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PROGRESS REPORT NO. 3

STUDY OF THE POST-IRRADIATION SYNDROME IN HUMANS

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56/P/88

INTRODUCTION

To date twelve persons have received total body irradiation. Three of these patients have received two courses. One patient is included who received total torso irradiation. Radiation was done with the 1 Mev General Electric generator, HVL=3. 3mm Pb, and was delivered through four ports, upper and lower half of the body, anterior and posterior. The target skin distance in all cases was about 290 cm and the dose rate was between 5 and 7 r/min. In general, coproporphyrin excretion, serum lipoprotein levels, and formed elements of the peripheral blood were measured. In a few instances one of the major studies was not performed.

All persons receiving total body irradiation were patients at the Memorial Center, with widespread cancer. However, no person showing biochemical evidence of renal or hepatic dysfunction was included in the investigative group nor was any person included who showed evidence of bone marrow impairment.

No person included in the series had received x-ray therapy or chemotherapy for at least six weeks prior to the present investigation. Patients were selected who had no complaints referable to their disease process.

The total dose delivered ranged from 20 r to 150 r; all doses were measured in air at the mid-plane of the body. Calculations of volume doses are not yet completed and at best will be approximations because of the varying anterior-posterior diameter from head to foot. Such calculations will be completed before publication of this work.

For purposes of organization, this report will be divided into four parts: 1. Clinical observations. 2. Hematological and Coproporphyrin studies. 3. Lipoprotein studies. 4. Electrolyte studies.

1. CLINICAL OBSERVATIONS

Patients receiving more than 50 r total body irradiation complained of weakness one to two hours following irradiation. This complaint persisted for the first twenty-four hours to thirty-six hours. It is difficult to know what role the physical and psychic rigors of the irradiation played in the production of this complaint. It is felt that this is not the only reason, because those receiving lower doses did not complain of weakness.

Only three persons experienced any nausea and vomiting. Onset of nausea was within one hour following completion of radiation. Vomiting usually started two to three hours thereafter. In one patient, (C.LeC.), the vomiting subsided spontaneously within eight hours but mild nausea and anorexia persisted for 24 hours. One patient, (M.D.), had rather severe nausea and vomiting for the first 48 to 72 hours post-irradiation. This subsided without medication. The patient again experienced severe nausea and vomiting on the 6th, 7th and 8th days; however, this followed the oral administration of tracer amounts of C^{14} labeled Trytophane, which had produced vomiting on a previous administration. The third patient, (B.C.), had much severe nausea and vomiting that on the third day it was deemed necessary to give Thorazine, 25 mgm, i.m. t.i.d. and, hence, nothing can be said of the subsequent symptomatology.

Six patients developed severe thrombocytopenia. The maximum effect occurring about 28 days post-irradiation (more detailed discussion will be found in the following section). Of these six patients, four developed hemorrhagic tendencies, (J.N., M.H.,* B.C.,* A.MacA.,* M.D., and W.J.*#). It is important to note that the first three of these had subsequent radiation therapy to large ports between the time they received total body irradiation and the time they showed a thrombocytopenia. The latter three patients had received either

*Hemorrhagic tendencies.
#Not otherwise included in this report, having been irradiated so recently that complete data are not available.

total body irradiation (A.MacA.) or nitrogen mustard therapy two months or more before total body irradiation (M.D. and W.J.). The latter three patients all had normal peripheral blood counts prior to receiving total body irradiation. With the exception of J.N., who received 75 r total body irradiation, all were given 120 r or more.

The incidence of symptomatology (1) and thrombocytopenia (2) in this series is less than that reported amongst those exposed in the Marshall Islands, even though the doses as reported are about comparable. These differences may be due to a real difference in volume dose, the time over which the dose was delivered, or the bone marrow depression from the isotope ingestion. The severe nausea and vomiting reported in this group could conceivably be due to, or be augmented by, the dust ingested.

Two cases of lymphosarcoma (A.MacA. and D.R.) had dramatic regression of their diseases. These were the only cases that showed any change in the neoplastic process.

