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Metallurgical Project

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DECLASSIFIED Per Letter Instructions Of TID-1085

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CALIFORNIA PROJECT

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NATIONAL CANCER INSTITUTE

R.R. Spencer - Chief

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REPORT FOR MONTH ENDING OCTOBER 31, 1944

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METALLURGICAL LABORATORY

Robert S. Stone, Director
Leon O. Jacobson, Assistant Director

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A B S T R A C T S

CLINICAL MEDICINE AND MEDICAL RESEARCH SECTION

The effect of $TO_2(NO_3)_2$ on tissue metabolism has been studied by injecting T into rats and killing them at intervals for study. Anaerobic glycolysis and the rate of oxidation of choline by the kidney were unaffected in T poisoning. The QO_2 values of the liver were also unaffected. However, the synthesis of glycogen, the synthesis of urea and the oxidation of pyruvate by the liver were inhibited to some extent in T poisoned rats. The minimum amount of $TO_2(NO_3)_2$ necessary to produce a rise in the blood N.P.N. values was found to be 0. 1 mg per Kg injected in a single dose. Potato phosphorylase activity, pyruvate dismutation, and pyruvate condensation (synthesis of acetoacetic acid) were markedly inhibited by T. Lactic dehydrogenase, polyphenol oxidase, trypsin, ribonuclease were not affected. Alpha hydroxyaspartic acid reactivated the enzyme activity of succinoxidase inhibited by T when used at a ratio of 10 to 1. A simple ultrafiltration method has been devised for our work on the combination of T and other metals allied to T with proteins.

During the month of September 15 to October 15, 1944, 5,800 clinical laboratory examinations were made; this included laboratory work on 92 new personnel, 256 controls and 848 in the work hazard group. Abnormalities considered on the basis of the white blood count alone show individuals with abnormalities in these groups of 30%, 26% and 25% respectively.

The injection of T into rabbits is followed by a prompt increase in urine catalase. No significant difference, however, has been found between the urine catalase of human control and T exposed groups in either the Chicago or Ames laboratories.

Biochemical studies of a 58 year old female who received, in a period of 18 days, a total of 156 r total body x-ray therapy for a metastasizing parotid tumor showed the following significant changes; a prompt sustained rise in the adenosine- triposphate fraction of the white blood cells immediately following the institution of therapy and lasting beyond its completion; a fall in the white blood cell count only after the entire dose had been delivered. (Note: Normal subjects stand this dose.)

A simple procedure has been devised for the micro-quantitation of T in 1/10 cc samples of urine. The method is sensitive to less than 0.0001 micrograms.

Coproporphyrin isomer studies in project personnel continue to reveal an elevated concentration of coproporphyrin III in T-exposed

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personnel as compared to control individuals. Studies of urorosein and other urine pigments show frequent elevated values in project personnel with various types of exposure. Little is known about the factors producing this undoubtedly non-specific increase.

MEDICAL INDUSTRIAL HAZARD SECTION

In general beta and gamma surveys in the New Chemistry building indicate very little change in the degree of contamination. A TX_2 hazard present in D-10A (New Chemistry) has prompted recommendation for installation of ventilation to diminish the hazard. Three rooms in the West Stands (8, 9 and 217) were returned to routine use after decontamination but widespread beta or soft gamma contamination again exists.

Approximately five hundred film meters are developed each week; during the period 8/21 to 10/8/44, 7 films of project personnel had readings greater than 0.6 r for one week. Of the pocket meter readings for the p eriod 9/7 to 10/11/44, 81 meters gave a daily reading exceeding 0.1 r. Of these 35 were felt to be valid readings.

BIOLOGICAL RESEARCH SECTION

Rats exposed to I_2^{131} vapor retained 10 percent of the inhaled material. The absorption from the lung was 95 percent complete at the end of a 30 minute exposure with a 10 percent deposition in the thyroid and 90 percent elimination in the urine in 24 hours.

Half of the retained yttrium (57d) chloride aerosol was eliminated from the lungs of rat in a day at tracer level. An average initial lung retention of 26 C per rat caused no detectable histological damage in one week for a lung exposure of 1500 r.

Zr^{93}-Cb^{93} injected into the heart or tail vein of the rat was deposited in lungs, 10 to 20 percent, liver, 10 to 20 percent and ske leton, 15 percent with a urine excretion of 30 percent at 10 days. The 50 percent lethal dose was 5 c/gm for 15 days survival.

The distribution of radiation intensity in the femur of a rabbit injected with Sr^{89} has been determined by a photographic film reproduced in the report.

The technique developed for assaying retained Sr^{89} in chronic animals by external counting of live animals shows that 4 to 5 percent of betas escape through the dorsal surface of the mouse.

The survival of rats after two exposures to x-ray indicate that the recovery from an initial exposure of 300 r is exponential up to three weeks with a half recovery time of one week.

A more complete experiment does not show the change in the growth of rats after a 50r exposure previously reported.

Dogs showed gradual increases in plasma histamine at all levels of X-irradiation from 50 r up.

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A dose of 57 n from the cyclotron was lethal to 50 percent of CF1 female mice in 30 days. This is considerably lower than the value from Clinton. It gives an X/n ratio of 8.1.

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I. Clinical Research Group - E. S. Guzman Barron

The laboratory has been engaged during this month in further studies on the mechanism of T poisoning, on the preparation of hydroxyaspartic acid to be used for the treatment of T poisoning, and on the metabolism of rabbit's bone marrow.
Tissue Metabolism on T poisoned Rats.—It has been shown in previous reports that the metabolism of the kidney is profoundly and progressively inhibited after the injection into rats of 0.5 mgs $TO_2(NO_3)_2$ per Kg. Rats killed on successive days showed a continuous diminution of O_2 uptake in the presence of succinate, pyruvate, and alanine as oxidizable substrates. This inhibition is not a general phenomenon for it has been found that the O_2 uptake in the presence of choline is not inhibited at all.
Liver Metabolism.—Shortly after $TO_2(NO_3)_2$ is injected into rats most of the metal is deposited in the kidneys, but some of it is found in the bone and in the liver. The metabolism of the liver of normal rats varies greatly unless the animals are starved for at least 48 hours. In the experiments reported in Table I both control and treated rats were kept without food for 48 hours, with water ad libitum. The animals were killed two and three days after injection of $TO_2(NO_3)_2$ (0.5 mgs per Kg). The O_2 uptake of the liver was practically the same as the control; in two out of three animals there was a slight inhibition of O_2 uptake in the presence of pyruvate as the oxidizable substrate, inhibition which was also manifest when the amount of pyruvate utilized was measured. The utilization of pyruvate in the absence of oxygen (in Ringer-bicarbonate with N_2:CO_2 as gas phase) was not affected.

Liver slices readily synthesize urea when incubated in Ringer-bicarbonate with arginine and lactate. This synthesis was inhibited 31 percent in the liver of rats poisoned by T. (Table II.)

When rats are starved for 48 hours the glycogen of the liver is completely depleted because liver glycogen is rapidly used by the animal to provide food for its energy requirements. The rate of this depletion was slightly affected by T poisoning as can be seen in Table III. Control rats after 24 and 48 hour starvation had lost 95.5 percent of the liver glycogen. The treated rats killed 24 hours later lost 99 percent. When food was not withdrawn, they lost 92 percent. Treated rats killed 48 hours after T injection lost 95.5 percent of the liver glycogen. When food was not withdrawn, the loss was 88 percent. These starved rats with no glycogen in the liver can be used for the study of glycogen synthesis in the liver by feeding them concentrated solutions of glucose and killing the animals at appropriate times: the glycogen found in the liver will be synthesized glycogen. In these experiments rats treated with 5 mgs $TO_2(NO_3)_2$ were used three days after the injection. When the animals were killed 4 hours after the introduction of glucose into the stomach there was an inhibition of glycogen synthesis of 14 percent. The inhibition rose to 44 percent in the animals killed 6 hours after introduction of glucose into the stomach (Table IV). In conclusion T poisoning produces not only kidney damage but also liver damage as shown in the slight inhibition of pyruvate metabolism, and urea synthesis, and in the inhibition of glycogen synthesis. It must be recalled that Lan (1) has found that muscle phosphorylase, the enzyme which catalyzes the reversible reaction glycogen + $H_3PO_4 \rightleftharpoons$ glucose 1-phosphate, is inhibited by T. Our in vivo experiments confirm Lan's in vitro findings.

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$TO_2(NO_3)_2$ required to raise blood N.P.N.—When 0.5 mgs $TO_2(NO_3)_2$ per Kg was injected into rats the blood N.P.N. rose steadily so that at the 5th day after injection it reached 161 mgs percent. 0.1 mgs per Kg raised the N.P.N. value to 60 mg percent at the end of the 4th day. 0.05 mg, (i.e. 24 micrograms T) per Kg in a single dose had no effect (Table V).

Effect of $TO_2(NO_3)_2$ on Pyruvate Metabolism.—It has been shown in previous reports that TO_2^+ inhibits some enzyme reactions while it has no effect in others. Further proof of this specificity is given in Table VI where the effect of $TO_2(NO_3)_2$ on the various metabolic reactions of pyruvate has been tabulated. Ground pigeon liver (washed and unwashed) was used in these experiments. Washed ground pigeon liver suspended in phosphate buffer containing small amounts of Mg^{++}, Mn^{++}, diphosphothiamine, and adenosinetriphosphate oxidizes pyruvate rapidly. This suspension was treated with the appropriate amount of $TO_2(NO_3)_2$ for 10 minutes; after this time phosphate buffer was added and the oxidation of pyruvate was determined by measuring the O_2 uptake. 0.001 M $TO_2(NO_3)_2$ produced a small inhibition (23 percent); 0.0002 M increased the O_2 uptake. This increase in the O_2 uptake must be due to a shift in pyruvate metabolism towards the oxidative pathway because in experiments where the amount of pyruvate utilized was measured, utilization was not affected (Table VII). In unwashed, ground liver, the pathways of pyruvate utilization are more complex because there is not only oxidation, but also dismutation and condensation leading to synthesis of acetoacetate, α keto glutarate and citrate. The effect of $TO_2(NO_3)_2$ on pyruvate dismutation, and on the synthesis of acetoacetate was therefore studied in this system. The dismutation of pyruvate, as measured by CO_2 formation in Ringer-bicarbonate with N_2: CO_2 as gas phase, was completely inhibited with 0.001 M $TO_2(NO_3)_2$, and 75 percent with 0.0002 M. The reaction is:

$$2CH_3COCOOH+H_2O=CH_3CHOHCOOH+CH_3COOH+CO_2$$

When pyruvate utilization was measured, the inhibition was 45 percent. The synthesis of acetoacetic acid was inhibited 54 percent with 0.001 M $TO_2(NO_3)_2$, while 0.0002 M had no effect. (Table VI). It can be seen from these experiments that in pigeon liver suspensions, the oxidation of pyruvate is only slightly inhibited by $TO_2(NO_3)_2$ the dismutation is strongly inhibited, while the synthesis of acetoacetic acid is half-inhibited; clear example of specificity.

Potato Phosphorylase—It has been shown by Lan (1) that muscle phosphorylase is inhibited by TO_2^{++}. Potato phosphorylase catalyzes the reaction starch + $H_3PO_3 \rightleftharpoons$ glucose 1-phosphate. Enzyme activity can be measured either by the amount of P liberated from glucose 1-phosphate or by the formation of starch. The experiments were performed at pH 6.2 in maleate buffer (added 5 minutes after the addition of $TO_2(NO_3)_2$ to the enzyme solution). The tubes were kept for 40 minutes at 23° after which CCl_3COOH was added and the P was determined colorimetrically. 0.001 M $TO_2(NO_3)_2$ produced 32 percent inhibition (Table VIII).

Trypsin.—The activity of crystalline trypsin was unaffected by the addition of 0.001 M $TO_2(NO_3)_2$; on the contrary there was a slight increase in activity (Table IX).

Polyphenol Oxidase.—$TO_2(NO_3)_2$ at a concentration of 0.001 M had no effect on the activity of this enzyme (prepared from mushrooms) as measured by the oxidation of catechol (Table X).

Ribonuclease.—10 micrograms of crystalline ribonuclease were treated with 0.001 M $TO_2(NO_3)_2$ at pH 6 in bicarbonate-CO_2 buffer. After 15 minutes the enzyme was tipped into the solution of yeast nucleic acid. The rate of hydrolysis of nucleic acid was followed manometrically. $TO_2(NO_3)_2$ produced no inhibition.

Lactic Dehydrogenase.—This enzyme activates lactic acid. The activated lactic acid is oxidized to pyruvate by diphosphopyridine nucleotide (DPN) which is reduced to dihydrodiphosphopyridine nucleotide (DPN) H_2.

