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HEALTH DIVISION

REPORT FOR PERIOD ENDING JANUARY 15, 1945

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L. O. Jacobson

Report Received: February 2, 1945; Figures Received: February 16, 1945

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ABSTRACTS

Clinical Medicine and Medical Research

The urinary catalase on rabbits shows large fluctuations daily. When rabbits having maximum catalase values of 20 are selected, the urinary catalase rises 4 to 8 hours after the injection of $TO_2(NO_3)_2$.

Phosphatase values in rabbit's urine are more consistent than those of catalase. There is a rise in urinary phosphatase a few hours after the injection of $TO_2(NO_3)_2$. There are however so many sources of enzyme contamination (bacteria, red cells, white cells, bladder endothelial cells) that it would be dangerous to rely on these enzyme measurements for the early detection of T poisoning. A periodic blood [illegible] test still remains the most reliable test for this purpose.

Biological Research Section

Rats which inhaled a smoke consisting largely of 500 day Ce together with its praseodymium daughter, retained in their lungs an average of 4[illegible] gc of material in terms of hard beta activity. Their initial calculated dose was 2460r/day to the lungs. Sixteen out of 20 are still alive after one month.

Rats which were injected with Sr^{89}, showed extensive destruction of bone marrow at one week after a dose of 2 mc/kilo and complete absence of hematopoiesis after a dose of 5 mc/kilo. Lymphatic tissue was damaged and some increase in production of blood cells by the spleen was noted.

Guinea pigs were treated with 175r. In the gastro-intestinal tract, epithelium showed slight damage. The lymphatic tissue showed considerable damage for two days; regeneration was complete by 7 days. Damage to bone marrow, thymus, spleen and lymph nodes lasted 10 days after which regeneration was complete. In the testis, immature cells showed a sequence of damage and regeneration like that in the bone marrow.

Mice which received daily x-radiation of 20 r during 180 days, showed an initial period of retarded growth, a second period of weight loss, and a final plateau period.

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I. Clinical Medicine and Medical Research

Leon C. Jacobson, Section Chief

Urine Catalase in T Poisoning

It was reported by the Rochester group that a rise in urine catalase was one of the earliest signs of T intoxication in rabbits. According to these reports there was also a rise in urine phosphatase. Both enzymes were found increased in some cases before the appearance of protein in the urine.

This remarkable finding - appearance of an enzyme of large molecular weight few hours after the injection of T - gave hopes of utilizing it for the early detection of T poisoning and at the last joint meeting in Chicago the catalase test was suggested as an early diagnostic method. However, the opinion regarding the validity of this test was not unanimous. Dr. Schmidts of the Health group of Chicago was unable to confirm the findings of the Rochester group; Dr. Watson warned about the possibility of [illegible] urine catalase the enzyme from red cells frequently present in urine and [illegible] warned against the danger of bacteria and white cell contamination. In view of these discrepancies it was decided to restudy the problem of the use of catalase as a test for early detection of T poisoning.

Methods

Catalase. - [illegible] determinations were performed manometrically with the Barcroft-Warburg manometer, first at 5° and later at 20°. To the main portion of the vessel there were added 2.3 cc of 0.1 M phosphate buffer pH 6.8, and 0.5 cc urine. The side arm contained 0.3 cc of 0.1 M H_2O_2 (Merck's 30 per cent superoxol). After temperature equilibrium and initial reading of the closed manometers, the H_2O_2 was poured into the urine and the evolution of O_2 was followed for 15 minutes. Readings were taken at 5 minute intervals. It is well known that H_2O_2 has a tendency to destroy catalase through opening of the porphyrin rings. The time of destruction obviously depends on the concentration of the enzyme. With catalase contents such as those found in rabbit urine the enzyme was destroyed in 20 to 30 minutes. In general the figures given for catalase represent our O_2 evolution in 15 minutes.

Phosphatase. - The method used for determination of urinary phosphatase was that suggested by the Rochester group, namely the hydrolysis of phenolphthalein phosphate, a sample of which was kindly furnished by Dr. Dounce. 10 cc of veronal buffer, pH 9.7 was pipetted into a test tube with 0.5 cc of urine. The tubes were introduced into the 38° water bath and after temperature equilibrium, 1 cc of a suspension of Ca phenolphthalein phosphate containing 0.5 grams per 100 cc water was added. The tubes were thoroughly mixed and were kept at 38° for 2 hours. The intensity of the red color produced on liberation of phenolphthalein was measured in the Coleman spectrophotometer at a wave length of 5500 A. The values given are the extinction values as given in the spectrophotometer with no changes.

