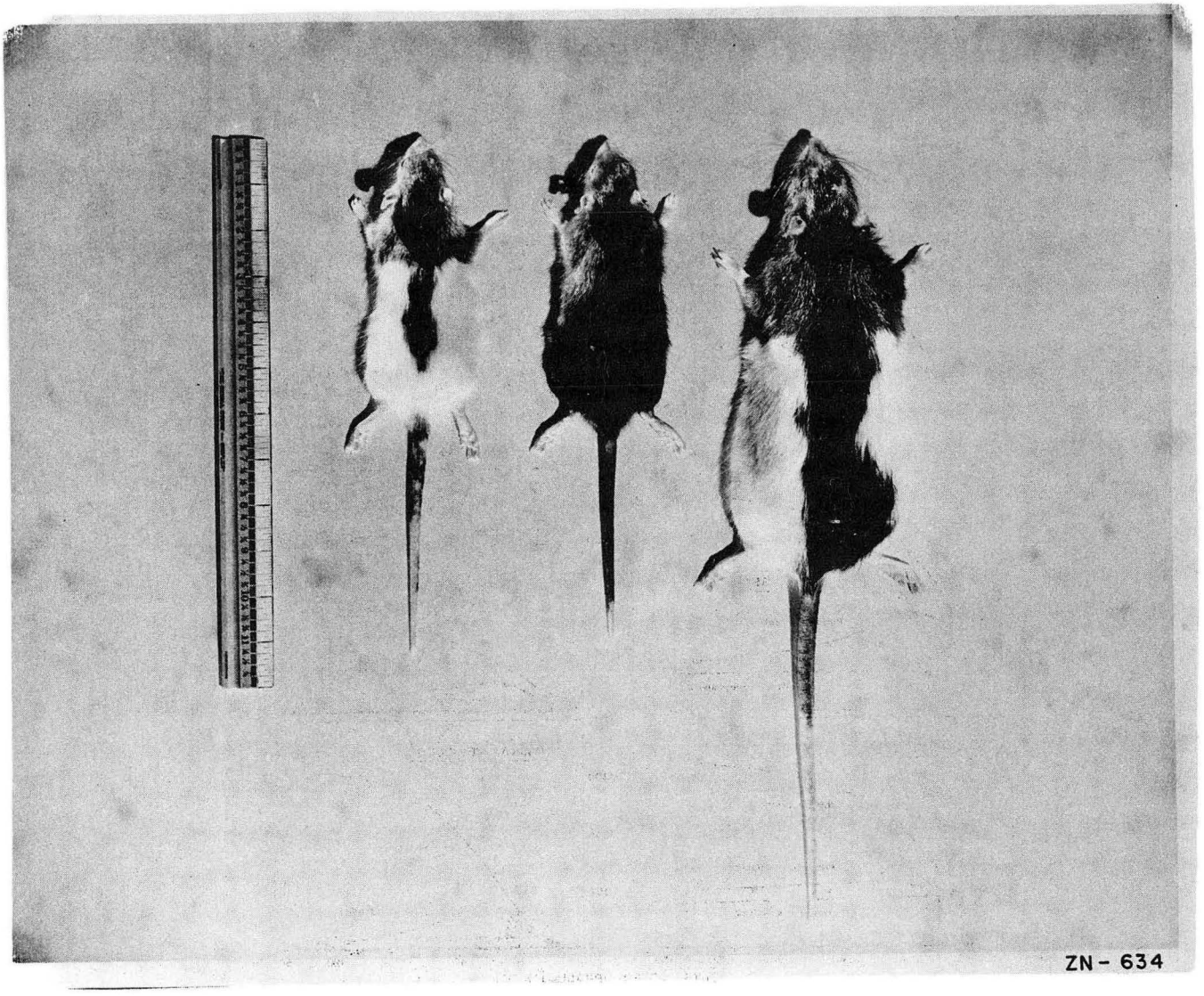

Fig. 11

A rat (middle) 5 months after irradiation of the pituitary with 30,000 rep, flanked by normal (right) and surgically hypophysectomized (left) controls of the same age. The irradiated rat was a typical hypophysectomized rat with a small well formed body with soft infantile fur and without obesity.

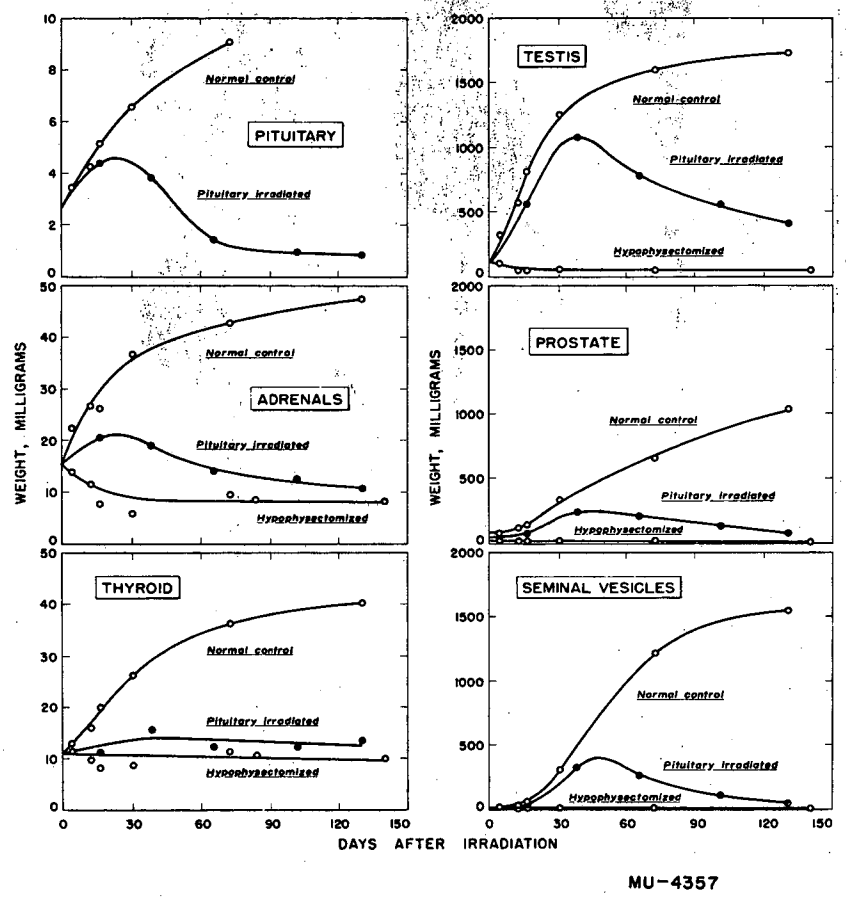

Fig. 12

Average weights of the pituitaries and some of the endocrine organs at various intervals after irradiation of the pituitary with 12,600 rep. Each point represents the average of 9 rats.

Table II

Representative Organ Weights of Rats Given High Doses of Irradiation to the Pituitary, Normal Rats, and Surgically Hypophysectomized Rats, 75 Days after Radiation.

Treatment	Body Weight g	Tail Length cm	Adrenals mg	Thyroid mg	Testes mg	Seminal Vesicles mg	Prostrate mg
18,900 rep	77	11.0	9	8	122	7	25
25,200 rep	75	11.6	6	6	136	6	28
Surgical Hypophysectomy	93	11.6	7	7	107	5	26
Normal	303	19.1	35	23	3181	833	624

Circulating red cell volumes were determined two months after irradiation of the pituitary with 12,600 rep in order to demonstrate whether the anemia which characteristically follows surgical hypophysectomy could be shown to develop after irradiation of the pituitary. The red cell volumes were determined by the Fe^{59} labeled cell dilution method in six irradiated rats, in five normal rats and in five surgically hypophysectomized controls. The hematological data are presented in Table III. It can be seen from the table that at this period after irradiation there was a small but definite anemia as judged by the hemoglobin, hematocrit, and circulating red cell volume. These determinations were done at a time when pituitary atrophy was only partial.