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$$\text{Lactate} + \text{DPN} \rightleftharpoons \text{Pyruvate} + (\text{DPN})H_2$$

This reversible reaction, goes to the right in the presence of a substance which combines with pyruvate and the rate of the reaction can be measured spectrophotometrically at 3450 Å where the $(DPN)H_2$ shows an absorption band. This enzyme was practically uninhibited by $TO_2(NO_3)_2$ 0.001 M (Figure 1). Dounce (2) has reported some inhibition. It must be pointed out however that the system used by Dounce is more complicated than that used by us. Dounce had lactic dehydrogenase and flavoprotein. Whether the reported inhibition was due to action on the first or the second protein in not known.

The Reactivation of Enzymes Inhibited by $TO_2(NO_3)_2$.—It has been shown in previous reports that the reaction between TO_2^{++} and enzyme is, under certain conditions, reversible. Reactivation of the enzyme was obtained on addition of certain organic acids among which citric acid was found the most powerful agent. It was postulated that reactivating agents would be those able to form cyclic compounds with TO_2^{++} which were not easily dissociable. In a list of compounds given in one of our reports it was predicted that hydroxyaspartic acid, an amino acid possissing the COO^- and OH groups so situated as to favor the formation of TO_2 cyclic compound, would be a good reactivating agent. The experiments reported in Table XI confirm our predictions. Succinoxidase inhibited 90 percent by $TO_2(NO_3)_2$ was reactivated 61 percent by citric acid and 47 percent by hydroxyaspartic acid. In both cases the organic acids were added at 10 times the concentration of $TO_2(NO_3)_2$. αhydroxyaspartic is the only amino acid which has been found to be a good reversal agent.

αhydroxyaspartic acid will be used as a therapeutic agent against T poisoning and its efficacy will be compared with that of citrate and carbonate.

Whether the enzymes inhibited by T possess in their molecule αhydroxyaspartic acid is not known. We are of course will aware that, up to now, this amino acid has not yet been found in proteins.

The Combination of TO_2 with Proteins.—After it has been shown that TO_2 combines with enzymes reversibly, the next step is to establish the nature of this combination and the groups in the protein molecule which bind to TO_2. These studies are going to be undertaken with crystalline proteins and in large scale experiments. For this purpose the colorimetric method of T determination was developed. A rapid method for the ultrafiltration of protein solutions has also been devised. The solution to be filtered is put into a cellophane bag which is suspended, inside a double-layer cheesecloth bag, in a 50 cc centrifuge tube. One hour centrifugation at 3000 r p m provides from 2 to 3 cc filtrate from a 10 cc protein solution.

In Table XII are given the results obtained when solutions of $TO_2(NO_3)_2$ in maleate buffer at several pH values were ultrafiltered according to the technique outlined above. At pH 6.5 or below, 80 to 90 per cent of T appeared in the ultrafiltrate. At pH 7 or above, from 10 to 20 percent of T were found in the filtrate. Analyses of T were made in 0.1 cc by the colorimetric method. Undoubtedly at pH 7 aggregate formation took place with subsequent plugging of the pores of the membrane.

Dialyzed human plasma almost completely prevented the appearence of T in the ultrafiltrate. (T at a concentration of 0.001 M $TO_2(NO_3)_2$).

Preparation of αHydrozyaspartic acid.—Complete details of the method of preparation of this amino acid based on Dakin's paper are available to persons in the project interested in this compound. Following this procedure a product was obtained of better than 98 percent purity.

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REFERENCES

I. Lan, T. H., from Progress Report No. 8, The University of Rochester. AC-7401-eng-49, July, 1944. Submitted by A. H. Dowdy.

II. Dounce, A. L., from Progress Report No. 9, The University of Rochester. AC-7401-eng-49 August 1944. Submitted by A. H. Dowdy.

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Table I

Liver Metabolism of Rats treated ith $TO_2(NO_3)_2$ (0.5 mgs per Kg).

Killed 2 days after: QO_2=(O_2 uptake per mg per hr in Ringer-PO_4 buffer, O_2 as gas phase; QO_2 pyr = O_2 uptake in Ringer PO_4 + 0.01 M pyruvate; $Q^{N_2}_{pyr}$= cmm CO_2 production in Ringer - $NaHCO_3$ with N_2:CO_2 as gas phase, $Q^{O_2}_{pyr}$ = cmm pyruvate utilized in O_2 as gas phase; $Q^{N_2}_{pyr}$ = cmm pyruvate utilized in the absence of oxygen. (1 mg pyruvic acid = 254.5 cmm).

Control	Control	Treated	Control	Treated	Control	Treated
QO_2	7.68	7.63	7.95	8.01	9.01	8.56
QO_2 pyruvate	9.43	9.69	10.04	8.45	10.01	9.58
$Q^{N_2}_{CO_2}$ pyruvate	3.0	3.16	3.73	3.43	3.27	3.24
Q^{O_2} pyruvate	7.97	7.77	12.6	8.9	14.19	12.35
Q^{N_2} pyruvate	3.93	4.07	3.66	3.83	5.66	6.12

Table II

The synthesis of urea by the liver of rats (slices) treated with $TO_2(NO_3)_2$ (0.5 mgs per Kg) Synthesis in the presence of arginine and lactate. Incubatime time, 1 hr. Figures give cmm urea formed in 1 hr per mg dry weight. All rats were starved 48 hours before death.

Rat	Control	Treated	Inhibition per cent
I	9.0	8.0	
II	12.2	7.8	
III	15.3	8.0	
IV	17.3	10.7	
V	12.6	9.6	
VI		12.1	
VII		9.0	
Average	13.3	9.2	31

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Table III

The b:yc ɔgen cont·nt of the liver of rats treated ith $TO_2(NO_3)_2$

The data repr sent av rage values of 20 r ts used in these experiments.

Conditions	Time after T injection days	Liver glycogen as mg glucose per g
Control at start of experiment	0	27.8
24 hours starvation	0	1.01
48 hours starvation	0	1.39
Treated 5 mgs x Kg starved	24	0.31
Treated 5 mgs x Kg starved	48	1.23
Treated food not removed until 24 hours.	24	2.29
Treated food n t removed until 24 hours	48	3.25

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Table IV

Synthesis of glycogen by the liver of rats after introduction of 3 cc of a saturated solution of glucose into the stomach. Rats starved 48 hours previously. Rats injected with 5 mgs $TO_2(NO_3)_2$ per Kg 3 days before glycogen test:

Time after glucose injection	Glycogen, mg. per g of liver		Inhibition
	Treated	Control	
hours			per cent
2	14.6	16.6	
4	32.5	37.8	14
6	25.1	44.6	44

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Table V

Blood N.P.N. in rats injected with varying amounts of $TO_2(NO_3)_2$. Normal values around 40 mgs per cent.

Days after injection	$TO_2(NO_3)_2$ mgs per Kg	Blood N.P.N. mgs percent
2	0.5	47
3	"	87
5	"	141
2	0.1	35
3	"	45
4	"	60
2	0.05	46
3	"	43
4	"	44

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Table VI

Effect of $TO_2(NO_3)_2$ on Pyruvate Metabolism

Pyruvate Reaction	$TO_2(NO_3)_2$ Concentrations M	Inhibition percent
I. Pyruvate Oxidation		
a) ground washed pigeon liver	0.001	-23
	0.0002	+25 increase
b) ground unwashed liver	0.001	-75
	0.0002	-28
II. Pyruvate Dismutation		
a) as measured by CO_2 production	0.001	Complete
	0.0002	-75
b) as measured by pyruvate analysis	0.001	-45
	0.0002	-46
III. Acetoacetate Synthesis	0.001	-54
	0.0002	0

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Table VII

Effect of small amounts of $TO_2(NO_3)_2$ on pyruvate oxidation by pigeon washed, ground liver, as measured by O_2 uptake and pyruvate analysis .

System		O_2 uptake Blank subtracted	Increase	Pyruvate utilization
		cmm	percent	micromoles
Control		291.7		25.4
$TO_2(NO_3)_2$,	0.0001 M	344	+18	25.5
	0.0002 M	347.2	+19	27.1
	0.0003 M	311.7	+ 7	25.6

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Table VIII

Effect of $TO_2(NO_3)_2$ on the activity of Potato Phosphorylase

System	E Value at 730 mu	P liberated in 40 min.	Inhibition	E Value at 630 mu (I_2-starch complex)
		micrograms	percent	
Control	0.540	386		0.492
0.001 M $TO_2(NO_3)_2$	0.415	263	32	0.026

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Table IX

Effect of $TO_2(NO_3)_2$ on the activity of Crystalline Trypsin

Experiment No	T Concentration	E*-Value	Effect
1	None M	0.477	Slight increase
2	0.001	0.520	
3	"	0.507	
4	None	0.400	
5	0.005	0.468	Slight increase
6	"	0.481	" "

E* value is directly proportional to enzyme activity under the conditions of these experiments.

Table X

Effect of $TO_2(NO_3)_2$ on the activity of Polyphenol oxidase.

System	O_2 uptake in 12 min.	O_2 uptake Blank substracted	Inhibition
Catechol-No enzyme	22	--	
" " " + 0.001 M $TO_2(NO_3)_2$	0	--	complete
Enzyme + Catechol	97	75	
" + " + 0.001 M $TO_2(NO_3)_2$	75	73	None

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Table I

α Hydroxyaspartic Acid and Citric Acid as Reactivating Agents of Enzyme Inhibitions Produced by TO_2^{++}

Succinoxidase prepared from pigeon breast, dialyzed free of phosphate. $TO_2(NO_3)_2$ was added to the enzyme suspension ten minutes before. Veronal buffer, pH 7.2 added afterwards. Duration of experiments, 40 minutes. Succinate, 0.01 M

System	O_2 uptake	Inhibition	Reactivation
	cmm	percent	percent
Control	96.5		
$TO_2(NO_3)_2$, 0.0005 M	9.8	90	
Control + citrate, 0.005 M	99.5		
$TO_2(NO_3)_2$ + citrate	64.4	35	61
Control + αhydroxyaspartate, 0.005 M	97.5		
$TO_2(NO_3)_2$ + αhydroxyaspartate	50.8	48	47

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Table XII

Ultrafiltration of 1×10^{-3} M T Solutions in Malcate Buffer at Various pH Values.

Experiments	Sample	pH	E-Value	Per cent Filtered
1	Control	5.1	0.214	
	Ultrafiltrate	5.1	0.193	89
	Ultrafiltrate	3.8	0.194	90
	Ultrafiltrate	7.0	0.032	15
2	Control	Average of 4 pH values	0.224	
	Ultrafiltrate	5.5	0.178	80
	Ultrafiltrate	6.0	0.194	87
	Ultrafiltrate	6.5	0.179	80
	Ultrafiltrate	7.0	0.038	17
3	Control	6.5	0.214	
	Ultrafiltrate	6.6	0.187	87
	Ultrafiltrate	6.5	0.180	84
	Ultrafiltrate	6.5	0.182	85

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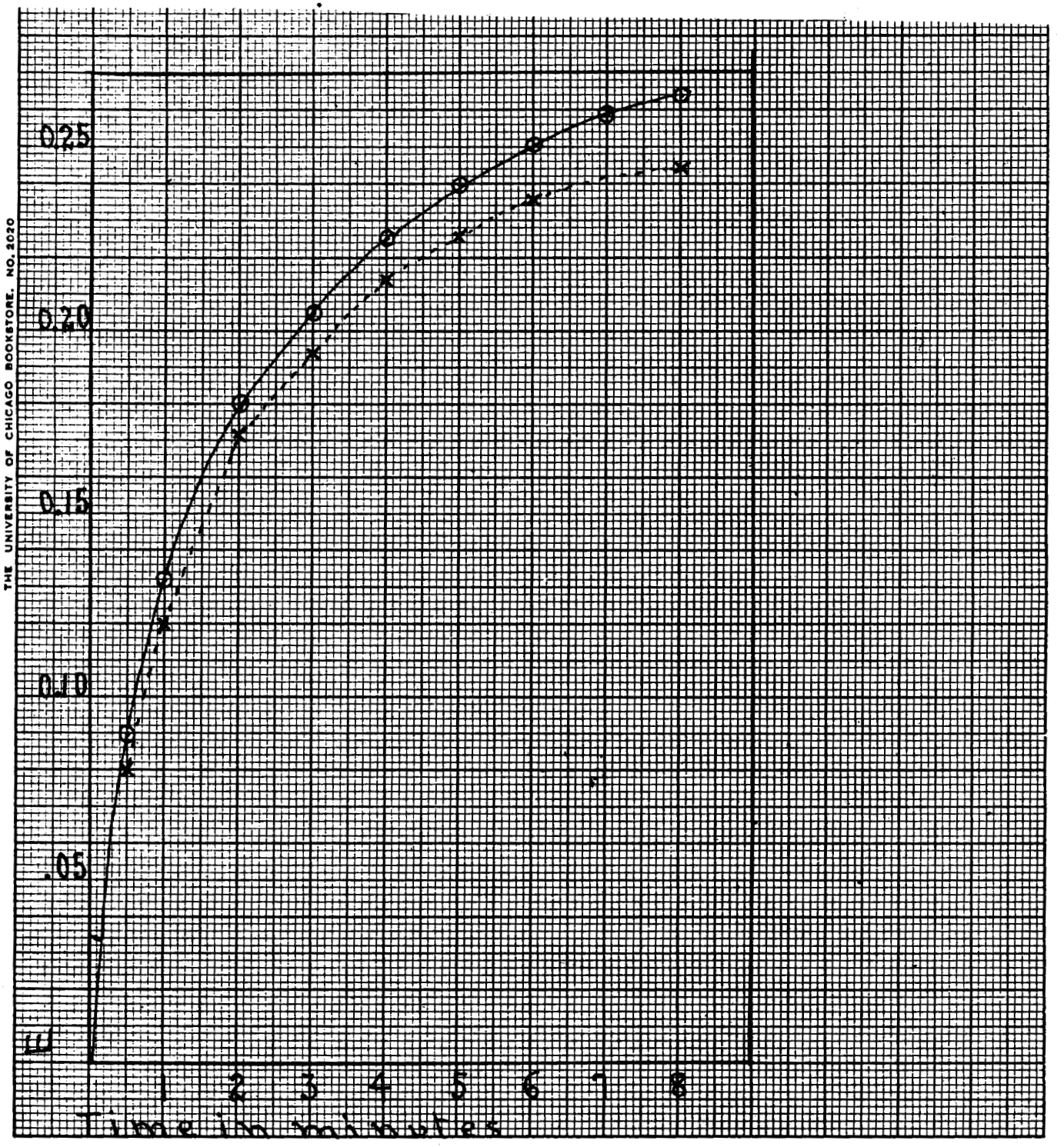
Figure 1