I. - Red cell catalase. - In order to answer Dr. Watson's question regarding the origin of the catalase present in urine the catalase content of washed laked red cells was determined under the same conditions as those used in the subsequent experiments. At the same time the absorption spectrium of oxy-hemoglobin from 1000 red cells per cmm was measured near the ultraviolet light where the maximum absorption of oxy-hemoglobin

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[illegible] in the Beckman spectrophotometer a value for extinction coefficient (log $\frac{I}{I_0}$) of 0.44 at 4150 Å, the point of maximum absorption (Fig 2). If catalase values of 42 [illegible] per 15 minutes were due to red cells it would be easy to detect by simply reducing the volume to one fifth. An extinction coefficient of 0.088 at 4150 Å would be obtained.

II. - Red cell catalase and urine.- It is well known that catalase in dilute solutions looses enzyme activity possibly because of the inhibiting effect of traces of heavy metals (Cu) or by denaturation of the protein. We have found that urine inhibits the activity of red cell catalase. In these experiments 1 cc of a solution containing red cell catalase was added to 1 cc of either water or urine. The activity of the enzyme was measured at 3° for 10 minutes. The urine was previously adjusted to the same pH as that of the phosphate buffer, pH 6.8. As can be seen in Table I urine added to catalase produced a definite inhibition which varied from 52 to 71 percent. The catalase values found in urine are not therefore true measure of the amount of catalase actually present in urine.

III. - Catalase of normal rabbit's urine. - Since the danger of bacterial contamination in the urine obtained from metabolism cages is great, it was decided to collect the urine for enzyme determinations by catheterization with a soft rubber catheter. The catalase content of normal rabbits was found to vary greatly not only from animal to animal but also in the same animal from day to day, as can be seen in Table I where urine catalase from some rabbits (those used in the T injection experiments) are given. The normal values fluctuated from 0 to 29 (Table III). It must be pointed out that rabbits which gave values beyond 40 were discarded.

When similar determinations were made on spontaneously voided urine and received from metabolism cages the values were always much higher, the increase ranging from twice to 300 times the value in catheterized urine (Table II). This was undboutedly due to bacteria growing around the walls of the funnel through which spontaneously voided urine flows to the collecting vessel. (These urine specimens were freshly voided).

IV. - Urinary catalase in Rabbits after the injection of $TO_2(NO_3)_2$. - It was reported by the Rochester group that the rise of urinary catalase was the earliest sign of T intoxication. When rats were treated with $TO_2(NO_3)_2$ it was found that a rise in blood N.P.N. appeared 24 hours after the injection of 0.1 mg $TO_2(NO_3)_2$ per Kg (October report). Dr. Lisco found also clear hystological changes on the kidney of those rats. It was therefore necessary to find changes prior to 24 hours; otherwise the blood N.P.N. would be a better sign of metal intoxication. The collection of urine was the most difficult obstacle because it was necessary to avoid any possibility of bacterial contamination. It was finally decided to use rabbits and to collect the urine by catheterization with a very thin flexible rubber catheter. The catalase content of the urine was determined daily for a period of about 8 days. Only those animals giving values not above 30 were used. $TO_2(NO_3)_2$ was injected either subcutaneously or intravenously at a dose of 5 mgs per Kg to avoid any uncertainty of intoxication. Samples of urine were collected 4, 8, 24 and sometimes 48 hours after injection. The results obtained have been collected in Table III. There are in this table the results of ten experiments. In considering only the catalase values obtained in the last four days previous to the injection of $TO_2(NO_3)_2$, 6 rabbits showed an increase in urine catalase higher than 100%; four of those rabbits showed the increase 4 hours later; two, 8 hours later. One rabbit showed an increase of 82% 4 hours after the injection; two showed slight increases (66 and 33%) 8 hours after the injection. Finally one did not show catalase increase until 24 hours later, the increase being 94%. It must be

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concluded from these experiments that there is certainly an increase in the urine catalase values demonstrable in most cases 4 and 8 hours after the injection of T. However if the catalase values obtained on 8 successive days is taken into consideration, rabbit No. 2 had on one day a catalase value of 20 while after T injection the maximum value was only 34, 24 hours after injection. Rabbits No. 3 had one day a value of 14; 3 hours after injection the value rose to 23. Rabbit No. 4 had a one day a value of 12; 5.5 hours after injection it rose to 21. Rabbit No. 6 had one day a value of 29; it rose to 34, 4 hours after injection. The increases were so small when compared to the highest values obtained before injection that the catalase test must be considered unreliable as a diagnostic test of early intoxication by T.