No gross injury to the cranial nerves in the path of the beam was seen and no functional aberrations were noted. The rats given the small total body irradiation (fast neutron background)equal to the highest background irradiation received by the experimental groups showed no significant changes in growth or in endrocrine organs even after 9 months.

The normal controls of this series survived 2 years or more. Half of the 100 hypophysectomized rats used as controls in this series died within 25 days after operation. Half of the animals given 25,200 rep also succumbed within 25 days; after 18,900 rep half survived after 45 days. After 12,600 rep most of the animals survived longer than 200 days but the rate of death was greater than in the normal control.

To determine whether the results obtained by pituitary irradiation could be explained by unintentional injury to surrounding tissue, several groups of rats were subjected to irradiation of the area surrounding the pituitary (a beam of the same size being directed above, anterior or posterior to the pituitary). It was found that doses of 12,600 and 18,900 rep to the surrounding area resulted in rapid death of all animals whereas doses of 3,150 rep did not cause death and resulted in no significant changes in pituitary weight, growth rate or state of the pituitary target organs.

Pituitary Histology

Pituitaries of 78 experimental and 44 control rats were examined histologically. The glands were fixed in Zenker-formol, sectioned at 4 micra after nitrocellulose embedding, and stained with a modified Mallory-Azán stain (Koneff, 1938).[23]

The effect of ionizing irradiation was studied on day 4, 12, 16, 25-30, 163-200 and 279-286 following irradiation with a 6,300 rep dose; on day 4, 12, 16, 25-30 and 72 after a 12,600 rep dose, and on day 25-50 following irradiation with massive doses of 18,900 and 25,200 rep.

Definite postirradiation effects could be seen even grossly at autopsy. Glands removed soon after irradiation (12-16 days) were already slightly smaller than their controls and were hyperemic. Pituitaries of rats sacrificed after a longer posttreatment interval, or those from animals subjected to the highest doses of irradiation, were considerably reduced in size, flattened, often pale, and without clear outline of individual lobes. The majority of rats subjected to 18,900 or 25,200 rep doses (5 of 8) showed considerable distention of the

Table III

Hematological Data on Normal Rats, Rats Given 12,600 rep to the Pituitary, and Surgically Hypophysectomized Controls. Irradiated or Operated Two Months Previously.

Treatment	Body Weight g	Hemoglobin g/100 ml	Hematocrit %	Red Blood Cell Volume/100 gm. Body Weight ml
Normal	291	14.2	48.4	2.18
	289	14.0	45.5	2.44
	334	13.8	47.0	2.57
	271	13.6	46.9	2.26
	321	11.2	42.5	2.02
	301	13.4	46.1	2.29
Irradiated	146	12.6	43.3	2.04
	140	10.6	37.0	1.66
	136	11.8	40.4	1.99
	135	13.6	43.5	2.12
	170	13.2	40.6	1.89
	120	12.4	38.6	1.90
	141	12.4	40.6	1.93
Hypophysectomized	110	10.0	33.9	1.58
	101	11.8	36.4	1.58
	86	10.2	31.5	1.36
	74	10.2	31.0	1.41
	76	10.6	31.7	1.42
	68	10.4	33.0	1.58
	86	10.5	32.9	1.49