The effect of $TO_2(NO_3)_2$ on the activity of lactic dehydrogenase as measured by the rate of reduction of diphosphopyridine nucleotide.

control

treated with 0.001 M $TO_2(NO_3)_2$



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II. Employees Health Service - J.G. Allen

During the month of September 15 to October 15, 1944, 1943 physical examinations were done; 32 of these were on individuals transferring to other sites. Of the 143 physical examinations, 44 were academic and 99 non-academic. Two persons were rejected because of findings on clinical or laboratory examination. Including the pre-employment examinations 463 individuals were seen because of sickness or accident during this period.

III. Clinical Laboratory Examinations - L.O. Jacobson and E. Marks

A resume of the clinical laboratory examinations conducted during the month (September 15 to October 15, 1944) follows:

No. of Laboratory Procedures		Abnormalities		
Hemoglobins	854		149	
Red counts	527		101	
White counts	1242	below 5000	82	
		above 10000	93	
Differentials	1242		223	
Platelets	192		35	(10 below 150,000)
Urines	873		195	
Retics	50		0	
Wassermans	243		0	
Misc.	10		0	
Sed. Rates	244		0	
Hematocrits	244		0	
Stippling			15	
Immature cells			63	

No. of individuals examined exam		No. with Clinical Laboratory abnormalities
New personnel	92	* 28 or 30%
Controls	256	* 66 or 26%
Work Hazard group	848	*211 or 25%

* Includes only abnormalities of the WBC (below 5000) reversal of Neotrophile, lymphocyte ratio and immature cell.

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IV. Clinical Research Group — S. Schwartz

I. Eff ct of Tuballoy Exposure on Urine Catalase Excretion
with Louise G. Holt*

Increased urine catalase concentration following ex osure of rabbits to tuballoy was recently described by A. L. Dounce and colleagues of Rochester, New York. A significant increase in catalase activity of urine of humans with heavy T exposure was also claimed .

During the past several months we have been investigating urine catalase in both experimental rabbits and in project members exposed to various compounds of tuballoy. The method used here is based on the iodometric titration procedure of Balls and Hale** with a few minor modifications of our own. We have chosen this procedure instead of a manometric method, such as is used by the Rochester group, because it was felt it was more accurate and more suited to routine laboratory work. The procedure, in brief, follows:

1. To 10 cc. of cold phosphate buffer (pH6.8), 3 cc of cold 0.02 normal H_2O_2 solution and 2 cc of urineare added. Mix gently. Incubate in an ice bath for 30 minutes. ($\frac{1}{2}$ cc urine and the equivalent of 20 cc or more peroxide solution was used in most rabbit studies because of the large amount of catalase present.)

2. Remove from ice bath and add 10 cc of 2 normal H_2SO_4, 10 drops of saturated molybdic acid solution, and 10 cc of 10% potassium iodide solution.

3. Let flask sit eight to fifteen minutes at room temperature. Titrate with 0.01 normal sodium thiosulfate solution, adding 1 cc of a starth solution as indicator after most of the initial brown color has disappeared. A sharp end point is reached with complete disappearance of the blue color.

Because urinary reducing substances other than catalase might decompose peroxide, we have attempted to control out studies with various types of blanks. The data covered in this report was obtained using one or both of the following urine blanks for each sample.

1. Urine blank with sulfuric acid added before the addition of peroxide (to inactivate catalase,) and titrated without incubation.

2. Same as #2, but incubate blank one half hour after addition of sulphuric acid.

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* We are indebted to Dr. E. S. G. Barron for his kind assistance in these studies.

** Journal of Assoc. of Official Agricultural Chemists, 15, 483, 1932.

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3. A water blank was also obtained in most of the studies.

Catalase is quantitated in terms of the difference between the thiosulfate necessary to titrate the unknown and the blank. 1/10 cc thiosulfate difference is equivalent to 0.011 cc of oxygen as determined manometrically.

A. Rabbit Studies

The samples stood, as a rule, from one to twenty-four hours without preservative before being studied. Part of the catalase activity was therefore, due to bacterial catalase. Determinations done on "fresh" urines or on urines collected under toluene, or in a bottle immersed in a dry ice-acetone mixture, however, showed the same range of activity. Urine blank #1 was used in most of these studies.

Hemochromogen studies on 3 cc urine samples were, with only a few exceptions, always negative. Because of the relative insensitivity of this test as compared to catalase, however, this does not exclude the possibility that red cells might be the chief source of the urine catalase.

In the following, all data is given in terms of thiosulfate difference per cc of urine.

1. Control rabbits

64 determinations on 13 control rabbits have been performed. Range of values: 0 (only once) to 34.6 cc thiosulfate (0 to 3.8 cc of oxygen). Average value: 4.94 cc thiosulfate (0.552 cc of O_2). If we omit seven determinations, which were much higher than others done, an average value of 3.2 cc of thiosulfate is obtained. The lowest average value for any rabbit was 0.8 cc of thiosulfate, (5 determinations).

2. T injected rabbits

6 rabbits were injected with 4 milligrams per kilogram of TNO_3, (generally a lethal dose). Urinary catalase excretion rose from an average value of 1.8 cc thiosulfate to 18.4 within 24 hours. In those rabbits that survived, peak values of about 70 cc thiosulfate were obtained on about the fifth day. In one to two weeks, catalase excretion returned to the normal range.

It might be pointed out that in all these rabbits, there was marked kidney damage as evidenced by the fall of urine specific gravity from control values averaging 1.047 to average values of 1.011 for the first week. These specific gravities returned to approximately normal only after 5 - 6 weeks. Only two of the rabbits showed more than 2 plus alburminuria; some had elevated urine catalase values with no increase in albuminuria. All had a pronounced diminution in urine volume.

B. Studies of Project Personnel

Regardless of the procedure used no significant difference has been noted in the urine catalase of control and T-exposed groups. The data is given in Tables I and II below. The data in Table I from the Chicago group includes 28 control individuals and 29 T-exposed individuals at Site "B". The Ames group consists of 13 control individuals and 41 T-exposed personnel.

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Table I. Catalase Activity Studies on Human Urine.

	Controls			T-Exposed		
Chicago Studies:	Tests	Range	Average	Tests	Range	Average
A with H_2O blank	19	0.05–0.25	0.11	24	0.03–0.22	0.11
B with #2 urine blank	12	0.04–0.11	0.06	17	0.03–0.15	0.05
Ames Studies:						
Chiefly #1 blank	14	0.0 –0.12	0.06	62	0.0 –0.17	0.09

A comparison of the titration prodedure used in our laboratory and a manometric analysis similar to that used by the Rochester group has been made. The manometric analyses were kindly done by Dr. E. S. Guzman Barron. It might be pointed out that the manometric studies were done at room temperature instead of ice bath temperature as used in our studies. A KCN treated blank was used. The data in both instances are given in terms of cc O_2 evolution per cc of urine after 30 minutes incubation with the peroxide solution. The accuracy of the procdures is roughly $\pm$ 0.004.

Table II. Comparison of Manometric and Iodometric Titration Procedures for Catalase Analysis.

	Manometric Analysis	Iodometric Titration	
T Exposed		H_2O Blank	Urine #2 Blank
1.	0.010	0.024	0.016
2.	.009	.007	.003
3.	.010	.013	.006
4.	.014	.013	.011
5.	.013	.013	.003
6.	.010	.016	——
7.	.003	.008	——
8.	.006	.022	——
9.	.016	.022	——
10.	.010	.008	——
	0.010	0.015	0.008
Controls			
Male			
11.	0.013	0.019	0.006
12.	.005	.013	.006
13.	.013	.016	.003
14.	.012	.008	.003
15.	.014	.019	.011
	0.011	0.015	0.006
Female			
16.	0.025	0.011	——
17.	.019	.013	——
18.	.023	.016	——
19.	.024	.022	——
20.	.008	.006	——
	0.015	0.014	

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It might be pointed out that thiosulfate differences of more than 1 cc have been found in hospital patients or control project members with albuminuria, but a negative hemochromagen test on 3 cc urine.

Conclusions:

1. The injection of a kidney-damaging dose of TNO_3 into rabbits is followed by a prompt marked increase in urinary catalase activity lasting one to two weeks.
2. All control rabbits studies have far more catalase activity in the urine than any humans studies.
3. No significant difference has been found in the urine catalase activity of the humans in our control and T-exposed troups.
4. Though our human exposed group has a considerable average increase in urine coproporphyrin excretion none of those studies have had an acute overwhelming dose of T which is in any way comparable to the dose administered to the rabbits studied. Such studies must be made before the value of this test can be finally ascertained. The possibility is suggested that the elevated catalase values found by the Rochester group, might be due to greater exposure of their subjects.
5. Studies of blood pigment concentration in urine based on hemochromogen analysis in a concentrated specimen are planned to help determine whether the catalase represents a leakage of red cells or actual sloughed kidney tissue.

II. Studies of Radiation Effects in a Patient Receiving Total Body X-Ray

Studies have recently been completed on various radiation effects in a 58 year old female, who received 156 r total body x-ray over a period of 18 days. The parotid tumor, for which she was treated was first noted in 1929. It was first excised in 1934, again in 1939, 1941, and in 1944. Radiation therapy (dose ?) followed the first excision. At the time of surgery in April 1944, the tumor had reached "volley ball" size. Pulmonary metastases were thought to be present. The patient had suffered a 40 lb weight loss in the past 18 months.

Treatment was divided into a series of almost daily doses, of 10 to 12½ r each. Vitamin B complex, vitamin C. feosol, barbituates, morphine and aspirin were given before, during, and after therapy.

Treatment was discontinued on the 19th day, following the first indication of a drop in the total white blood count.

Urine studies were made on consecutive 24 hour urine collections. Blood studies were made on oxalated samples of blood, using essentially the technique described in the report of June, 1944 for the isolation of white blood cells.

The average values of various phosphorus fractions in the white blood cells during the control period, radiation period, and post-radiation period, are given in Table I below. The ration of adenosinetriphosphate to total acid soluble phosphorus, is included as a check on the procedure used, since this value would tend to correct for any loss in white cells or error in the white blood count.

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Table I. White Cell Phosphorus Fractions in Patient Receiving 10-12.5 r Total Body X-Ray Almost Daily. (Total Effective Dose: 156 r.)

	Control Period	Radiation Period	Post-Radiation Period
Total phosphorus	2.18*	2.66	2.19
Nucleoprotein plus Lipoid P	1.46	1.90	1.50
Acid soluble P	.76	.76	.64
Adenosinetriphosphate P	.06	.19	.11
ATP - P x 100 / Acid soluble P	7.9%	24.2	17.0
Hexosediphosphate plus phosphopyruvate P	.08	.07	.04
"Inorganic" P	.20	.17	0.13
WBC	8300	7244	2500

* Values are given in terms of gamma phosphorus/million white blood cells. Total phosphorus is not equal to the sum of the nucleoprotein -- lipoid phosphorus plus acid soluble P in these average values since one or the other of these determinations was not done in every instance.

Unfortunately no attempt was made to subdivide the nucleoprotein plus lipoid phosphorus fraction in this study. Procedures are now being worked out to quantitate the white blood cells nucleic acids both chemically and by means of Beckman absorption studies to determine whether they might be the cause of the rise shown.

As noted in these figures, the most spectacular finding was the prompt, marked, and sustained rise in the adenosinetriphosphate content of the white blood cells. Of great interest also, was the very marked rise in the excretion of urine coproporphyrin during the last week of life, associated with increased values for urobilinogen and other urine pigments.