Phosphatase content in the urine of rabbits injected $TO_2(NO_3)_2$. - The urinary phosphatase values from normal rabbits were far more constant than the catalase values, the average of all determinations being 0.051 ± 0.013. In no case a phosphatase value above 0.09 was observed. (Table IV). Four hours after the injection of $TO_2(NO_3)_2$ the urinary phosphatase rose to an average value of 0.105 ± 0.43. Of the ten rabbits used in these experiments, five gave phosphatase values above 0.1 four hours after the injection of $TO_2(NO_3)_2$; two gave values (0.08) twice as much as the normal values in these animals; three gave values not significantly above the normal values. Eight hours after injection seven animals gave values above 0.1; one gave a value of 0.09, 2.5 times as much as the control; two gave no significant increases. It must be concluded from these experiments that an increase in urinary phosphatase is a more reliable sign of early T intoxication.

Microscopic findings in the Kidneys.- It was intended to sacrifice the animals as soon as an increase in catalase an phosphatase appeared in order to see in this increase was accompanied by visible microscopic damage of the kidney. Unfortunately only three on these animals were selected for this purpose, giving no significance to the findings that might be reported.

From the two enzymes found to be increased in the urine a few hours after T injection into rabbits, phosphatase gave a more reliable indication because the normal values did not fluctuate as much as those of catalase. It is quite possible that greater increases in catalase values would have been observed were it not for the fact that urine inhibits catalase activity, a sort of quenching effect. The source of these enzymes has not yet been found. They might come from few red cells passing the glomerular endothelium at the same time as T is being excreted. In fact 70 red cells per cmm would give catalase values comparable to those found few hours after T injection. They might come from necrosis of some endothelial cells from the proximal tubules, due to reabsorption of T excreted through the glomeruli. These findings apply to rabbits, and it is our opinion that they might not apply to humans. From a careful study of these experiments we have to state that in our opinion neither the phosphatase nor the catalase test are sure signs of T intoxication. Since the kidney is the organ that first suffers T injury we believe that a periodic examination of blood N.P.N. remains still the most reliable sign of T intoxication.

Figure I

The absorption spectrum of oxyhemiglobin contained in 1000 red cells per cmm.

(normal human).