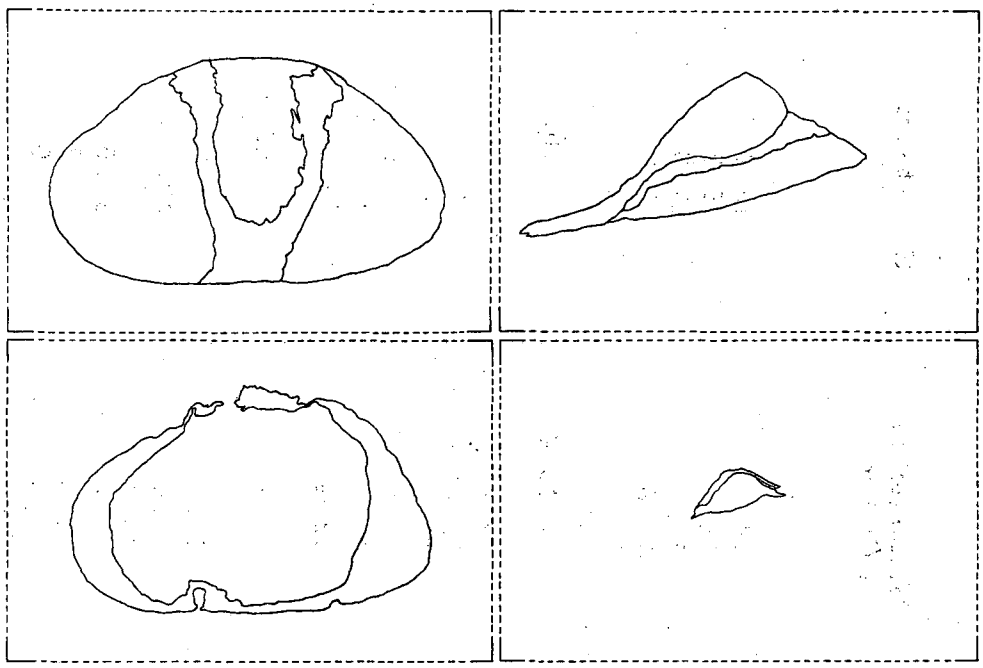

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Fig. 13

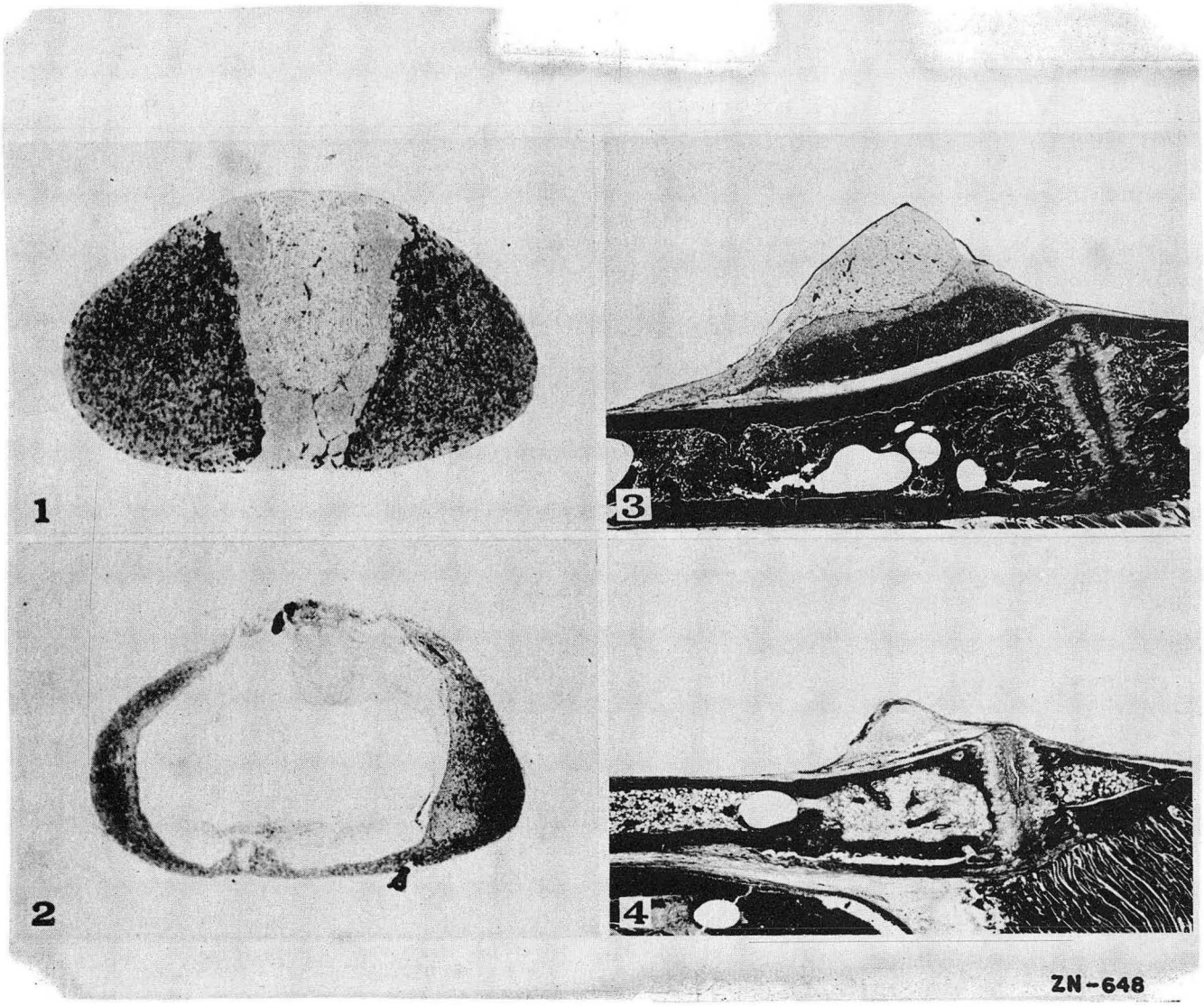

Fig. 13

Horizontal and sagittal sections of pituitaries at magnifications x 23 and x 18 respectively; Mallory-Azan stain.

1

Pituitary of normal 53-day-old rat.

2

Pituitary of an experimental rat 25 days after irradiation with 18,900 rep. Note distention of cleft and reduction of the anterior lobe tissue.

3

Sagittal section through the pituitary of a 259-day-old, normal rat in its normal position. The stalk and all parts of the hypophysis are clearly shown.

4

Sagittal section through the pituitary of an experimental rat 256 days of age, 227 days after irradiation with 18,900 rep. The parenchyma of the pituitary has been replaced by scar tissue.

cleft, which was filled with colorless or blood-tinged fluid (Fig. 13b). It was also noted that after prolonged post irradiation periods, there was a firm adherence of covering membranes to the pituitary tissue.

The effect on pituitary weight of different doses of ionizing irradiation is shown in Table IV.

Table IV

Weights of Irradiated Pituitaries at Different Postirradiation Intervals

Days after irradiation	4	12	16	25 to 39	49	72	163	200	279 to 286
Normal controls (mg)	3.5	4.3	5.2	6.5		9.1	10.6	11.2	12.8
6,300 rep	3.4	4.2	3.6	4.0			1.1	1.0	1.4*
12,600 rep	3.3	3.4	3.5	3.0		2.8			
18,900 to 25,200 rep				2.1	1.1				

*Actual weight of the pituitaries was probably less than the figures of the table show because some fragments of the covering membranes could not be removed.

The effects of irradiation with 6,300 rep will be described in some detail as the effects of this dose on the pituitary were studied at intervals throughout a considerable span of time.

Morphological changes in the pituitaries 4 days after irradiation were slight though detectable. Some pyknosis in chromophils, increased numbers of mitoses, abnormal mitoses and degranulation in acidophils were seen.

By day 12 the effects were more pronounced. Mitoses were found in all lobes of the hypophysis. Degranulation of chromophils, especially of acidophils, was obvious. Vacuolated basophils and degenerating acidophils were seen throughout the anterior lobe.

By day 16 the glands were hyperemic. Brilliance of staining was lost. Acidophils were decreased in number. They were degranulated and were degenerating. Some basophils were markedly degranulated; others showed cytoplasmic vacuolation resembling that found in "castration" and "thyroidectomy" cells or showed fragmentation of the cytoplasm and pyknosis of the nuclei. Atypical mitoses were found in all lobes and apparently were responsible for the appearance of cells with varying degrees of nuclear and cytoplasmic hypertrophy or atypical nuclear shape*. It was also noted that by this

*Attention should be drawn to the excellent demonstration of Wm. Bloom and R. E. Zirkle (Proc. Am. Assoc. of Anatomists, Anatomical Record, 1953) of the disordered mitosis induced by irradiation in vitro of the chromosomes of the newt resulting in daughter cells with abnormal nuclei.

time there was a definite lack of uniformity of cell and nuclear size as well as staining reaction of individual cells of anterior and intermediate lobes. Pools of homogenous material, stained blue by Mallory, were found among the parenchyma cells in several glands.

After 25 to 30 days all the alterations noted earlier were still present, but were more marked. Young scar tissue had begun to appear in the parenchyma, evidently at the site of destruction of the parenchyma.

It is of interest to note that the damaging effect of irradiation on the parenchymal elements was similar in all three lobes of the pituitary. Abnormal mitoses, multinuclear cells, giant cells, "foam cells", hypertrophy and abnormalities of the nuclei were common in all parts of the glands. Cells of the various lobes, thus changed, could not at times be distinguished morphologically. Alterations found in different cells of the pituitary parenchyma are shown in Fig. 14.

After 163 to 286 days the average pituitary weights were reduced to 1.2 mg. In 18 rats the weights ranged from 0.8 mg. to 1.7 mg. The covering membrane was thickened by scar formation and fibers were continuous with the scar tissue within the parenchyma (Fig. 16d). The lack of distinct subdivision of the glands into individual lobes, noted at autopsy, was due in part to thickening of the covering membrane, and in part to localized irregular invasion of tissue from one lobe by that from another. The anterior lobes were often markedly hyperemic. Histologically, small localized hemorrhages were gound in the glands, especially in the anterior lobe.

All structural changes and degenerative processes, previously described for individual cells, were also found at this postirradiation period. The continued presence of abnormal mitotic divisions may be interpreted either as due to the initial injury of resting cells, which was not evidenced until the infrequent mitoses occurred, or may be interpreted to mean that the injurious effect of the radiation was carried down through succeeding generations of daughter cells during the 6 to 9 months postirradiation.

Although degenerative changes in the three different parts of the pituitary were similar and frequently led to the formation of indistinguishable cells, the three lobes still maintained sufficient characteristics so that they could usually be recognized. The anterior lobe, in addition to the hemorrhages and scar tissue previously mentioned, consisted almost entirely of nongranular cells. Some of them, in contrast to typical chromophobes, varied considerably in size, were sharply outlined and often showed a negative image of the Golgi apparatus. Among these atypical chromophobes occasional abnormal acidophils and basophils were distinguishable. In a small region, the so-called gonadotropic area in the anterior aspect near the pars intermedia, clumps of easily recognizable basophils were present. The majority of these cells, however, were signet ring forms or had darkly stained peripherally-located granular material. The intermediate lobe had lost its usual uniformity of cellular and nuclear size and staining reaction, but could be recognized by occasional groups of typical cells. In the posterior lobe the localized hemorrhages and degenerative changes in the cells constituted the usual alterations.