Urorosein determinations, unfortunately, were not done during the first two weeks following institution of therapy: hence the break in the curve shown.

The method used for the fractionation of the white blood cell constitutents was suggested to us by Dr. E. S. G. Barron. It is based upon solubility in 1.5% trichloracetic acid, and on fractional hydrolysis at severn minutes and one hour in a one normal HCl colution. The so called adenosine-triphosphate fraction is the difference betweenthe seven minute hydrolysate and the unhydrolyzed fraction. More specific identification of this fraction is, of course, needed.

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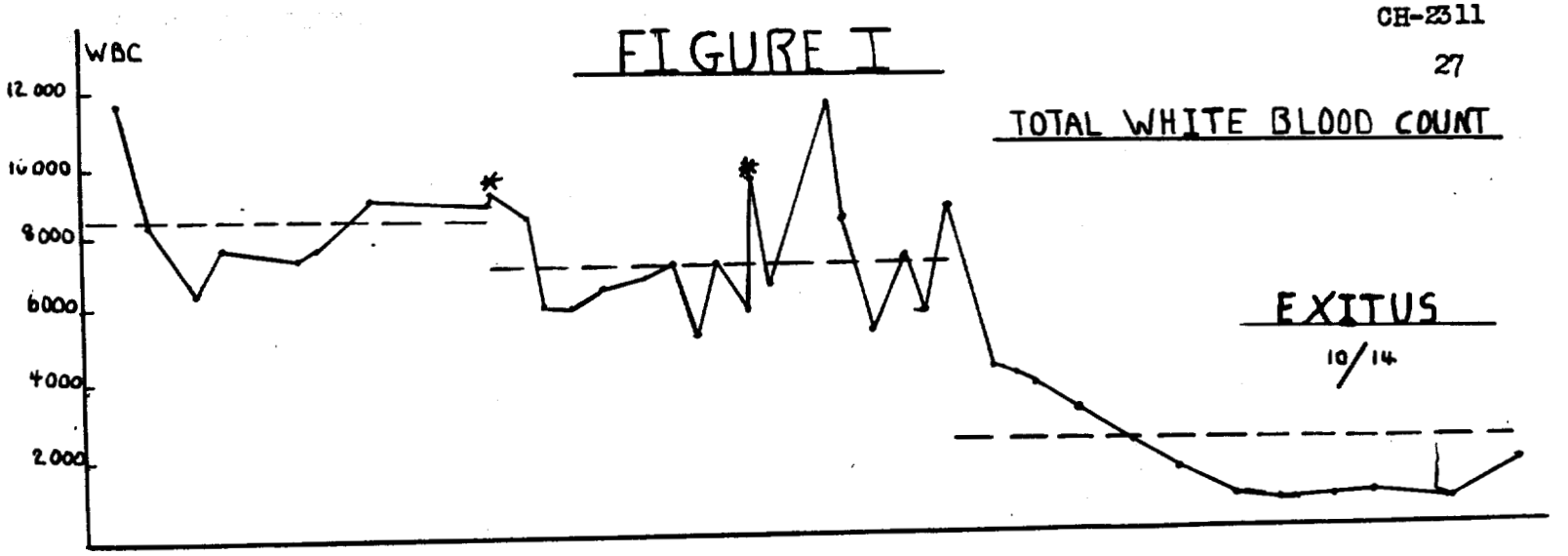
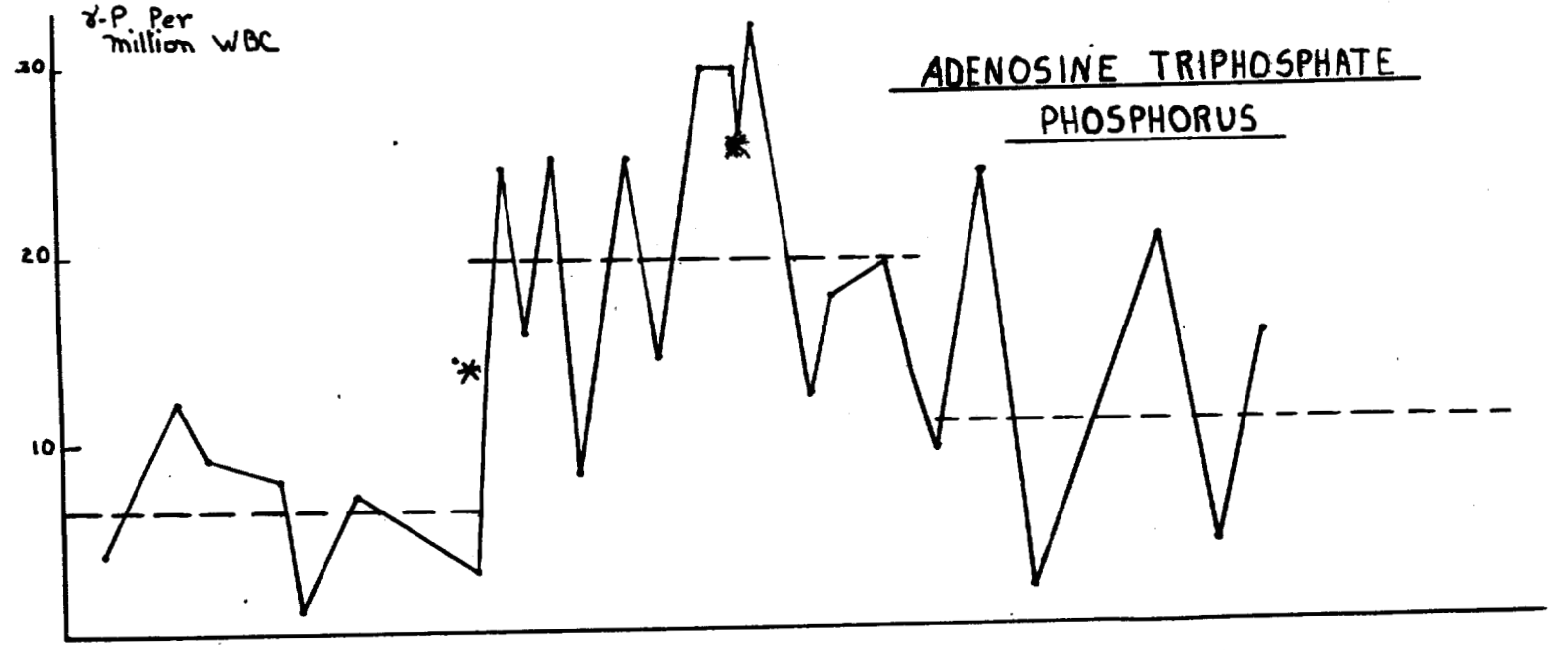
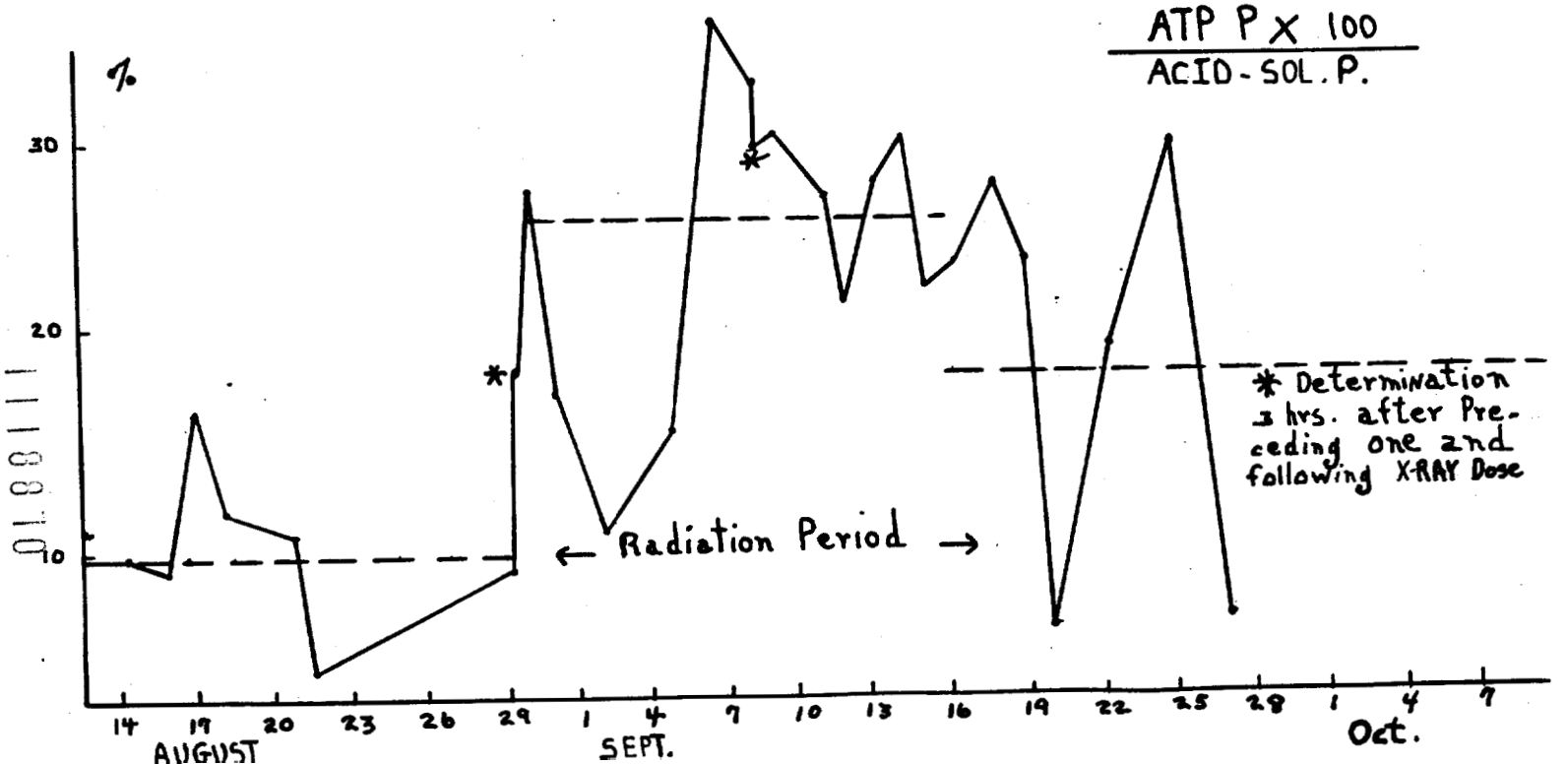
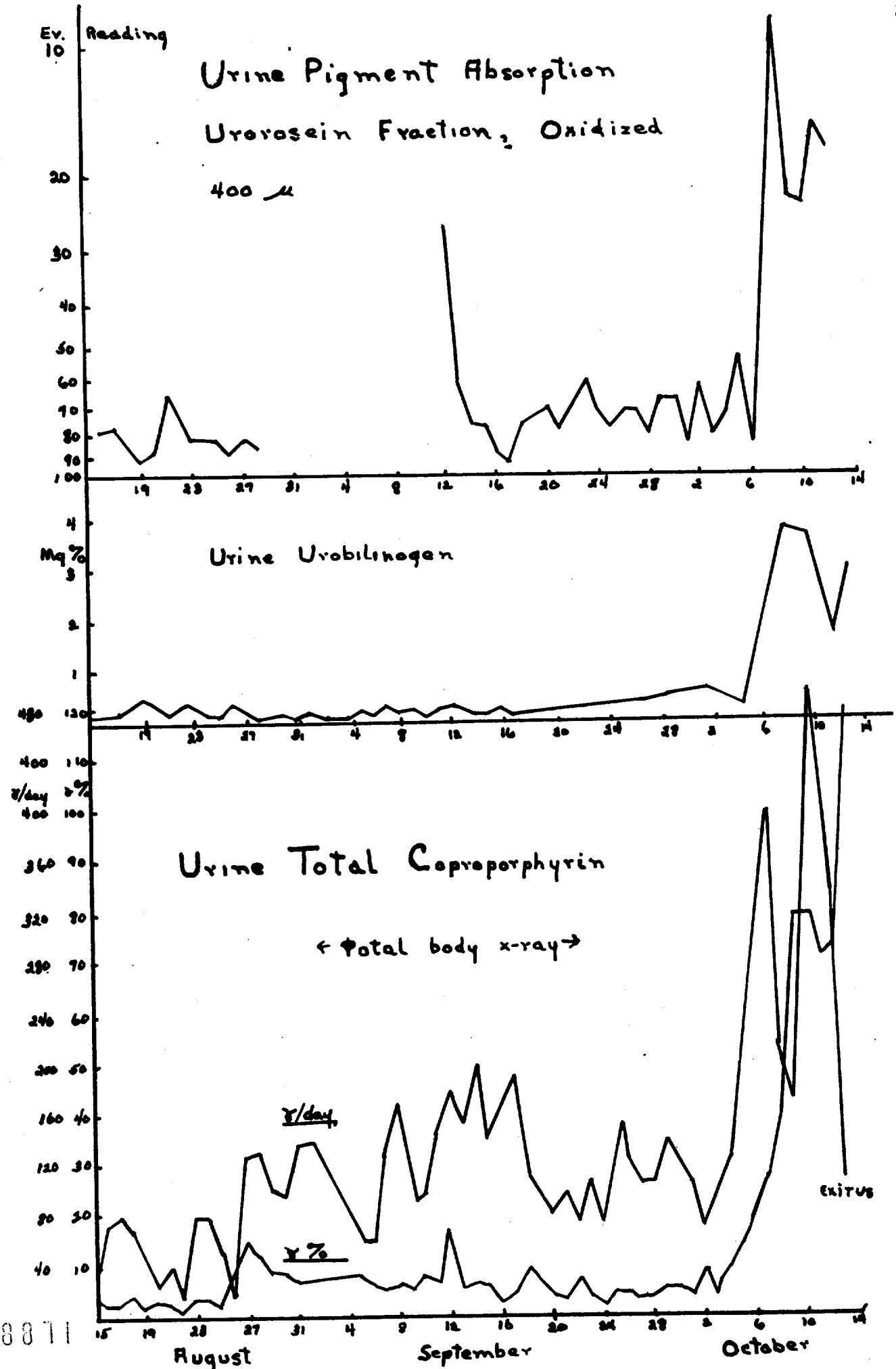
FIGURE I