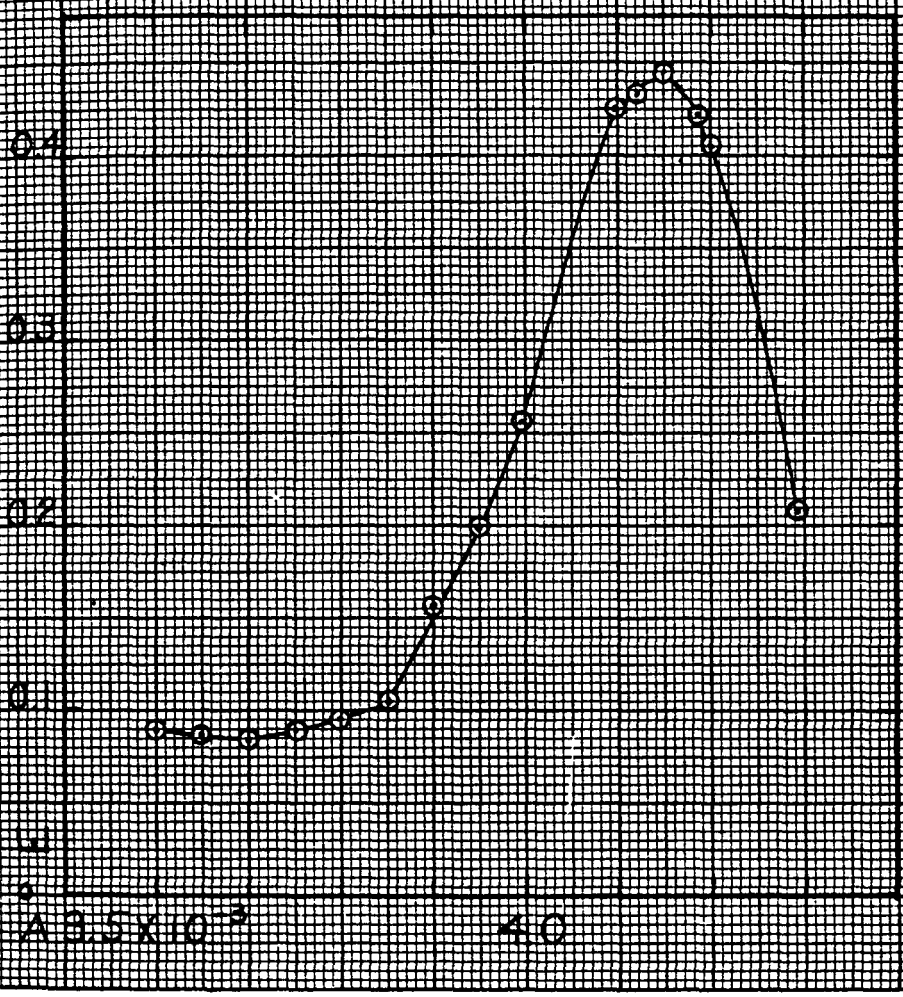

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Figure II

Catalase content of 100 red cells per cmm - 1cc of dialyzed red cells - pH 6.8. Alrcissa, time in minutes; ordniate, cmm O_2 evolution.