The effects on the pituitary of a dose of 12,600 rep will not be described separately as there were only slight quantitative and no qualitative differences from the effects of 6,300 rep doses.

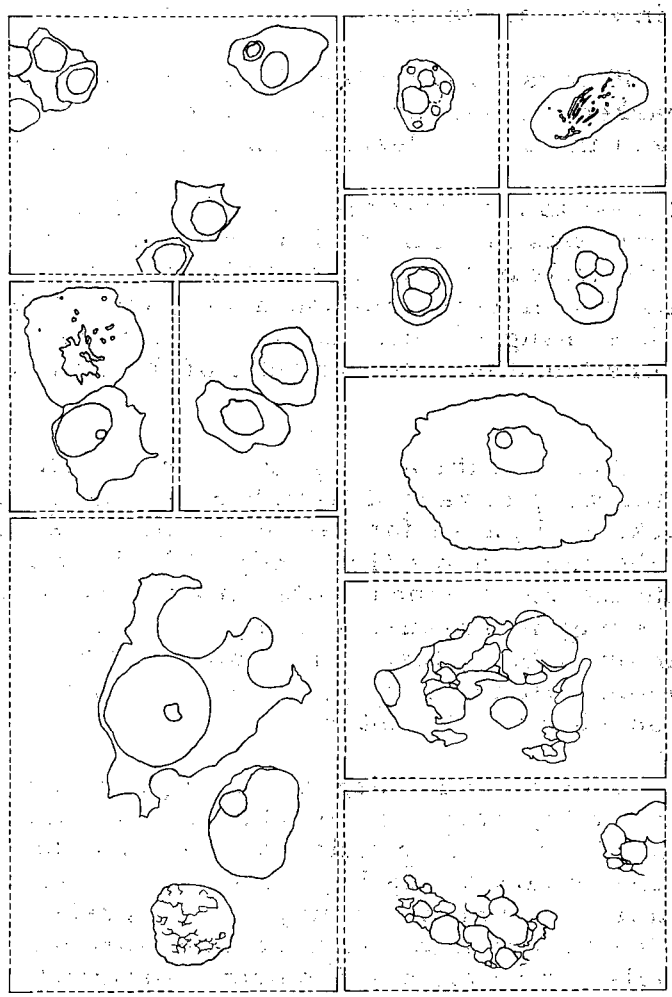

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Fig. 14

Photographs taken with oil immersion objective at magnification x 1000; Mallory-Azan stain.

1

Anterior pituitary of a normal rat 72 days old, showing normal appearance of a acidophils; b basophils; and c chromophobes. The remaining figures (2 to 11) show the changes found in different hypophyseal cells 12 to 286 days following irradiation.

2 and 6

Anterior pituitary cells in abnormal mitotic division. Note scattered position and abnormal shape of individual chromosomes.

5, 7 and 8

These figures show abnormal number of nuclei in single cells. Fig. 5 (intermediate lobe) shows a cell containing (within the 4 micra section) 8 nuclei of different sizes. Figs. 7 and 8 show anterior lobe cells containing 2 and 3 nuclei.

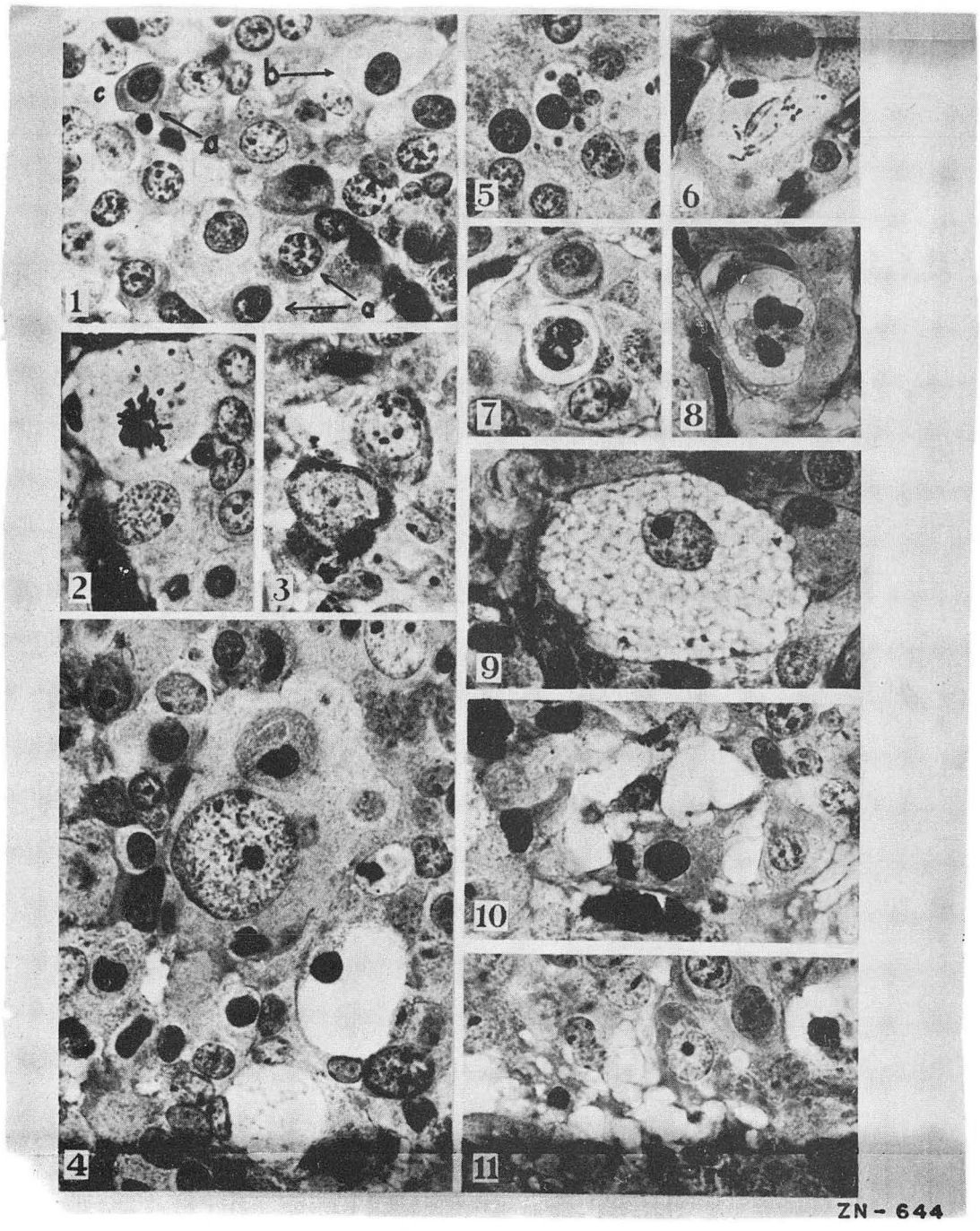

Fig. 14

3

Two anterior lobe acidophils which show general nuclear and cytoplasmic size increase, each containing large Golgi apparatus.

2, 4 and 9

These figures show hypertrophied anterior lobe cells (basophil, acidophils and chromophobes) from irradiated pituitaries. Note that cytoplasm, nucleus and nucleolus all show hypertrophy. Also note that in some cases, hypertrophied cytoplasm has surrounded neighboring smaller cells.