WBC
12,000
10,000
8000
6000
4000
2,000
TOTAL WHITE BLOOD COUNT
EXITUS
10/14


γ-P. Per Million WBC
30
20
10
ADENOSINE TRIPHOSPHATE PHOSPHORUS


%
30
20
10
ATP P X 100
ACID-SOL. P.
* Determination 3 hrs. after Preceding one and following X-RAY Dose
← Radiation Period →
14
19
20
23
26
29
1
4
7
10
13
16
19
22
25
28
1
4
7
AUGUST
SEPT.
Oct.


Ev. Reading
Urine Pigment Absorption
Urorosein Fraction, Oxidized
400 μ
Urine Urobilinogen
Mg %
Urine Total Coproporphyrin
← Total body x-ray →
γ/day
γ %
Exitus
August
September
October

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III. Urine Coproporphyrin Isomers in Human Subjects.

Studies of urine coproporphyrin isomers in T-exposed project personnel continue to indicate that the increase found is due chiefly to the type III isomer. A complete report of these studies will be presented next month.

IV Studies of Urorosein and Other Urine Pigments.

Studies of project personnel continue to reveal elevated values of urine urorosein and other pigments. The method used has been described in the report of February 1944. The pertinent data is summarized in tables I and II below.

Table I Absorption Studies of Urine Urorosein Fraction

	520 mu				400 mu			
Exposure	Unoxidized		Oxidized		Unoxidized		Oxidized	
	No.	% 2-4+	No.	%2-4+	No.	% 2-4+	No.	% 2-4+
Controls	33	3	33	6	33	6	33	6
Slight mixed exposure	62	9	57	32	62	16	58	28
Radiation	44	20	42	38	50	20	52	48
T-Metal	82	17	80	29	83	14	74	24
Non T-Metal	46	17	45	40	44	16	46	26

Table II Urine Urorsein(Absorption Band 543 mu) in Project Personnel.

Exposure	No.	% Unoxidized positive	No.	% Oxidized positive
Controls	33	3	33	36
Slight mixed exposure	57	12	52	69
Radiation	41	24	37	92
T-Metal	80	9	80	55
Non T-Metal	51	4	51	69

The reasons for the obviously non-specific increases in urine pigment are still unknown. It is hoped that light may be thrown on this problem by absorption studies using the Beckman spectrophotometer.

V. Studies of T in Urine
With George R Price and Renato Ferretti.

Studies aiming at the development of a relatively simple, rapid, sensitive and accurate method for the micro-quantitation of T in biological materials are continuing. Our efforts to date have been concentrated almost entirely on urine. Changes both in the apparatus and in procedures are still continually being made. We have been handicapped, too, by the lack of space where adequate precautions might be taken. In spite of this a sensitivity of 0.0001 microgram of T has been achieved using a fluorophotometric method of analysis for T in fused NaF.

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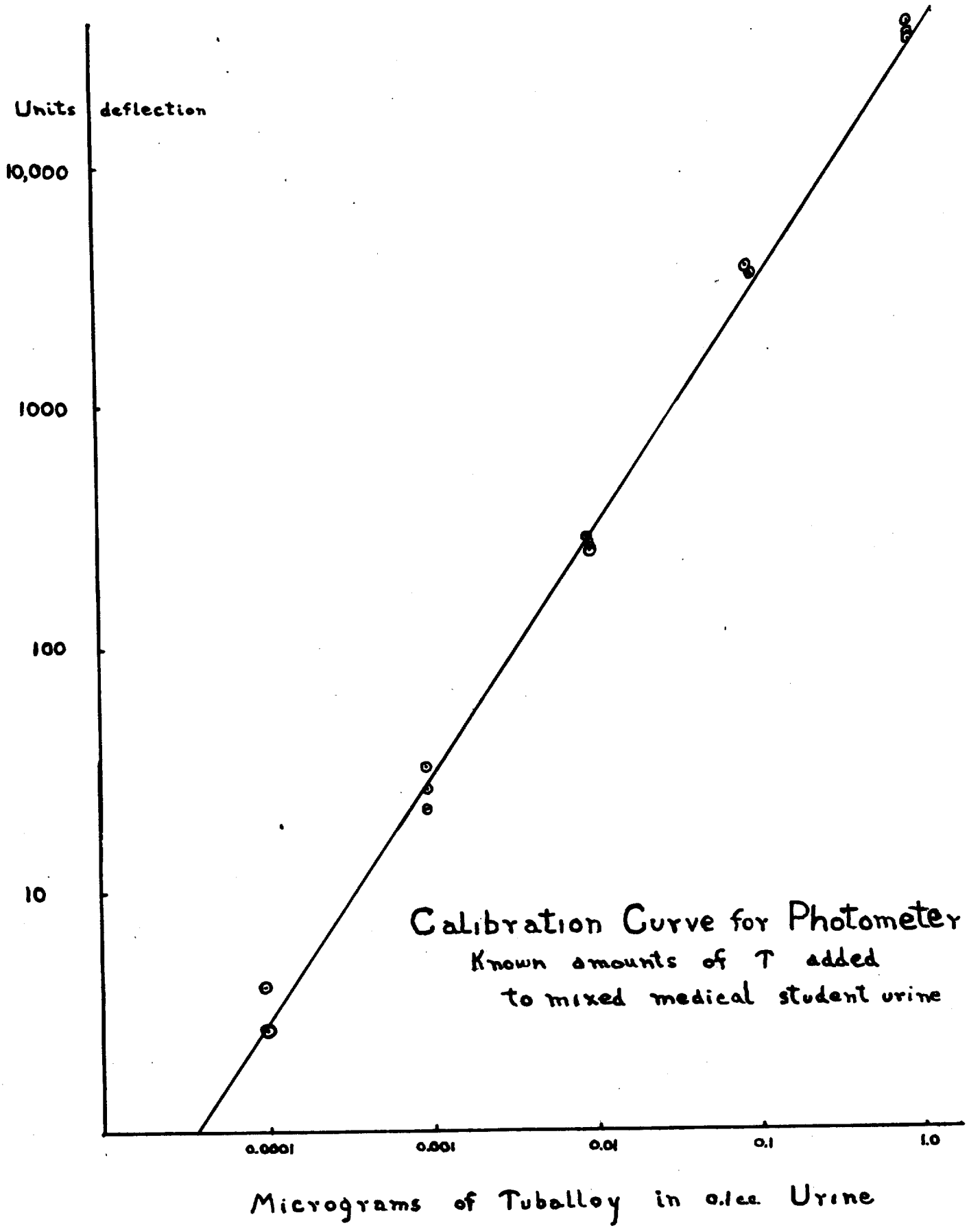

Calibration Curve for Photometer
Known amounts of T added
to mixed medical student urine

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a. Apparatus

1. A very sensitive 931A electron multiplier photocell. This photocell is stabilized by cooling with dry ice.
2. A galvonometer with sensitivity of 0.0006 micro amperes per mm deflection, equipped with a variable shunt.
3. A special lens system.
4. A high voltage source of direct current.
5. A 4H General Electric mercury arc lamp.

b. Method

Add about 0.1 colume concentrated HNO_3 to a small sample of urine. Evaporate 0.1 cc urine to dryness in a platinum dish on a warm hot plate. Ash by heating in a flame. Add about 150 milligrams specially pure NaF. Heat again to fuse the NaF. Quantitate b comparison with one or more fluorescent standards.

Different 0.1 cc samples of urine studied to date have been found to produce from 30 to 60 percent quenching of T-NaF fluorescence. The curve shown in figure I was obtained by adding various amounts of T to portions of a combined sample of urine from medical students.

One unit on the curve corresponds to approximately 0.001 micro amperes which will give a galvonometer deflection of 1 2/3 devisions when set for maximum sensitivity.

c. Urine T Studies in Humans

Studies of urine T concentration in Ames personnel show a reasonable correlation with T exposure (chiefly TF_4) as indicated in Table 1 below. These urine, according to instructions, were passed at home into a clean bottle immediately after bathing. A nitric acid rinse of the bottle in which the urine was shipped to us was added to the urine to make sure that no T was lost b adsorption on the glass.

In this table personnel are divided into three main groups according to exposure:
Group I: Chemists exposed to little or no T.
Group II: Personnel in the plant supposedly having only incidental T exposure.
Group III: Moderate to heavy T exposure.

All urines were studied at least in duplicate. The average value was multiplied by 1.4 to abtain a partial correction for quenching. (See table II for details.) The micrograms of T so obtained were multiplied by 10,000 to convert from 0.1 cc to one liter.

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Table I: Correlation of Urine T with Exposure

T Exposure	Micrograms T/liter urine
Group I	
1. A. V.	
2. H. B.	less than 3
3. R. B.	7
4. J. P.	less than 3
5. R. E.	less than 3
6. [illegible]	10
	Average-less than 5
Group II	
1. C. M.	18
2. O. E.	16
3. C. R.	15
4. R. T.	33
	Average--20
Group III	
1. S. L.	44
2. B. S.	48
3. J. T.	29
4. A. J.	43
5. L. B.	25
6. L. S.	12
7. C. A.	50
8. D. M.	73
9. D. L.	31
10. N. M.	58
11. D. S.	28 6
	Average--40

In order to correct for the variation in the quenching produced by different urines, experiments were run in which urines from Ames personnel having different amounts of exposure to T were read both as originally obtained and after the addition of .01 micrograms of T to each one-tenth cc sample. The difference between the two groups of readings is due to the added .01 microgram, and by comparison with the readings obtained with this much T in the absence of urine, or in the presence of mixed medical student urine, a rough measure of the amount of quenching can be obtained. Assuming that the % loss through quenching is independent of the amount of T present (as indicated in Figure 1), we derive a correction factor that will correct the Ames urine to the calibration curve obtained with a sample of mixed medical student urine.

In table II the columns headed "Original Urine" and "Urine plus T" give the average readings of a number of determinations, "N". The standard deviation, $\sqrt{\frac{\Sigma(\ldots)^2}{N-1}}$ is listed in the columns headed "s". Δ indicates the difference obtained [illegible]

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addition of .01 micrograms: % quenching refers to the percent of 367 that is lost. The correction factor corrects to 276, the value for .01 micrograms in the mixed medical student urine. The next column gives the corrected reading obtained by the use of the correction factor. The amount of T read from the calibration curve multiplied by 10,000 gives the results in liters instead of 0.1 cc.

Table II: Studies of Fluorescence Quenching Produced by Urines of Ames Personnel.

T Exp.	Urine	N	s	Urine T	N	S	Δ	% quenching	corr. factor	Corr. Read.	Micro grams T/ liter
A.											
1.							367				
B.							276				
C.											
1.	3.4	5	4.2	229	4	24	226	38	1.2	4.1	<3
2.	4.1	3	2.5	223	2	11	218	41	1.3	5.3	<3
3.	4.0	4	3.4	220	2	29	216	41	1.3	5.2	<3
D.											
1.	122	6	19	380	2	42	258	30	1.1	134	44
2.	80	6	16	236	2	11	156	58	1.8	144	48
3.	58	5	5	238	2	5	180	51	1.5	87	29
4.	93	3	18	292	2	1	199	46	1.4	130	43
								Average	1.4		

Group A: Pure T (No urine)
Group B: Medical Students
Group C: Negligible Exposure
Group D: Heavy Exposure

The average correction factor of 1.4 is used for all the urines not included in this table. Its use on the above urines, instead of the derived correction factor, would not have caused any change amounting to as much as 30%.