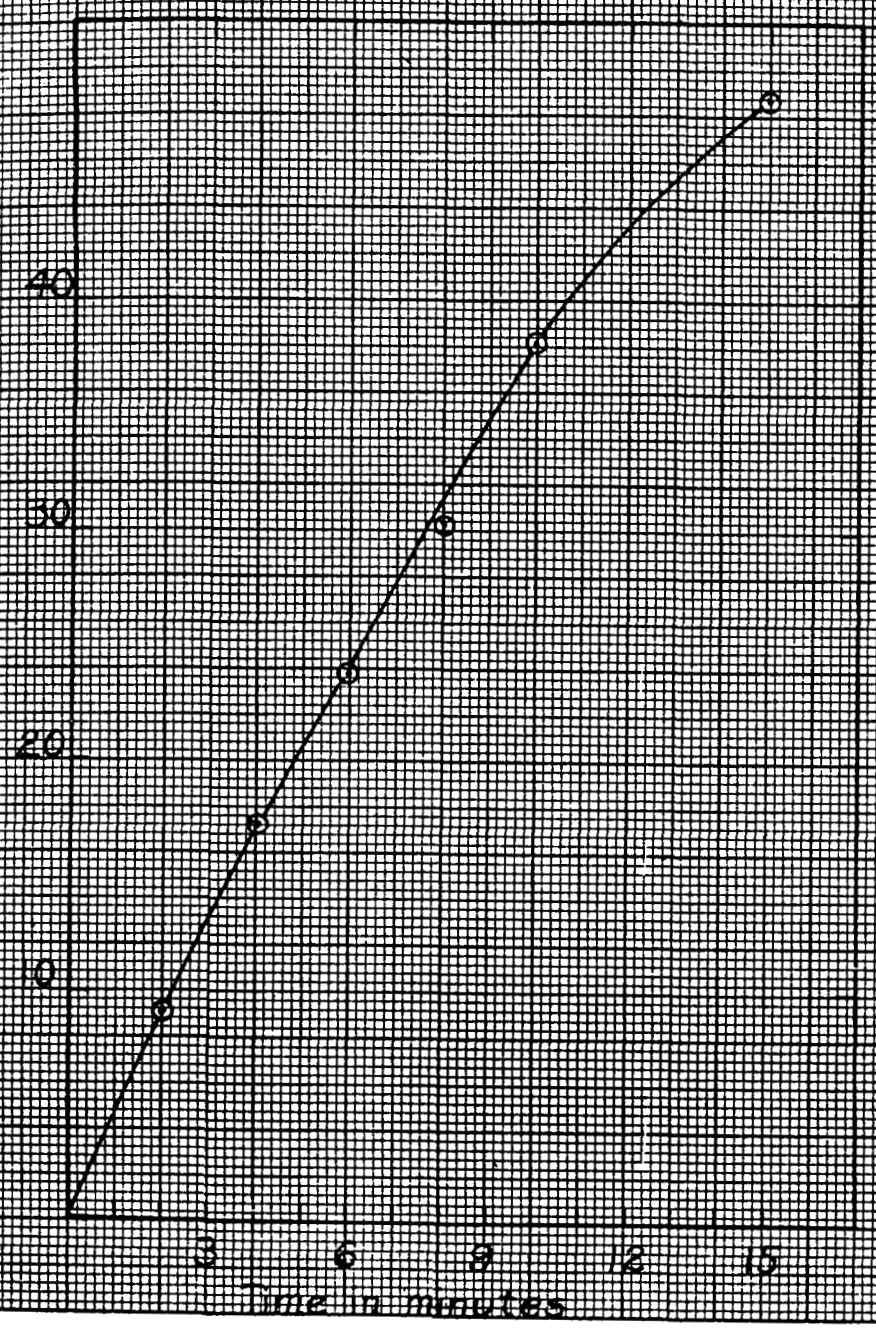

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Table I

Effects of Urine on the Activity of Red Cell Catalase

1.8 cc, 0.1 M phosphate, pH 6.8; 1 cc red cell catalase: 1 cc H_2O or urine. Side arm 0.2 cc 0.1 M H_2O_2. Temp. 3°.

Sample	O_2 output in 10 minutes	Inhibition
	cmm	per cent
I. Enzyme + H_2O	143	
Enzyme + urine	79.2	52
II. Enzyme + H_2O	197	
Enzyme + urine	90	54
III. Enzyme + H_2O	119	
Enzyme + H_2O	34	71

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Table II

Catalase Content of Urine Obtained by Catheter and of Voided Urine

Catalase content is given as cmm O_2 output in 10 minutes per 1 cc of urine.

Rabbit	Catalase Content	
	Catheter	Voided Urine
I	10	245
II	4.4	58.5
III	4.0	48.8
IV	1.9	54.0
V		52.5
VI	6.5	232.0

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Table XIX

Catalase in the Urine of Rabbits Before and After the Injection of $Th_4(UO_2)_2$ (5 mgs per kg). The catalase content is given as cms O_2 output in 15 minutes. Temp. 20°. Urine obtained with catheter.

Rabbit No.	Injection ThO_2UO_2	Catalase content: Before Injection — catalase in different days	After Injection — hours	After Injection — catalase
2	Intravenous	4;1;20;3;10;2;3;9	4	0
			8	15
			24	34
3	Intravenous	5;3;4;14;3;1;9;8	4	5
			8	23
			24	23
4	Subcutaneous	3;1;1;7;12;0;1;0;0	4	18
			5.5	21
			8	15
			24	0
			48	28
6	Subcutaneous	4;3;20;15;29;2;0;10;[illegible]	4	34
			5.5	19
			8	17
			24	7
			48	3
10	Intravenous	17	4	21
			8	16
			24	33
11	Subcutaneous	8	4	36
			8	34

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Table III Continued

Rabbit No.	Injection TO_2NO_2	Catalase content: Before Injection	After Injection	
		catalase in different days	hours	catalase
13	Subcutaneous	20	4	33
			8	87
14	Subcutaneous	24	4	--
			8	32
16	Subcutaneous	0	4	33
			8	32

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[illegible]

[illegible] Content of [illegible] Value of Rabbits Treated with $NO_2(NO_3)_2$

[illegible] per Kg. The values given are E values obtained in a Coleman spectro-[illegible]

[illegible]	$NO_2(NO_3)_2$	Phosphatase Values		
		Before Injection	After Injection	
		Phosphatase in diff. [illegible]	Hours	Phosphatase
[illegible]	Intravenous	0.08;0.05;0.07;0.06;0.07;0.06	4	0.19
		0.08 = 0.067 ± 0.009	8	0.25
			24	0.34
[illegible]	Intravenous	0.05;0.02;0.06;0.05;0.02	4	0.16
		0.03;0.03;0.05 = 0.039 ± 0.013	8	0.57
			24	0.31
4	Subcutaneous	0.03;0.03;0.03;0.02;0.05;0.03	4	0.04
		0.03;0.05 = 0.034 ± 0.007	5.5	0.12
			8	0.09
			24	0.29
6	Subcutaneous	0.06;0.04;0.05;0.03;0.03;	4	0.08
		0.03;0.04 = 0.04 ± 0.008	5.5	0.11
			8	0.09
			24	0.13
			48	0.20
11	Subcutaneous	0.07	4	0.10
			8	0.11
12	Subcutaneous	0.04	4	0.08
			8	0.07
13	Subcutaneous	0.03	4	0.24
			8	0.20