4, 7, 8, 9, 10 and 11

These figures show different stages of cytoplasmic degeneration. Fig. 9 shows shrinkage of the cytoplasm. Fig. 8 shows early cytoplasmic degeneration. Fig. 4 shows late degenerative change. Fig. 9 shows multiple uniform-sized vacuoles ("foam cell"). Figs. 10 and 11 show late degenerative changes in basophils.

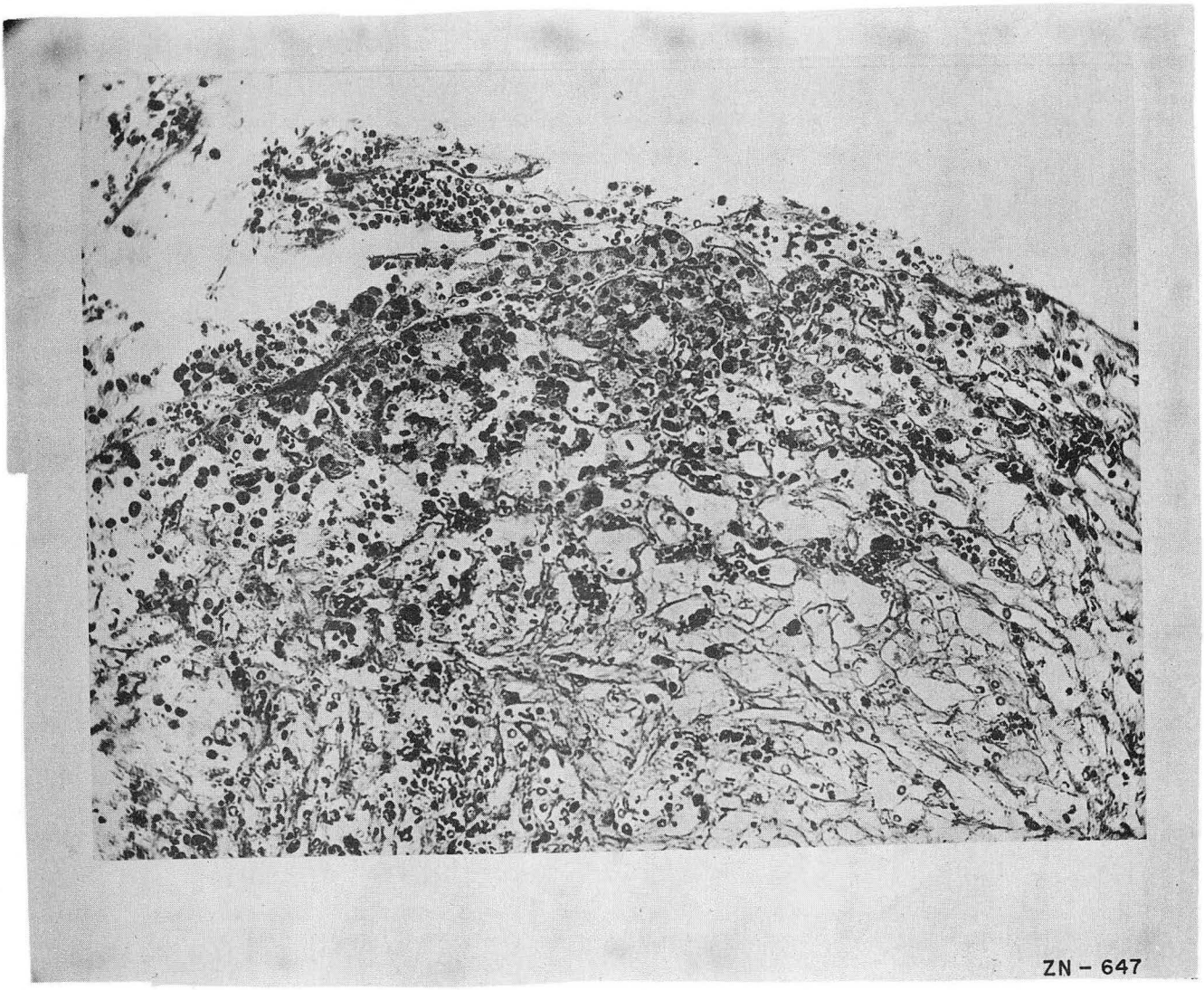

Fig. 15

Photograph taken with 8 mm objective, magnification x 200; Mallory-Azan stain.

Anterior pituitary of a rat on the 49th day following irradiation with 25,200 rep. Anterior pituitary parenchyma has been replaced by scar tissue except a few scattered small nests of cells (upper part of the photograph). In such regions some chromophobes, acidophils and basophils could be recognized. (For details, see Figs. 16 and 17.)

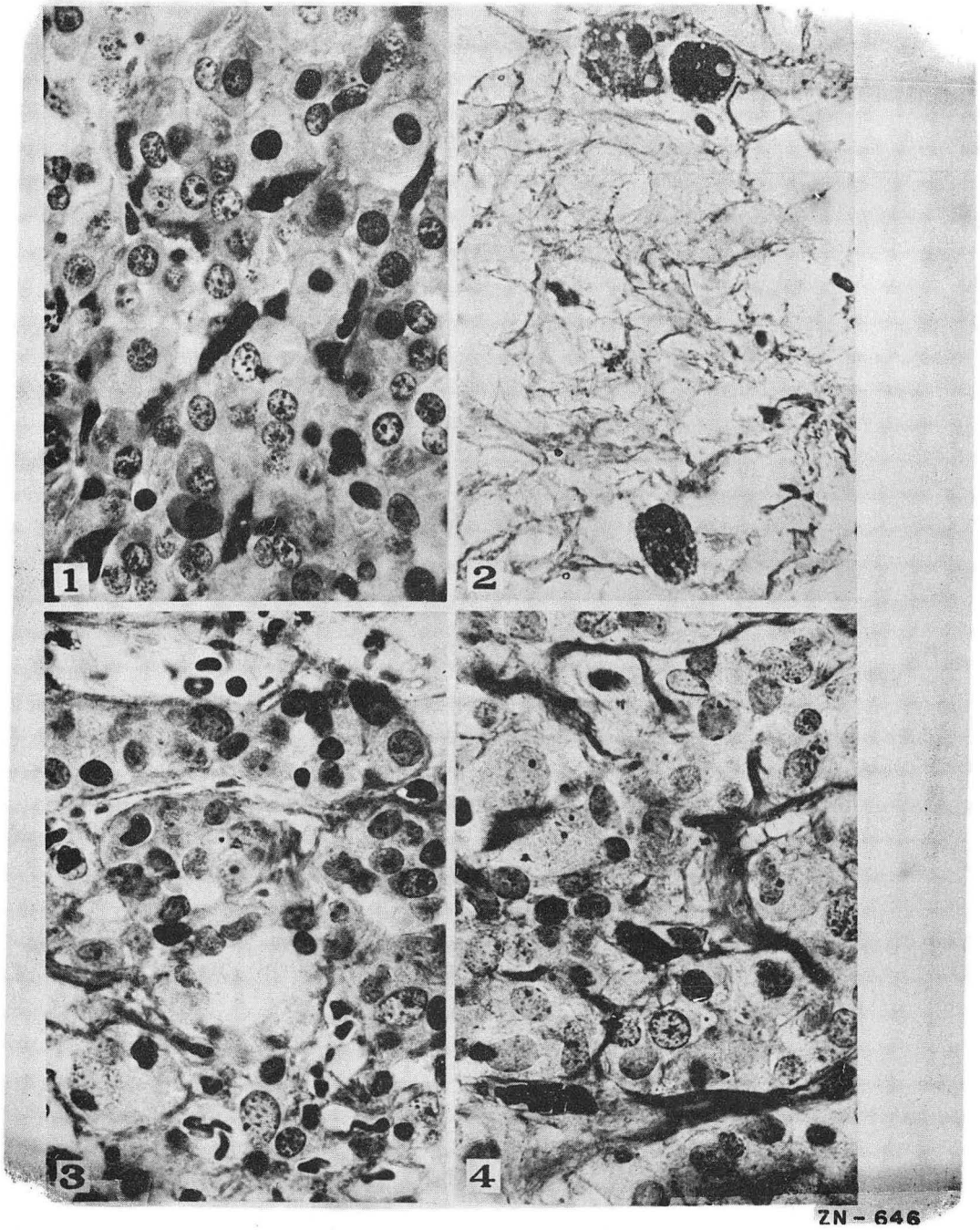

Fig. 16

Photographs taken with oil immersion objective at magnification x 800; Mallory-Azan stain.