The very large deviations among the lowest level dishes can be greatly reduced when special precautions are taken, as was done in the case of the calibration curve. These precautions require considerable extra work. In the absence of adequate laboratory space, some occasional contamination is almost unavoidable.

Preliminary studies have been made employing various organic solvents to achieve further purification by extraction of T from urine. These procedures are promising as regards other biological materials. At the present time, however, we feel that the method proposed in this report is adequate for routine urine T studies.

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Metallurgical Laboratory

BIOLOGICAL RESEARCH SECTION

K. . Cole, ection Chief

A. Radioactive Materials - Mammals

1. I_2^{131} vapor.

A group of 21 rats were exposed to air containing a very low concentration of carrier-free I_2^{131} vapor (8 x 10^{-7} curies/liter) for 30 minutes to obtain rate of absorption through the lungs and subsequent distribution of material administered by this route. The results may be summarized as follows: (1) Roughly 10 percent of the iodine inhaled by a rat was retained. (2) Rate of absorption from the lung was rapid (like iodide absorption from the gut), with over 95 percent of the iodine absorbed during the 30 minute exposure. (3) Fate of inhaled iodine was semi-quantitatively similar to fed iodide - within 24 hours 10 percent was in the thyroid and the other 90 percent had been excreted in the urine. Chaikoff's figures in the literature for fed iodide indicate a much larger thyroid deposition, 65 percent, and a correspondingly smaller excretion.

2. Aerosol exposure methods

A good deal of time has been spent in construction of a static chamber and methods of producing aerosols whereby we can increase in the efficiency of utilization of material. One of two general lines along which we are working is spraying of solutions in volatile solvents with subsequent evaporation of destruction of the soluvent. In this connection work has been done on the preparation and properties of organic complexes (cupferride and oxine, in particular) of product and various fission products. Attempts have been made to dissolve the materials in low boiling non-toxic substances such as the freons and allow the solution to atomize through an orifice. Also, we have tried to atomize ethyl acetate solutions through a spark which combusts the solvent to CO_2.

The second line along which we have worked is the electric arc as a means of putting up material. A number of different types of electrodes have been tried in atmospheres of varying oxygen contents, the arc usually being operated at about 50 volts DC. It was found that all the active material could be burned off a carbon electrode in a few seconds but violent thermal impingment deposited activity on the chamber walls.

Both the general methods outlined above have so far given 15 to 20 percent of the starting material in the final aerosol. Work is continuing to step up this efficiency.

3. Fission mixture (mainly cerium) aerosol.

Animals were exposed to dry aerosol of a fission mixture preparation which was mainly long lived Ce. Sufficient absorber was used in counting so that our data can probably be interpreted as Ce data. Loss from the lung was slower than with yttrium but faster than zirconium. After 56 days, 20 percent of the activity originally in the lung was in the skeleton, 15 percent still in the lung, 5 percent in the liver, and the other 60 percent was no longer in the animal.

4. Yttrium (57d) aerososl.

a. Tracer esposure

A tracer run with 57 day yttrium as a dry aerosol has been carried out but data are not yet complete. Half the activity in the lung was gone in less

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than a day and 90 percent was gone in 8 days.

b. High level exposure.

A high level yttrium run was carried out which deposited an average of 26 μc in the lungs of each rat. Rats killed after one week had histologically normal lungs after a total lung dose of 1500 r.

5. Zr^{93}-Cb^{93} metabolism and toxicity.

A solution of Zr^{93} - Cb^{93} was injected into fifteen rats. Five of the rats received the solution intraperitoneally, five by heart puncture, and five by injection into a tail vein. Ten to twenty percent retentions in lungs and liver were found after ten days, in animals injected intravenously and intracardially. In these animals retention of the radioactive substances at the site of injection was about 1%. In the case of intraperitonealy injections absorption from the site of injection was about 75 percent, 6 percent of which was retained by the liver and about 0.5 percent by the lungs. By all routes, 15 percent of the absorbed activity was taken up by the skeleton, and 30 percent excreted in the urine.

The 15 day mid-lethal dose of Zr^{93} -Cb^{93} solutions is about 5 μc/gm in rats. This dose approximates the mid-lethal doses of Sr^{89} and Ba^{140}-La^{140}. But unlike these alkaline earth, injected Zr^{93}-Cb^{93} caused extensive damage to soft tissues. Upon gross examination after autopsy, hemorrhages were found throughout the gastro-intestinal tract. The livers were unusually pale.

6. Sr^{89}

a. Radioautographic dosimetry.

A femur from a rabbit injected with Sr^{89} was imbedded in methyl methorcrylate, cut in half and placed in contact with photographic film. The exposed film was developed under standard conditions and the radiation intensity determined by comparison with film exposed to a calibrated standard. The numbers on the autograph - (Plate I) represent r/hr received by the tissues at the various points indicated. The relative values are fairly accurate. The absolute values are subject to revision.

b. Chronic effects of Sr^{89}.

ABC and CF_1 mice that received injection of radioactive strontium in April are showing tumors in both the controls and experimental animals. Thus far 2 bone tumors, 2 mediastinal lymphomas, several lung tumors and a breast tumor have been seen. One of the bone tumors was in a control animals. Two tumor masses from treated animals were tested for radio-activity. One was a mediastinal lymphoma which was inactive and the other was bone tumor which showed considerable activity.

c. External Count of Animals Containing Radio- Strontium.

Calibration of an external counter for mice has been made by comparing external counts with ash counts on 100 animals injected with Sr^{89}

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b. Chronic effects of Sr^{89}.

ABC and CF_1 mice that received injections of radioactive strontium in April are showing tumors in both the controls and experimental animals. Thus far 2 bone tumors, 2 mediastinal lymphomas, several lung tumors and a breast tumor have been seen. One of the bone tumors was in a control animal. Two tumor masses from treated animals were tested for radio-activity. One was a mediastinal lymphoma which was inactive and the other was bone tumor which showed considerable activity.

c. External Count of Animals Containing Radio-Strontium.

Calibration of an external counter for mice has been made by comparing external counts with ash counts on 100 animals injected with Sr^{89}. Using an electroscope for the ash measurement and a mica end-window tube and wire screen box for the external counting a conversion factor of 8.7×10^{-4} μc/external count/

min. was obtained, that is, a mouse containing 1 μc of Sr^{89} would give an external count of 1150 counts/min. The external count is 4 percent of the ash count on the same animal counted on the open counter. A calculation of a factor which combines geometry and absorption by mouse, screen etc. gives for this external counter a factor of .052 percent for the mouse and 13 percent for a capsule. The mica window tube has recently been replaced by an elongated thin walled G-M tube and the screen chamber replaced by a cellulose acetate holder. This set-up has been calibrated by comparing the external count and ash count on 12 mice. The conversion factor is 6.4×10^{-4} μc/external count/min. which gives a geometry and absorption factor of .067 percent. By proportion the present set-up gives an external count equivalent to 5.1 percent of the ash count under the comparable geometry.

It was found in a large series of mice counted with the skin on and after the skin was removed that 50 to 40 percent of the activity detected by the external counter was absorbed by the skin.

No correlation could be found between the size of mice injected intraperitoneally with Sr^{89} and the percent of the Sr^{89} retained, as determined by an ash count, after two to three weeks. However, the external counts tend to be lower for the larger mice but with no constant size factor.

7. Fission recoil products.

Radioautographs have been prepared and histo-pathology studied on rats which were exposed to fission recoil products in the Clinton pile. A complete report will be submitted from the Clinton Laboratory.

B. External Radiation

1. Single doses of X-radiation

a. Weight

Study of the weight of rats receiving 10 r, 25 r, and 50 r, shows no significant effect on their subsequent growth. These results do not agree with a previous report but are more reliable.

b. Histopathology

The gut of rats exposed to 900 r showed changes in the surface epithelium and dense connective tissue infiltration of the lamina propria, neither of which effects were marked in the previously reported 600 r rat series.

Six rabbits exposed locally (one leg or anterior third of body) to 2000 r and 3000 r showed only such slight changes in shielded organs as might be explained on the basis of incomplete shielding.

2. Paired doses of X-radiation - survival.

Paired dose studies on rats indicate that recovery is exponential the first three weeks after 300 r and that one-half recovery is accomplished in about 7 days. There is no evidence that the recovery rate changes during the period studied.

Paired dose studies on rabbits seem to indicate that there is a residual effect from doses between 500 r and 900 r after 100 days, but we have been unable to quantify the residual damage.

3. Daily doses of X-radiation - survival.

Three of ten rats exposed to 12.5 r per day remain alive after accumulating 3,400 r.

4. Acute effects of irradiation on dogs. (Single Dose)

Dogs receiving 300 r or higher have shown (1) shock reactions (prostration, vomiting, diarrhea) during the first 2 hours after irradiation, (2) a late period of depression (rise in temperature, rise in heart rate and fall in blood pressure) at 10-14 days, (3) a gradual fall in hematocrit and plasma protein and (4) death in 10-17 days. Those animals which showed late adema, salivation or diarrhea also showed a late rise in hematocrit and plasma proteins. Dogs which received doses between 50 r and 200 r showed none of the above reactions although they did show a leukopenia. Dogs showed gradual increases in plasma histamine at all levels of irradiation from 50 r up. One dog which received 50 r showed an increase of about 10 times in plasma histamine on the 42nd day.

5. Therapeutic Effects of High Water Output.

Four normal rabbits with high urine outputs (200 cc/day) have survived more than 28 days after receiving 800 r. Two rabbits were given 1.0 cc/Kg of 15 percent NaCl and 1.0 cc/Kg of 10 percent glucose respectively intravenously daily for two weeks after 800 r. The urine output was increased, both rabbits showed good weight recovery and are in good condition at 28 days. These results agree with previous reports on the therapeutic value of agents which tend to flush out toxic materials.

6. Fast neutrons - survival.

The median lethal dose at 30 days for cyclotron fast neutrons on CF1 female mice is approximately 57n. This is considerably lower than the value obtained by Zirkle at Clinton. The x/n ratio for these mice is 8.1, a value somewhat higher than that obtained for rabbits or for Drosophila eggs.

C. Toxicity of Non-Radioactive Materials.

1. Slug impurities - histology.

Cold slug metal impurities were made up in solution so that 50 cc contained the equivalent of one slug. There were four groups of rats, each containing one female and one male with the exception of one group which had only one male:

A)	5.0 cc by stomach tube		1 M	1 F	0.1 of a slug
B)	1.0 cc by stomach tube		1 M	1 F	0.02 of a slug
C)	1.0 cc	I. P.	1 M		0.02 of a slug
D)	0.2 cc	I. P.	1 M	1 F	0.004 of a slug

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At seven days the kidney was damaged by all of the above dose levels. With the exception of focal necrosis in the adrenal of the animal showing the most severe kidney damage, no other organs showed damage. It was not possible to make quantitative comparisons of the extent of damage at the various dose levels.

2. Tuballoy nitrate - histology.

Damage to the kidney was detected in rats given 0.5 mg of $TO_2(NO_3)_2$ by subcutaneous injection as early as 24 hours after injection.

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Plate I

Fig 1 Photograph Fig 2 Autuograph of one half of the femur from a rabbit which had been injected with 1 millicurie/kilogram of Sr^{89} and died 13 days later. The figures in the autograph represent dosage in r/hr in the various regions of the bone. The bone is imbedded in methyl methacrylate. 2.5 X