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Table IV - Continued

Rabbit No.	$TO_4(EO_3)_2$	Phosphatase Values		
		Before Injection	After Injection	
		Phosphatase in diff. days	Hours	Phosphatase
14	Subcutaneous	0.04	4	—
			8	0.05
15	Subcutaneous	0.04	4	0.13
			8	0.13
16	Subcutaneous	0.05	4	0.07
			8	0.12

Employees' Health Service - J.G. Allen

During the period from January 1, 1945 through January 14, 1945 58 physical examinations were performed; 6 of these represent transferred personnel, 5 terminal examinations, the balance being pre-employment examinations. One individual was rejected for employment because of physical abnormalities. The total number of patients seen at Drexel House during this period was 331.

Clinical Laboratory Examinations - Edna K. Marks

More than 4,000 clinical laboratory examinations were performed between January 1, 1945 and January 15, 1945. Of the abnormalities noted none were considered to be due to the special hazards.

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H-II Biological Research Section

K. S. Cole, Section Chief
C. Ladd Prosser, Asso. Section Chief

Radiotoxicity of Inhaled Radioactive Cerium, 244 MLH 2134.

On December 21 a group of 28 rats were exposed to an arc smoke of 300 day Ce. The rats breathed for one hour from a chamber where the initial Ce concentration in the air was 1.25 mC/li. External counts were made on all the animals after exposure. Eight of the animals, which were dead or moribund at the end of the exposure, were sacrificed for dose measurement. It was found that the lungs on the average contained 41 μc of hard beta activity. Thus the initial dose rate is given by:

$$D = 60 \frac{QE}{W} \text{ r/day}$$

where Q = μc of Ce in lung = 41 μc
E = Average beta energy in MeV = 1.5
W = Lung weight = 1.5 gm

$$D = \frac{60 \times 41 \times 1.5}{1.5} = 2460 \text{ r/day}$$

Of the 20 rats, 16 are still alive after 27 days. During that time they have received a total lung dose of approximately 30,000 r. Two rats, which had lost 30 to 40 gm. in weight, were sacrificed after 8 days. Two others, which were moribund after 12 and 16 days (weight dropped 80 gm), were killed and tissues fixed for histological examination. Several others at present have respiratory difficulties. Histological reports not yet available.

Histological Effects of Radio-Strontium in Rats, 246 MLH 2531.

This series consists of 6 rats injected intraperitoneally with Sr^{89}. Three doses were given intraperitoneally, 2 mC/kilo, 5 mC/kilo, and 10 mC/kilo. At one week after injection, one animal was sacrificed from each dose; at two weeks a 10 mC/kilo rat was sacrificed and at eight weeks two 2 mC/kilo rats were sacrificed.

The bone marrow was the most severely damaged organ. With 2 mC/kilo, hematopoiesis in the marrow cavity was greatly decreased at one week; with 5 mC/kilo, and 10 mC/kilo, there was complete absence of hematopoiesis. Partial recovery was seen at 8 weeks after 2 mC/kilo. Gelatinous marrow and widened sinuses filled the marrow cavity at one week with all doses; at 2 weeks (10 mC/kilo), and at 8 weeks (2 mC/kilo), fatty marrow predominated. There was some fibrous marrow in the 2 mC/kilo rats.

The lymphatic tissue of the thymus, mesenteric lymph node, splenic white pulp, and gastro-intestinal tract showed "clumping" of lymphocyte nuclei and in some cases also of reticular cell nuclei. Mitotic activity was increased and abnormal mitotic figures were seen in all these areas.

Extramedullary erythrocyte formation in the red pulp of the spleen was increased 2 weeks after 10 mC/kilo and at 1 and 8 weeks after 2 mC/kilo. There was no increase in erythrocyte formation one week after injection of 5 mC/kilo or 10 mC.kilo. There was an elevation of granulocyte and megakaryocyte formation in the spleen with all doses; the latter was most striking at 2 weeks after 10 mC/kilo.