1

Section of anterior pituitary of a normal 72-day-old rat. Note general appearance and distribution of cells.

2

Section of anterior pituitary of a rat on the 49th day following irradiation with 25,200 rep (same pituitary as shown in Fig. 15). Note complete absence of parenchymal cells; presence of young scar tissue, three macrophages.

3

Same pituitary as Fig. 2. This figure shows remnant of surviving anterior lobe parenchyma. Note abnormality of size and shape of the chromophobes. Also note degenerating cells. No recognizable acidophils or basophils have been found in this particular field.

4

Anterior pituitary of a rat 286 days following irradiation with 6,300 rep. Note that only chromophobes are present. Also note heavy scar tissue.

Changes in pituitaries of rats subjected to massive doses, 18,900 and 25,200 rep, were studied only between 25 and 50 days following the irradiation. At 25 days, owing to the great distention of the vestigial cleft, the intermediate lobe was usually stretched and often fragmented. Scarring was more pronounced a month after irradiation than it was after the lower doses, and the characteristics of the scar tissue suggested that it had appeared earlier. There were small hemorrhages in the anterior lobe in close proximity to the scarred areas. Surviving anterior pituitary tissue consisted mostly of abnormal nongranular cells with occasional chromophils recognizable by a few characteristically stained granules. Intermediate and posterior lobes showed profound degenerative changes accompanied by the appearance of abnormal cells and giant cells.

Two months (50 days) after irradiation with 25,200 rep all that remained of the pituitary was a small, pale, transparent fragment of tissue in which the individual lobes were not distinguishable. Histological examination showed obliteration of the general pattern of subdivision into lobes. This was due to the extensive degeneration of the parenchyma and heavy scar formation which was in continuity with the surrounding membranes. Small numbers of surviving parenchyma cells still recognizable occurred in nests measuring at most 170 x 580 and 200 micra. Even these cells were atypical and usually nongranular (Fig. 15).

Alterations in all lobes of such pituitaries are shown in Figs. 13, 15, 16, and 17.

In summary, the injurious effects of deuterons on the pituitary were detectable within 4 days after treatment with 6,300 rep. Changes were more definite on the 12th day and well advanced by day 16. Thereafter the damage to all parts of the pituitary appeared to be progressive throughout the remaining period of observation (up to the 286th day). The earliest and most extensive damage was observed in the acidophils. Basophils were less affected by the deuterons and after 286 days could still be found in nests at the anterior aspect of the pituitary in close proximity to the intermediate lobe. They showed varying abnormalities in their structure, but were possibly capable of function.

Massive doses of deuterons (18,900 and 25,200 rep) produced the same qualitative damage to the individual cells as the lower doses. However, the effects were shown more rapidly and were more extreme.

It should be noted that although mitoses were present 6 to 9 months after irradiation, they were abnormal in appearance and abnormal cells resulted. There was no evidence within the period of these observations of recovery of the parenchymal cells.

Discussion

Only by giving extremely high doses of irradiation (18,900 rep or more) was it possible to bring about complete and immediate destruction of the pituitary of the rat, as judged by growth stasis comparable to that after hypophysectomy and by inhibition of differentiation of the testis, the most sensitive of the target organs to small amounts of functional pituitary tissue. At lower doses (12,600 rep or less) the pituitary continued to increase in size for some time and then proceeded to undergo atrophy. The greater the dose the more rapid the atrophy.

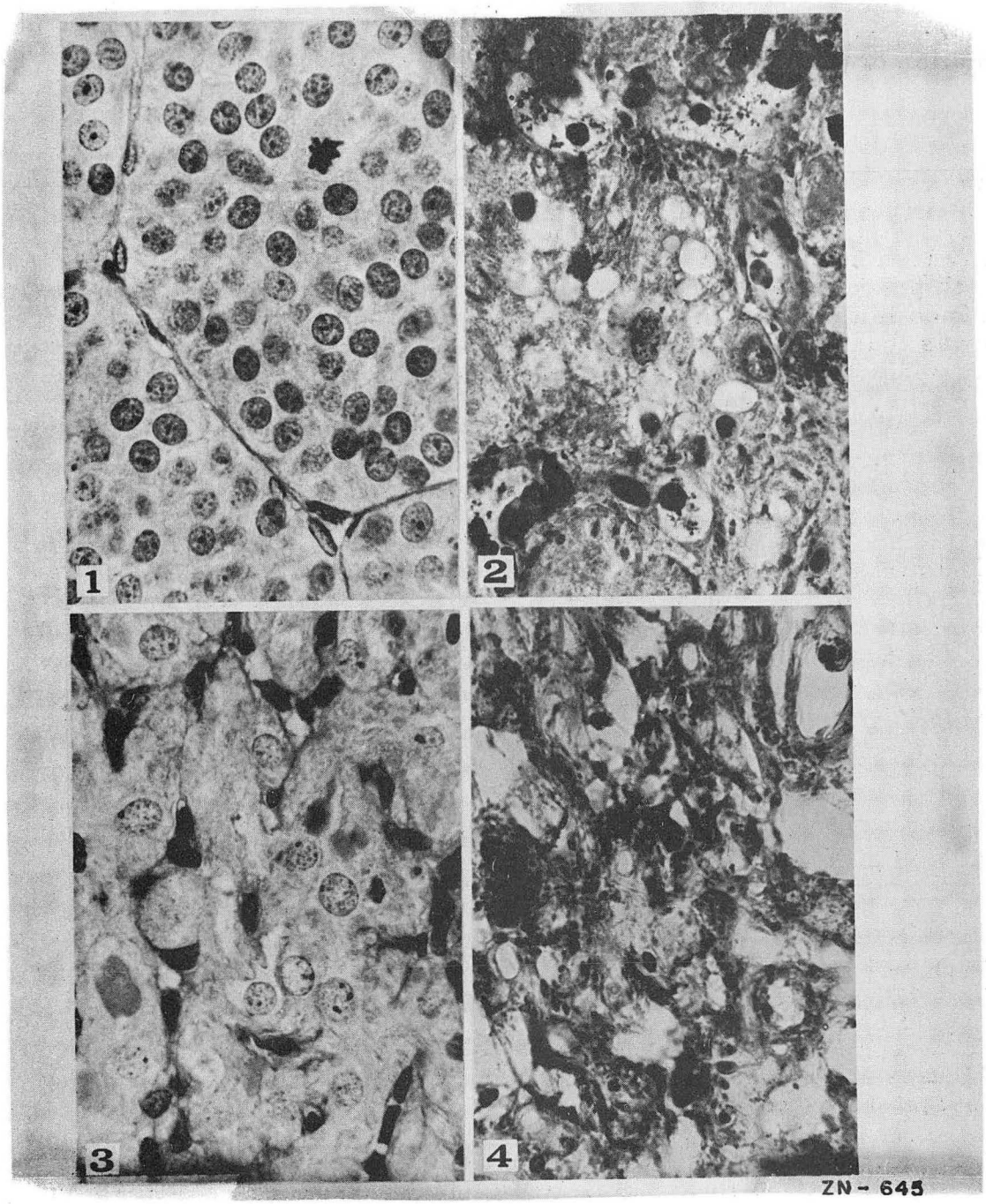

Fig. 17

Photographs taken with oil immersion objective at magnification x 800; Mallory-Azan stain.