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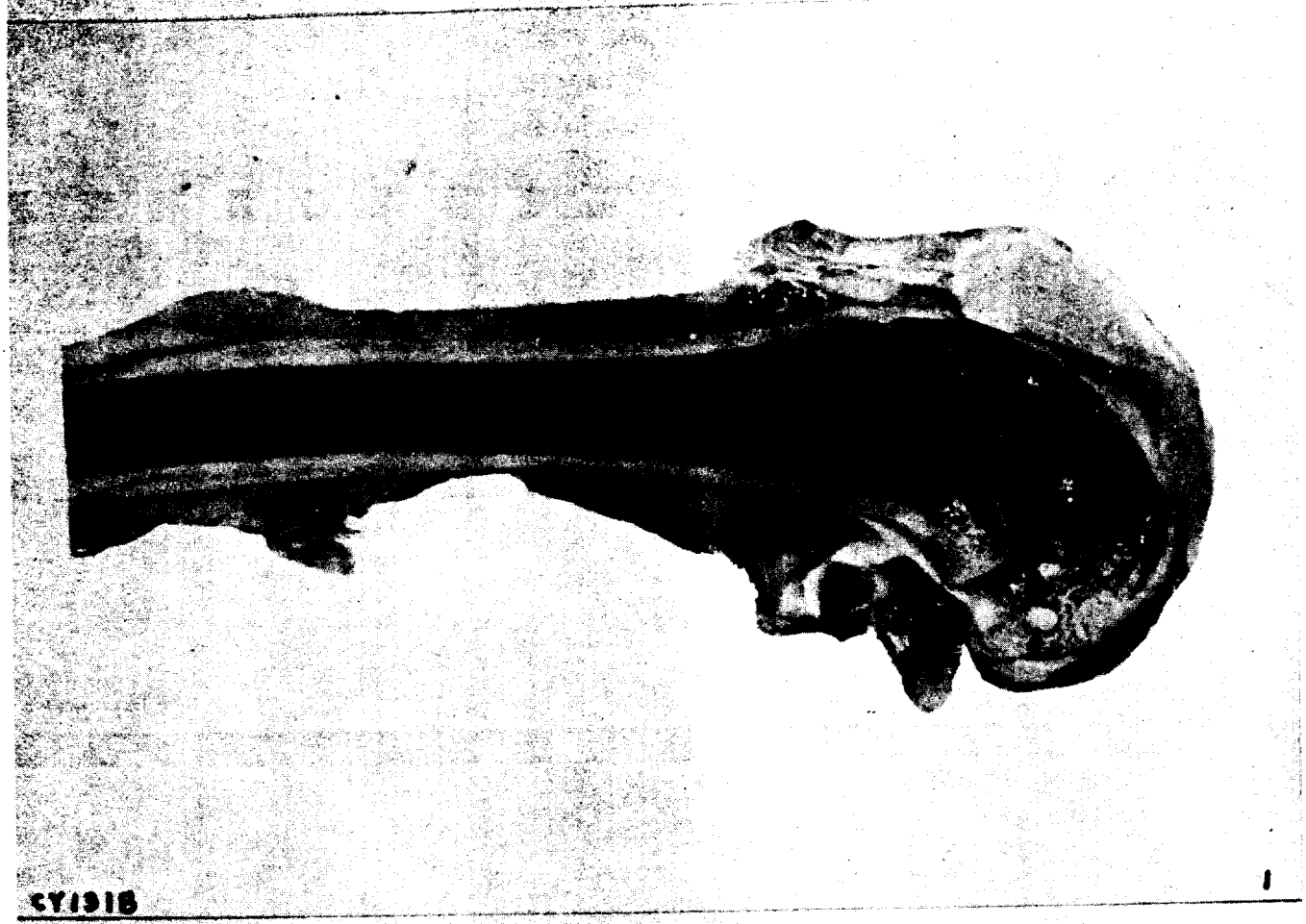

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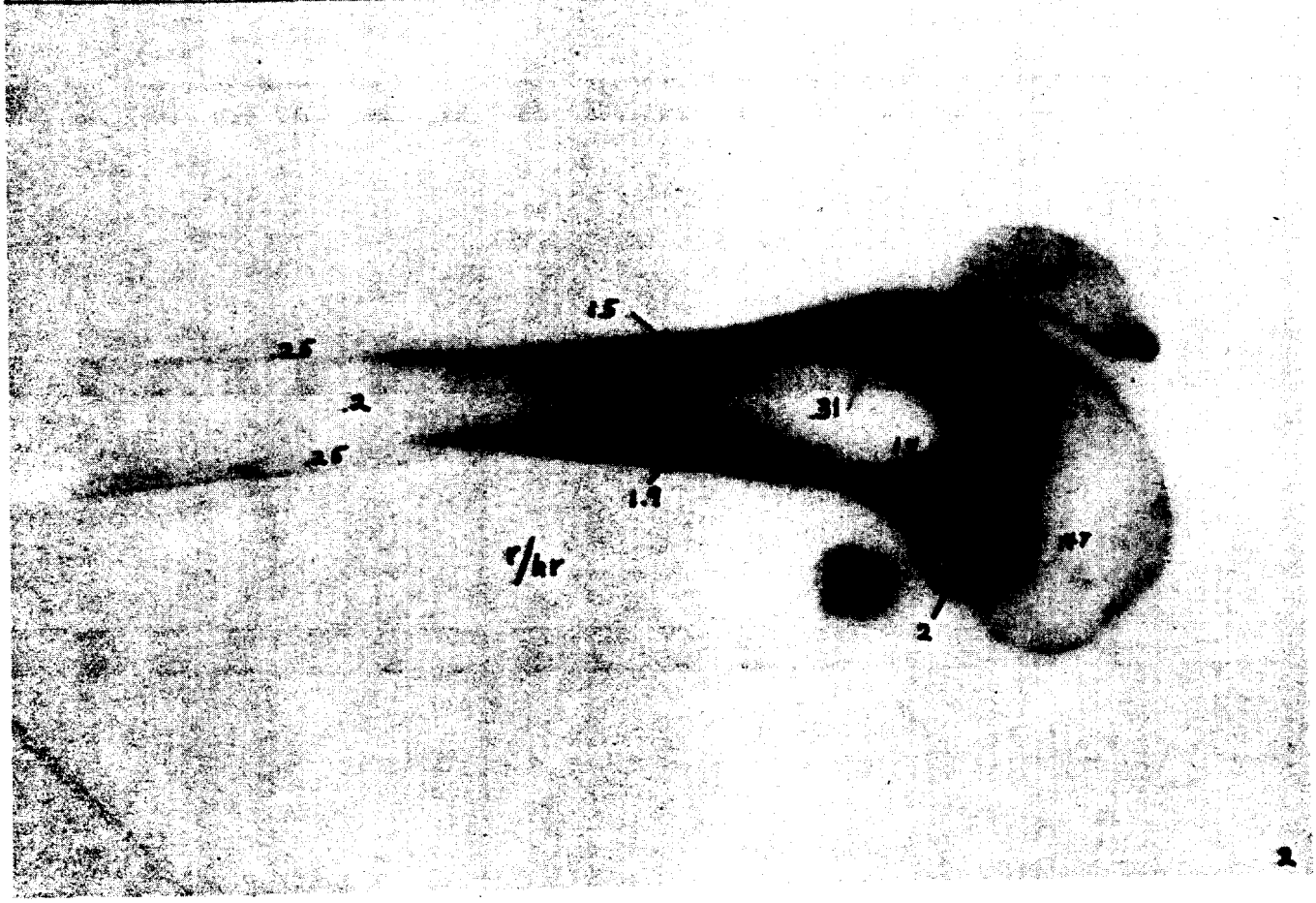

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Metallurgical Laboratory

MEDICAL INDUSTRIAL HAZARD SECTION
J. J. Nickson, Section Chief
J. E. Rose, Associate Section Chief

New Chemistry

Beta and gamma contamination in this building continues at much the same level as during the previous month. For the most part the contamination is on small articles. Little gamma radiation has been noted.

A TX_{II} hazard has been discovered in room D-10A. Recommendations for installation of ventilation to diminish the hazard, have been made. Mail shipments of active material from two outside areas to the laboratory ceased recently.

West Stands

Rooms 8 and 9 were returned to routine use after decontamination. Widespread beta or soft gamma contamination again exists. Few areas of hard gamma are found. This is in contrast to the conditions observed before the decontamination. Room 217, the hot lab, was used for several runs during the month. The room again shows wide spread areas of radioactivity. As above, they are for the most part beta or soft gamma. Both instances illustrate the fact that we have not developed satisfactory techniques for handling active materials. The "reactivation" of these rooms occurred promptly after work was initiated.

Construction of the solvent extraction process columns continues. The possible hazards involved have been the subject of numerous discussions with the Technical Division.

Site B Annex

Fission product contamination of equipment has been satisfactorily controlled during the past month. Storage has also been satisfactory.

Armory

Unsuspectedly high activities in special materials occurred only once. An aluminum jacketed specimen was found to give approximately 2 r at its surface. It had been planned to ship it by courier to Clinton. After the activity was discovered, it was sent with a lead shield by truck.

Eckhart-Ryerson

The x-ray installations in Ryerson 153, have been shielded satisfactorily after the discovery that up to 1300 mr per hour (soft x-rays) was possible in certain working areas.

Site B

As a result of the findings discussed last month, recommendations were made to the Director of the Technical Division for the installation of some means of preventing the escape of the TX_{II} into the air. It is hoped that this installation will be completed in the near future.

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A recent survey using the techniques described last month indicated that approximately one-third of the rooms still showed undesirable levels of T activity.

Cyclotron

Operations were suspended in this building during the past month. Equipment being shipped out is being survayed for activity. A general survey of the area reveals widespread low activities throughout.

Badge Meters: for the period 8/21 - 10/8/44.

Approximately 500 films are developed each week. 7 films of project personnel had readings greater than 0.6 r for one week. 4 of these occurred on persons working in Ryerson 153. This room is used for x-ray diffrection work. The conditions responsible for the over-exposure have been corrected. 2 over-exposures occurred at the West Stands. The last over-exposure was on a member of the Health Physics group.

Pocket Meters: readings for the period 9/7 - 10/11/44.

81 meters gave a daily reading exceeding 0.1 r. Of these 35 were felt to be valid readings. 18 of these were from the West Stands, 11 from the New Chemistry area, and 6 from the Cyclotron area. The blood counts were normal in all but one person. This individual had a 4400 white blood cell count. He is a member of the semi-works group.

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E. ASSOCIATED PROJECTS

I. N. C. I.

R.R. Spencer, Chief

BIOLOGICAL ACTION OF X AND GAMMA RAYS

MICE: Total doses: 4800 r (8 r/day), 2400 r (4 r/day), 1200 r (2 r/day), 600 r (1 r/day), 63 r (0.1 r/day). Experiments started in the first months of 1943. The blood picture of the surviving experimental and control mice is confusing as far as the control groups, those given an acute exposure only and those given 0.1 r per 8 hours per day are concerned. The majority of the males and some of the females show high white counts between 10,000 and 20,000 (counts between 3,000 and 8,000 are considered as normal for LAF_1 mice). The reason for these high counts is obscure. These high white counts are almost totally absent in the mice of the higher levels (1 r, 2 r, 4 r, and 8 r per 8 hours per day). Red count and platelet count are normal in all mice with the exception of some mice of the 4 r per 8 hours level and all mice of the 8 r per 8 hours level in which it is low. Twelve more mice were found with malignant lymphoma bringing the total up to 46 at present. The distribution of the lymphomas among controls and experimental mice is given in a table. It follows from this table that only in some of the mice of the 2 r, 4 r and 8 r per 8 hours level the incidence of lymphomas was enhanced by the irradiation. The total white count of inbred strain A mice (started in May 1944) exposed on the 8 r per 8 hours level (total dose 1350 r) which at the early stages of the experiment was considerably lower than that of the controls has gradually risen and is at present similar to that of the controls.

Hybrid Guinea pigs: Total dose 2400 r, 1200 r, 600 r and 63 r. Experiments started in the first months of 1943. The blood picture of the hybrid guinea pigs, exposed on the 0.1 r and 1 r per 8 hours levels as well as that of those given acute exposures only or limited exposures is essentially the same as that of the controls. The hybrid guinea pigs on the 2 r per 8 hours level, show all counts lowered, especially the platelet counts. The surviving hybrid guinea pigs of the 4 r per 8 hours level show a considerable lowering of all counts (R.B.C. down to 1.3×10^6 and platelets down to 50,000). The inbred guinea pigs of family 13 exposed on the 8 r per 8 hours level since April 1944, (dose 1,500) show a lowered total white count and platelet count. The red count is normal contrary to findings on hybrid or inbred guinea pigs of family 2 which have either died of aplastic anemia before this dose was reached or had low red counts for this dose.

Rabbits: total doses 3900 r, 1950 r, 975 r, 490 r and 48 r (exposed since July 1943). There is no change in the blood picture of the rabbits from that previously reported: lymphopenia with increasing total dose.

Inbred strain C3H females and hybrid LAF_1 females differ markedly in the response of their ovaries to irradiation. Whereas C3H females are sterile after a total dose of approximately 400 r given at the rate of 4 r per day, some LAF_1 females bred (although with reduced litters) and other were sterile after 800 r at 8 r per day, and all bred with normal sized litters after 700 r at 8 r per day. On the 4 r day level LAF_1 mice bred normally after a total dose of 500 r but showed a reduced litter size after 700 r. This difference between the 700 r at 4 r per day group and 700 r at 8 r per day group may be owing to the older age of the 700 r at 4 r per day group.

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As yet the breeding tests have failed to show a marked difference between the effects of the continuous exposure and the 8 hour/day exposure when the total dose is the same. After a dose on 800 r at 8 r per 8 hours some LAF_1 females will breed although with reduced litters while others are sterile. This is comparable to the results of 800 r at 8 r per day.

Male mice are more resistant to sterility effects of chronic exposure than female mice. Whereas females became sterile with doses around 800 r at 8 r per day and 8 r per 8 hours, sterility appeared in the males after 1600 r t 8 r per 8 hours. Further more the males recovered whereas the sterile females have not.

Tests for translocations have thus far failed to give evidence of chromosomal changes in progeny of these chronically irradiated mice.

Tissues from one control and fifteen experimental mice were available for histologic examination since the last report. Gonadal atrophy, ovarian tumors, and lymphomas were observed. Their occurrence is related (only to a certain degree for lymphomas and also probably ovarian tumors) to level and total dose of irradiation as previously reported on other animals. Other findings, includi g hepatoma, subcutaneous hemangioendothelioma, subcutaneous sarcoma, aquamous carcinoma of the vagina, benign uterine polyp, pulmonary adenomas, chronic nephritis with amyloid disease of the liver and spleen, were encountered and considered to be unrelated to the irradiation.