There was slight damage to epithelial cells in the bases of the crypts in the intestine. Liver, kidney, lung, ovary, testis and pancreas showed no significant change.

Comparison of Effects of Radiation and War Gas Exposure, 362 MLH 2581.

Nitrogen mustard dissolved in diphenyl ether was injected by ear vein into 8 rabbits. The dose was 0.65 mg/kilo (somewhat less than half the median lethal dose). One rabbit was sacrificed at each of the following intervals after injection: 3 hours, 33 hours, 3 days, 5 days, 9 days, 14 days, and 21 days.

The effects noted in these animals were minimal compared to those observed after intravenous injection of a midlethal dose (3 mg/kilo) of mustard. Mitoses were inhibited in the spleen and lymph node at 3 and 8 hours. There was increased formation of lymphocytes in the node from 33 hours to 9 days, and greatly increased transport of small lymphocytes in the sinuses of the node.

There was no significant change in the bone marrow, thymus and gastro-intestinal tract.

Histological Effects of Single Doses of X-radiation, 142 MLM 2510.

Twelve guinea pigs were given total body x-radiation of 175 r. Twelve controls were boxed but not irradiated. Two experimentals and two controls were sacrificed at intervals of 1, 3, and 8 hours, and one each at 1, 2, 4, 7, 10, and 18 days.

The lung, liver, pancreas, heart, kidney, and adrenals showed no effects of treatment. In the gastro-intestinal tract there was only mild degeneration in the epithelium, but severe degeneration of the lymphatic tissue for two days; by 7 days complete regeneration had occurred. There was severe depletion of all forms of immature cells (except reticular) in the bone marrow, thymus, spleen and mesenteric lymph node between 3 hours and 10 days. After this time there was complete regeneration, and in thymus and mesenteric lymph node a 10 per ent to 20 percent overgrowth by 18 days. There were indications of a transient secondary phase of degeneration in the hematopoietic organs between 4 and 7 days. The mature cells of the bone marrow, i.e., red blood cells and granulocytes, were not directly affected by treatment, but their numbers decreased because of lack of replacements. In the testis, the immature cells (primary spermatocytes and spermatogonia) showed a degenerative and regenerative sequence very like that observed in the cells of the bone marrow, but they regenerated faster; the sustentacular and interstitial cells did not degenerate. As a consequence of the changes in the numbers of immature cells, there was a temporary decrease in the number of mature forms (spermatids and spermatozoa).

Effects of Daily X-radiation upon Growth in Rats, 142 MLH 2371.

Analysis of weight data on mice and rats, which received daily x-radiation, is nearing completion. The data on rats and mice which received relatively high daily doses, show a short period after the beginning of daily treatment during which growth continues and thereafter a precipitate loss of weight until the group dies out.

The weight and mortality responses of mice (ABC male), receiving 20 r per day, present a good picture of chronic injury. Examination of the growth curves of 20 r animals and controls (12 in each group) shows three phases of chronic X-ray response. Results are presented in figure 1.

The first phase is typically one of somewhat retarded normal growth. This phase lasted from 65 to 90 days after the beginning of treatment. The second phase, which was then entered quite abruptly, was a period of slow weight loss, during about 60 days. During this phase, the irradiated animals lost about one gram per month. The third phase, was a plateau period in which the animals held a constant weight. It is not known how far this plateau may extend, because the records have now been carried to only 180 days. During the entire 180 day period, the control animals have gained weight.

The observations reported above hold for 3 of 4 experimental groups of 12 animals each. The figures presented here are for one of the 3 groups and are typical of all. The fourth group, is atypical in not showing the second phase of weight loss and also in that the controls show abnormally retarded growth.

Figure 2 gives the regression of experimental on control weight. On the basis of a comparison of the initial slope of the post-irradiation period with the expected slope in normal populations, we estimate that the experimentals are growing about 80 per ent as rapidly as the controls. That is, for every gram gained by the controls, the experimentals gain 0.8 gm. in the first 60 treatment days. The arrow in figure 1, indicates the point at which the transition from the first to the second phase occurs. This point was determined from the regression curve.