1

Field selected to show typical appearance of the intermediate lobe of a normal 72-day-old rat.

2

Intermediate lobe of a rat on the 49th day following irradiation with 25,200 rep. Note that the normal pattern of tissue is lost; remaining cells show varying degrees of degeneration; scar formation.

3

Field selected to show posterior lobe of a normal 72-day-old rat.

4

Posterior lobe of a rat on the 49th day following irradiation with 25,200 rep. Note complete replacement of parenchyma by scar tissue; a few macrophages present.

It was hoped at the onset of this work that a differential radiation sensitivity of the various cell types of the anterior pituitary might be demonstrated. However, at the doses employed, all cell types and all lobes were markedly injured. There was some evidence that the pituitary basophils were slightly less sensitive to irradiation than acidophils, as a few survived doses at which practically no acidophils could be identified. The only outstanding difference in the effect of pituitary irradiation on the different pituitary functions was that there was some effect on body growth and thyroid morphology manifested within a few days, whereas the testis was the last target organ to atrophy at any given dose. This might be interpreted as a differential effect on pituitary function; however, it is more probable that the irradiated pituitary gives the same end result as incomplete hypophysectomy, although the entire pituitary was irradiated. Smith[20] showed that only minute fragments of the anterior pituitary need remain for stimulating effects to be shown by the testes and adrenal. The thyroid and body growth were far less sensitive to minute fragments. Pituitary weight and histological examination have shown us that at lower doses marked destructive changes in the pituitary become manifest only after long periods. During the earlier postirradiation period the degranulated anterior lobe cells were still producing hormone in amounts that were adequate to maintain the more sensitive target organs. Later, when the destructive changes in the pituitary were more advanced, even the more sensitive target organs ceased to respond. Although it has been reported by Ghilarducci[10] (1922) and Lawrence[6] (1937) that regeneration of the pituitary occurs after small doses of irradiation, no evidences of regeneration have been observed at the doses and time intervals employed in these experiments.

The early reports suggesting stimulation of pituitary function after irradiation appear to have employed much smaller doses than the smallest used in this series. It is possible that these workers who reported stimulation of body growth after pituitary irradiation (Rahm[7], 1922, and Epifanio[8], 1932) could have been observing the development of obesity resulting from irradiation damage to the hypothalamus, since the criteria of increased growth was increased weight gain. There was no tendency to develop obesity in any of the rats of this series.

It must be remembered that the conclusions here are all based on doses of irradiation which resulted in eventual serious damage to the pituitary. Further work must be done at lower doses and longer postirradiation periods.

Conclusions

(1) The use of deuteron particles for alteration or destruction of a localized volume of tissue deep within the body has been demonstrated by irradiation of the pituitary of the rat employing doses of from 3,150 to 25,200 rep.

(2) It has been shown that the degree of destruction observed following irradiation of the pituitary is a function of time as well as dose.

(3) Only by giving high doses of irradiation (18,900 rep or more) was it possible to bring about rapid destruction of the pituitary. With lower doses (6,300 rep) the destruction was not complete for many months after irradiation (9 months).

(4) There was no evidence of stimulation of any function of the anterior pituitary with the doses and periods of observation employed in this experiment.

(5) There was no evidence that the pituitary was able to recover after being damaged by this type of irradiation at dose levels employed.

(6) The different cells of the anterior pituitary were found histologically to be almost equally sensitive to irradiation.

(7) Body growth and thyroid growth were impaired soon after irradiation at each dose level, but degeneration of the testes occurred much later.

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HEALTH CHEMISTRY

N. B. Garden

PROGRESS REPORT

Equipment Development Group

The first high-level bombardment from the Idaho Falls reactor, equipment for which has been described in detail in the Quarterly Report for July-September, 1952, and in subsequent monthly reports, was received and successfully processed in the six-inch lead cave and in boxes in the two-inch lead shields. This equipment and its operation have subsequently been evaluated as to efficiency; the two-inch shields and associated boxes and equipment have been organized as standard units, and the six-inch cave setup is undergoing the same treatment.

An ionium sample bombarded in the Idaho Falls reactor was also received during this period and is being processed.

Several shipments of Hanford waste solution from which americium is extracted have been received and processed in the equipment set up in Bldg. 5A.

The major efforts of this group have been on the plans and creation of equipment to be used in processing large quantities of special soil. This operation will take place in a building behind Bldg. 5. This equipment includes a two-inch lead cave and requisite manipulators, etc., for chemical separation on a large-batch scale. This setup may be of use later on in continuous cow operations, where americium is essentially continuously extracted from plutonium.

This group has developed a hydraulic mechanism for loading and unloading samples into the Idaho Falls reactor; this device is cheap, easily constructed and operated; it is made of materials with low neutron-capture cross section and short decay periods.

Incorporation of our own fabricated plastic liners and shapes at various points in equipment set up to process radioactive materials has been used extensively during this period to reduce the cost and increase the corrosion resistance and ease of decontamination of these parts.

The one-hundred-twenty-curie cobalt source, located in Bldg. 4A, is now in use; at the present time it is being used in the irradiation of organic compounds, furnishing a gamma field in excess of 10^5 r per hour.

Considerable time has been spent on the creation of special equipment for use in gloved boxes for special filter-paper analytical experiments to be done at Livermore.

The development of fuming apparatus for highly corrosive remote-control operations has been worked out and tested. The results are encouraging but possibly not universally acceptable.

Activity Handling Group

Success has been achieved in shipping out some 500 kilograms of uranium in the oxide form to a central recovery depot. This material has been converted from shavings and turnings to the oxide through a burning process in order to provide a form more amenable to shipment. Containers are being prepared for shipping the remainder of the turnings - some 2100 pounds - to Hanford; these containers will be equipped with lids having breathers similar to those used in shipments between Los Alamos and Oak Ridge.

A summary of the experience in converting metallic uranium chips to the oxide form is furnished by reference to the Health Chemistry Quarterly Reports for April-June and July-September, 1953.

In the first quarter the method of conducting this operation was described as washing the chips in carbon tetrachloride and burning them in a specially constructed furnace with a filtered exhaust. This method was chosen after considerable experience in trying lots under different conditions of oxidation. During the early stages of operation, the conversion went fairly smoothly, but in the course of time the high temperatures involved in burning the uranium introduced failures into the furnace equipment, and the exhaust gases were at such high temperatures that the duct and filter system became very troublesome--that is, the first hundred pounds were processed with relative ease; after 500 pounds has been processed, complications began and by the time 1000 pounds has been processed it was estimated that the replacement of parts, filters, etc., would be such as to make further conversion unwarranted. As reported in the July-September report, after two months of working to eliminate the bugs it was deemed advisable to discontinue local conversion and plan to transfer the scrap metal to a large installation where ample provision has been made for dealing with this problem. We would strongly recommend against anyones drawing conclusions on a satisfactory method of converting metallic uranium to oxide based on results of using a small (say five-pound) lot.