I Continuous exposure for 8 hours daily

Daily dose: 8 r, 4 r, 2 r, 1 r, and 0.1 r. Approximate total doses up to date of mice and quinea pigs (with the exception of the guinea pigs of the 8 r per 8 hours level which are all dead): 4800 r, 2400 r, 1200 r, 600 r and 63 r (acute exposures of 12.5 r and 50 r not added). These are the original experiments started between February and March 1943.

Since that time a number of new experiments were started; some of which are finished.

(1) Female rabbits exposed on all levels since July 1943 approximate total dose up to date 3900 r, 1950 r, 975 r, 490 r and 48 r (acute exposures of 12.5 r and 50 r not added).
(2) Female LAF_1 mice exposed to 5 r in 1 hour daily, one group receiving in addition 0.1 r in 8 hours daily since May 1943. This experiments was finished in February 1944.
(3) Hybrid ruinea pigs (male and female) exposed on the 8 r per 8 hours level since December 1943. This experiment was finished in August 1944.
(4) Inbred guinea pigs (male and female) of family 2, exposed on the 8 r per 8 hours level since November 1943 (male) and February 1944 (female). This experiment was finished in May and August 1944 respectively.
(5) Inbred guinea pigs (male and female) of family 13 exposed on the 8 r per 8 hours level since April 1944; approximate dose 1500 r.
(6) Inbred guinea pigs (male and female) of family 13 exposed on the 8 r per 8 hours level since May 1944. Approximate dose to date 1400 r.
(7) Hybrid guinea pigs (male and female) exposed on the 8 r per 8 hours level since August 1944. Approximate dose up to the present 500r. The purpose of these additional experiments was outlined in previous reports (Month of start of the individual experiment).
(8) Inbred Strain A mice 8 r per 8 hours level. As the first mice received on blood counts, another group of strain A mice was set up on this level for blood counts in May 1944. Approximate dose up to date 1350 r.

Since the last report 26 mice were killed and autopsied. They were killed for various reasons such as: palpable tumors or rapid loss of weight or severe eczema. Twelve showed grossly malignant lymphomas. Other lesions found were ovarian tumors, hepatomas, lung tumors etc. These lesions were considered not to be due to the irradiation. The animals are 21 to 23 months old at present. The incidence of ovarian tumors has not been tabulated as yet. The distribution of the malignant lymphomas among the different experimental and control groups is as follows: 2 from the 8 r per 8 hours level (4500 and 4700 r), 1 of the same level which was exposed to a total 600 r between the age of 3 to 5 months and then removed from the field, 3 of the 4 r per 8 hours level (dose 2250 to 2300 r), one of the 2 r per 8 hours level (dose 1200 r), one of the 1 r per 8 hours level (dose 600 r), one of the 0.1 r per 8 hours level (chronic dose 60 r plus acute dose of 50 r), one that had received an acute exposure of 12.5 r in 5 hours at the age of 4 months, and 2 controls.

Up to the present the total number of mice with malignant lymphoma is 46. The distribution among the different levels is given in the following table. A number of these lymphomas have not yet been examined histologically, however, only those were included in the table in which gross examination left no doubt

Data on Malignant Lymphomas

Group	Original number of mice	Number still living	Age at present in months	Total chronic dose to date	Acute dose	Mice with malignant lymphoma			
						No.	age range	total chronic dose	acute dose
Control	32	22	21-23	---	---	3	20-22	----	----
Control (acute dose)	16	10	23½	---	12.5 r	1	23	----	12.5
Control (acute dose)	16	7	23½	---	50 r	1	19	----	50
0.1 r per 8 h. per day	48	39	22½	63 r	16 mice each 12.5 r & 50 r resp.	1	22	60	50
1 r per 8 h. per day	48	37	21	600 r	"	2	19-20½	530-580 r	acute exposures
2 r per 8 h. per day	48	31	21-21½	1200 r	"	7	15-21½	800-1200	negligible small in comparison
4 r per 8 h. per day	48	25	21½-22	2400 r	"	8	18-22	1700-2300 r	to total
8 r per 8 h. per day	48	8	23	4800 r	"	20	12-22 3/4	2500-4700 r	dose
8 r per 8 h. per day for 1 month	16	11	23	300 r	---	2	17-20	300 r	----
8 r per 8 h. per day for 2 months	16	13	23	600 r	---	1	21	600 r	----

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as to the character of the lesion. There are 3 mice of the control groups which have developed spontaneous malignant lymphoma. This indicates that probably all malignant lymphomas in the experimental groups are spontaneous with the exception of some of the 2 r, 4 r and expecially 8 r per 8 hours groups.

Since the last report 1 guinea pig of the 0.1 r per 8 hours level was killed because of rapid weight loss and one guinea pig of family 13 was killed accidentally. One rabbit that had received an acute dose of 12.5 r, 12 months previously died after losing 1200 gm in 3 months. Grossly the autopsies of these animals showed nothing noteworthy.

II Breeding Experiments

The early experiment, in which the total dose necessary to produce sterility in C3H mice was sought, showed that a total dose of approximately 400 r given at the rate of 4 r/24 hours was sufficient to produce sterility in female C3H mice. However, this dose was not sufficient to produce sterility in male C3H mice. This experiment has been repeated and the findings are in accord with those of the original experiment. The histological picture of the ovaries of these females is essentially that found previously. The ovaries are devoid of developing follicles, there are numerous anovular rings, some of which have hyaline centers and the organ is composed almost entirely of luteal-like tissue. The testes show normal spermatogenesis and are within the limits of the controls. There is perhaps a slight reduction in the number of sperm present in the epididymis.

The results of the experiments using LAF_1 mice indicate that a total dose of 400 r given at the rate of 4 r/24 hours is not sufficient to produce sterility. The first indication of any effect was seen in the females which received a total dose of 800 r at the rate of 8 r/24 hours. When these animals were mated, five of the fifteen animals failed to produce any littles. The remaining ten animals produced litters with an average litter size of 2.6. LAF_1 females, which received a total dose of 700 r at the rate of 8 r/24 hours, mated and produced litters with an average litter size of 7.1 which falls within the limits of the controls. Females receiving doses up to 500 r on the 4 r/24 hours level showed normal litter size. Those receiving a total dose of 700 r at the rate of 4 r/ 24 hours show a definite reduction in litter size, the average in this case being 2.3. This reduction in litter size is probably in part an age effect due to the fact that they were not mated until they were approximately eight months of age. The few results available indicate an effect in the case of females which received a total dose of 800 r at the rate of 8 r/8 hours. Breeding results show an average litter size of 2.0 for these animals. The data seem to indicate that very little difference if any exists in the results obtained for the animals receiving a total dose of 800 r at the rate of 8 r/24 hours and those receiving 800 r at the rate of 8 r/8 hours. However it is impossible at this time to say that this is the first indication of any damage because of the meagre results vailable at present and also because of the fact that no breeding results are available for the group receiving a total dose of 700 r on the 8 r per 8 hours level. These latter animals have only recently entered the field.

In order to test for the presence of translocations in the offspring of irradiated animals, two breeding experiments are being run. The first of these, in which the LAF_2 progeny of irradiated LAF_1 males which received 1000 r at 8 r/8 hours

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are being tested, has been in progress since June. To date there is no evidence of chromosomal changes in the offspring. The second experiment in which the LAF_2 progeny of irradiated LAF_1 females which received 700 r at 8 r/day are being tested, has been set up recently. There are no results available in this experiment to date.

III Hematology

Mice: The blood picture of the control and experimental mice having received an acute exposure of 12.5 r and 50 r respectively or a chronic exposure at the rate of 0.1 r per 8 hours per day is somewhat confusing. Up to a few months ago the total white count of these animals fluctuated between 3000 and 8000, with the females usually having lower counts than the males. As already pointed out in the last 2 reports but increasing at present, the majority of the male mice and some females show high total white counts between 10,000 and 20,000 with differential counts either normal or with high percent neutrophil counts. It is not possible to say whether this is due to an infection or whether it is an old age effect. The animals eat normally, are active and show fairly constant weights. Some of them may be developing malignant lymphoma but, as stated in previous reports, the typical blood picture of lymphogenous leukemia is present in less than 50% of the cases with malignant lymphoma. Red count and platelet count of these animals are within normal limits. These high total white counts are much less frequent or absent in the animals of the higher levels (1r, 2 r and 4 r per 8 hours per day) and the total white count is within the above normal limits. The red count, and the platelet count are within normal limits with the exception of some animals of the 4 r per 8 hours level (total dose 2400 r) in which the red count is lowered. Most of the remaining mice of the 8 r per 8 hours level (dose 4800 r) show a normal white count (5) and the remaining 2 (males) counts between 10,000 and 20,000. Some show a high neutrophil count. The red count and platelet count are lowered in all. The mice of the 8 r per 8 hours level which were removed after having received a dose of 300 r or 600 r respectively show approximately normal counts.

The strain A mice exposed on the 8 r per 8 hours level (dose 1350 r) show total white counts ranging between 3100 and 8000, that of the controls lies between 4000 and 8000 except for 2 having counts above 10,000. It may be recalled that at the beginning of the experiment the white count of the experimental animals showed a rapid drop resulting in an average count of 50% of that of the controls. At present average counts of experimental and control animals are approximately the same.

The hybrid guinea pigs exposed since the beginning of last year of the 0.1 r and 1 r per 8 hours level as well as those given acute exposures only, show essentially normal counts as compared to the controls. Some of the animals of the 2 r per 8 hours level (total dose 1200 r) show lowered total white counts (some with relative lymphocytosis) and in some considerably lowered red and platelets counts. The surviving animals of the 4 r per 8 hours level show all counts lowered in varying degrees, R.B.C. between 4 and 1.4×10^6 and platelets between 200,000 and 50,000. The inbred guinea pigs of family 13 exposed on the 8 r per 8 hours level (total dose 1500 r) show low total white counts (average 4000) and low plutelet counts between 200,000 and 80,000. The red counts are normal. It is interesting to note that at this dose on the same level 59% of the hybrid guinea pigs died of aplastic anemia, the remaining showing low red counts. Also at this dose on the same level the majority of inbred guinea pigs of family 2 had died of aplastic anemia

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The hybrid guinea pigs exposed on the 8 r per 8 hours level since August 1944 (dose 500 r) show lowered white and platelet counts with red counts essentially normal. (2 experimental and 2 control guinea pigs of this series were killed and autopsied at the beginning of October by Dr. H. Lisco. The two experimentals have received an approximate dose of 350 r).

There is no essential change in the blood picture of the rabbits from that reported previously (lumphoma with increasing total dose).

IV Pathology

Mice--Tissues from 16 mice were examined histologically since the last report.

Seven of these had been exposed on the 8 r per 8 hours level (total dose 4200 to 4400 r). In three males there was extreme tubular atrophy of the testes yet with several masses of immature spermatogenic elements in two and spermatozoa in one. Lymph nodes were small in size but contained giant follicles. There was no atrophy or fibrosis of the bone marrow. The four female mice all had malignant lymphomas and ovarian tumors. The latter were composed of granulosa-like cells in solid masses in which there were invaginate downgrowths of the germinal epithelium. In all females the uterus was atrophied. In these seven mice numerous other changes were observed and considered to be unrelated to the irradiation, these included a hepatoma, a hemangioendothelioma of the chest wall, pyelonephritis and amyloid disease of liver and spleen.

Two female mice that had been exposed on the 8 r per 8 hours level (total dose approximately 300 r) at 2½ to 3½ months of age had ovaries devoid of follicles when autopsied at 20 months of age. There were extensive invaginate downgrowths of the germinal epithelium in these ovaries. One animal had a malignant lymphoma apparently arising in the mesenteric lymph nodes. The other had a benign polyp of the uterus and a subcutaneous sarcoma in the suprapubic region, which were not considered to be related to the irradiation.

One male mouse had been exposed on the 4 r per 8 hours level (total dose approximately 2100 r). There was perhaps slight tubular atrophy of the testes but there was active spermatogenesis in all tubules and mature spermatazoa in the epididymus. The mouse had malignant lymphoma arising in the meseteric lymph nodes.

Two male mice had been exposed on the 2 r per 8 hours level (total dose approximately 1080 r). The testes appeared normal. Both animals had malignant lymphomas. Other organs appeared normal.

One male and two female mice had been exposed on the 1 r per 8 hours level (total dose approximately 540 r). The testes of the male appeared normal while the ovaries of the females were devoid of follicles and there were invaginative downgrowths of the germinal epithelium. One female had a malignant lymphoma. The bone marrow of these animals was not atrophied.

One control female mouse came to autopsy at 19 months of age. The ovaries contained numerous developing follicles. This animal had a malignant lymphoma and a squamous cell carcinoma of the vagina.

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