III Medical Industrial Hazards Section

Personnel Monitoring: Beta (241 MLH 3350); Gamma (241 MLH 3360)

Badge Meters: Measurements from 1970 individual films indicated no individual with greater than 0.6r per week.

Pocket Meters: 3841 readings for the month of which 16 indicated exposures greater than 100 mr/day but did not exceed the weekly tolerance.

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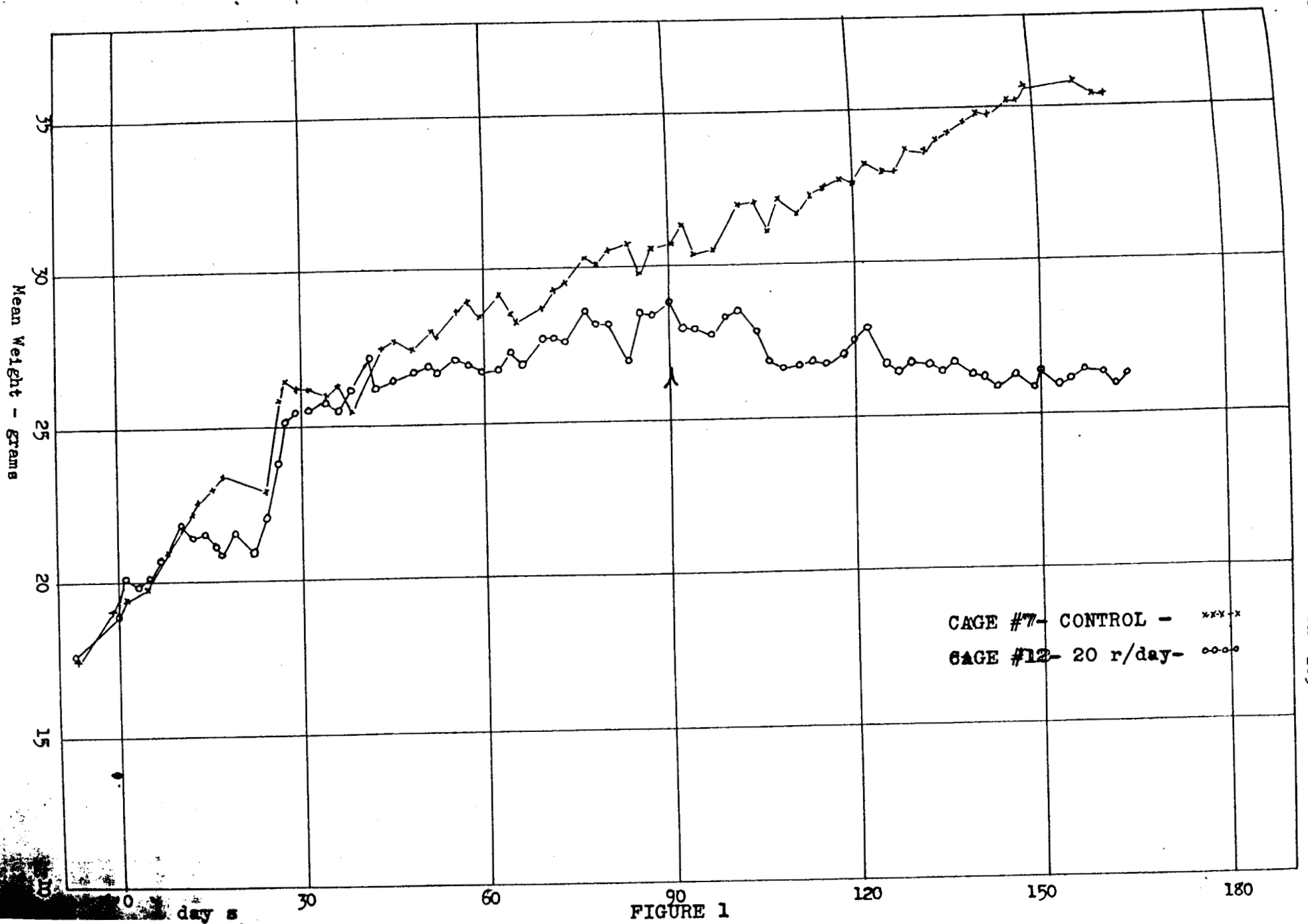

FIGURE 1

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FIGURE 2

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