Consideration of wet oxidation was given but quickly abandoned as being too expensive.

A new Chevrolet Carryall for use in the increased radioactivity transportation work, both at Berkeley and for movements to Livermore, has been acquired by Health Chemistry Activity Handling group. This vehicle has been lined with polyethylene and at present a plywood floor covered with mastopave is being used for floor covering, which surface will be decontaminated with ease in case of escaped activity.

A problem has arisen concerning large amount of liquid radioactive wastes from Livermore inasmuch as such quantities are not disposed of with ease, as would be obvious. The Director's Office and the Chemistry Office are backing Health Chemistry in invoking the cooperation of the Livermore experimenters in taking heed of the distinction between (1) radioactive liquid wastes, (2) non-radioactive chemical wastes, and (3) innocuous wastes (cooling water, etc.); it has been strongly requested that Category no. 1 be carefully kept from no. 2 and no. 3, the latter two being in great quantity.

A technique has been devised for emptying and then rendering harmless gas cylinders which have the main cylinder valve broken or frozen shut, or have the main cylinder valve frozen open with an auxiliary needle valve frozen shut. It has been of special value in emptying gas cylinders that have become so corroded that transportation back to a supplier or to a remote area would be considered hazardous.

The technique utilizes a specially designed assembly consisting of a high-pressure chamber than can be secured to the head of the safety plug on the valve assembly, a drill which operated through the chamber to drill a hole through the safety plug, and a needle valve for controlling the emptying of the gas cylinder. Since only the safety plug is damaged, the cylinder can be reused after freeing the frozen valve and inserting a new safety plug.

Numerous chlorine and hydrogen chloride, cylinders, and one for hydrogen sulphide, have been successfully drilled and emptied.

Berkeley Box Group

During this period the trend toward quantity production in group lots has increased. This is because of the need for complete installation for Livermore needed on short notice.

More attention has been given to the dust problem than in the past. The presence of quantities of room and atmospheric dust is a continuous nuisance. In several instances the group has been able to apply experience, equipment and materials available at hand to eliminate or reduce this problem. Continued improvement may be expected.

Requests for inert atmosphere enclosures continue to be received. An improved model has been placed in use and results are being watched; this is a double-box protection technique of placing the inert atmosphere pot in an "animal box" under the usual negative pressure.

Considerable reconditioning and reworking of out-of-date stock used by this group have been successfully carried out during this period.

Monitoring

The monitoring program has remained essentially the same during this quarter with the exception of an increased training program for personnel destined to be assigned to the Livermore area.

An improved procedure for preparation, issuance, monitoring and custodianship of radioactive sources has been put into effect. These sources have been prepared and issued through Health Chemistry on request; requests have lately increased considerably.

Decontamination

Experience during this quarter with the decontamination of gloved boxes with plastic liners in good condition has shown that certain decontamination can be effected without using the decontamination chamber. Generally speaking, if the loose activity in these boxes is removed and the remaining

activity tied down with spray paint, the panels of the box can be removed without being recontaminated. This action has been tried many times successfully; in using this technique, less time and personnel are involved, and personal ingenuity can be used to advantage.

Considerable time has been spent by this group, with the aid of the monitors, in preparing some of the hoods in Room 105, Bldg. 5, for removal and disposal. (These hoods were used for many years as high alpha activity areas, where several spills had necessitated their being painted over several times; they were thought to be too contaminated either to be used any longer or to be decontaminated). It was necessary to create an improvised "gloved box" out of the hood itself by attaching a lucite front and filter outlet to the front of the hood; a flexible-shaft grinder operating through a spring-hinged door effected mechanical detachment of dangerous surface and under-paint contamination, which was removed by an electrolux cleaner. The operation resulted in no contamination in the immediate area.

New equipment or articles acquired by this group include (1) a fully vented storage cabinet for contaminated articles pending processing, and (2) an exclusive vacuum system, with acid scrubber and traps, for transferring and storing active liquid waste.

Airborne Activity Control Group

Considerable time and effort has been spent on consultations regarding the following problems:

1. The dissolver-shack problems at Livermore commanding considerable attention owing to the specific ventilation requirements, which, generally speaking, may be described as quite challenging because of substantial quantities of radioactivity, vigorous (occasionally violent) chemical procedures, limited time of operation, scrupulous air-cleaning efficiency demands, etc.;

2. A detailed recheck of Bldg. 70 bid specifications leading to change orders in contract details made;

3. Design of the exhaust system for the building in which the special soil processing described on page 114 will take place.

New equipment developed during this quarter includes:

1. Slight modifications of the saran fiber box exhaust scrubber, described in the last quarterly report;

2. A box exhaust filter comprised of two layers of different grades of fibreglas (B-fiber and PF-105) incorporated in the same housing with the CWS-6 cleanup filter; the efficiency of this filter seems good; whether this assembly, termed "graded" filter, will replace entirely the two-unit PF-105 CWS-6 assembly will depend on further field experience;

3. Fast chemistry boxes for special low-level short-lived work without glove fronts, which have been installed in Bldg. 161, Livermore, employing 225-cfm. "Cambridge" exhaust filters on each. Although chemists

using this setup are enthusiastic, it is possible that filter life will be short, leading to expensive replacements. A large exhaust system was required to handle the effluent air;

4. Collaborative work with Plant Design regarding substitute materials for hood canopies. A plastic glass laminate appears promising, both cost- and maintenance-wise. About 100 new hoods, in Bldg. 70 and in Livermore, will be needed in 2 to 18 months, and this development is being tested to fill these needs.

A system was devised whereby half of Room 105, Bldg. 5, could remain in use during the preparation for removal and removal of certain hoods, described in the section on Decontamination. The room was subdivided by means of a temporary wall and an air lock.

General

Many man-hours have been expended on the problem of Source and Fissionable Material Accountability imposed on Health Chemistry because of the Livermore operations, in addition to those already existent in this regard at Berkeley.

HEALTH PHYSICS

B. J. Moyer

STATISTICAL SUMMARY OF MONITORING PROGRAM

Survey Instruments Maintained

1.	B-γ Ionization Chamber	43
2.	Victoreen 263 Meters	19
3.	I.D.L. Portable Survey Instruments	20
4.	Cutie Pies	3
5.	Recording γ-Intensity Meters	21
6.	Victoreen Proteximeter	3
7.	Fast Neutron Proportional Counters	6
8.	Slow Neutron Proportional Counters	15
9.	Fast Neutron Proportional Counter (Portable)	9
10.	Slow Neutron Portable Unit	3
11.	Balanced Chamber - Fast Neutron - Portable	1
12.	Special Tissue Wall Survey Instrument	1

Personnel Meters in Use

1.	Total Personnel Covered with Film Badges	2,375
2.	Total Man Days Coverage with Pocket Chamber	2,980
3.	Total Man Days Coverage with Pocket Dosimeters	2,981
4.	Total Man Days Coverage with Pocket Chambers (S.N.)	3,001

Cases of Weekly Exposure above 0.3r

Weekly Film Expos. above	184" Area	60" Area	Lin. Acc.	Chem.	Other	Total
0.3	0	7	12	48	6	73
0.5	0	1	6	17	1	25
1.0	0	0	3	9	1	13
1.5	0	0	0	4	1	5
2.0	0	0	0	0	1	1
2.5	0	0	0	0	0	0

Information Division
6-19-53 bw