2. HEMATOLOGY AND COPROPORPHYRIN STUDIES

INTRODUCTION. The effect of ionizing radiation on the formed elements of the blood has been the subject of investigation for many years and the results of studies in this area have formed the principal criteria for radiation damage. From the studies cited in the literature and from our own observations, the earliest evidence of radiation damage would appear to be a decrease in the number of circulating lymphocytes. The blood platelets are next in the order of sensitivity, followed by a decrease in the number of circulating granulocytes. Finally, the red blood cells decrease as the pancytopenic picture develops.

It would appear that the fall in the numbers of circulating mature cells is secondary to damage to the reticulo-endothelial system and to the bone marrow. Conversely, there is little evidence to support the hypothesis that the decrease is caused by a direct effect on the circulating elements themselves.

RESULTS. From the experiments conducted in this investigation, it is apparent that the time lag between the administration of total body radiation and the fall in the circulating cells is greater in humans than it is in experimental animals (2). The decrease in the white cell count is seldom apparent before the third week post-irradiation. In most cases it does not reach maximal depression before the fourth or fifth week.

The lag in platelet depression is even greater than that seen in the white cells. When it occurs, however, its duration is longer, usually lasting weeks rather than days. Detailed graphs of the hematologic alterations in the thirteen patients studied are presented in Figures 1 to 13. It should be noted that ordinate scales are different on the various graphs.

The long life span of the mature red blood cell is undoubtedly responsible for the very late occurrence of anemia following ionizing irradiation. Unless total aplasia of the red cell precursors takes place, there must be a lapse of one red cell half-life (60 days) before obvious clinical anemia could be expected to occur.

Previous radiation insult to the bone marrow, even in the presence of a normal hematological picture, is a real hazard to the individual exposed to total body radiation. Several of the patients studied in this group had received local x-ray therapy or had been exposed to so-called "radiomimetic agents" such as nitrogen mustard or triethylene melamine prior to total body

irradiation. Although all of them had a normal peripheral blood and bone marrow picture prior to exposure, their response to the dosage administered was definitely exaggerated both in duration and extent.

This danger cannot be too strongly emphasized since we have no objective criterion to date by means of which we can predict the degree of hematological response in patients who have received previous irradiation.

INTRODUCTION. In an effort to find a more sensitive index of radiation effect than the formed elements of the blood, a study of the response of the precursors of hemoglobin to ionizing radiation was begun in our laboratory several years ago. During the course of this study it was found that the excretion of coproporphyrin in the urine and in the feces was increased following therapeutic dose levels of isotopic or local x-radiation(3). This increase occurred at dosage levels which did not produce a change in the formed elements of the blood.

The present study was begun to evaluate this observation more critically and to quantitate, if possible, the radiation dosage in terms of coproporphyrin excretion.

It is not the intention of this report to give a detailed description of the metabolism of the porphyrins. However, a short discussion of the synthesis of protoporphyrin IX is essential to the understanding of the possible mechanisms by which the observed increase in coproporphyrin excretion is produced.

From *in vitro* and *in vivo* experiments it has been shown that the synthesis of the porphyrin ring and the introduction of the prosthetic groups which characterize the specific porphyrins follow immutable and irreversable lines.

In the process of synthesizing heme Fisher[4] found that he could remove carboxyl groups from uroporphyrin to produce coproporphyrin but that the reverse reaction of recarboxylation was not possible. Similarly, to produce heme, two carboxyl groups are removed from the coproporphyrin ring before the introduction of iron. The reverse of this process has been an attractive hypothesis for the explanation of the presence of the coproporphyrin stereoisomer III during the degradation of hemoglobin after hemolysis. The work of Lemberg[5] has conclusively shown, however, that the porphyrin ring of the hemoglobin molecule is opened in the alpha position before it loses its iron. Thus, an increase in coproporphyrin excretion following hemolysis cannot be explained in this way.

RESULTS. Two independent and physiologically dissimilar hypotheses may be considered as possible etiological agents in the increased coproporphyrin excretion which follows ionizing irradiation.

The first is the so-called dual hypothesis of Fisher[4] which states that for every moiety of porphyrin stereoisomer III formed, a small but definite quantity of stereoisomer I is concomitantly synthesized. According to this theory, the excretion of coproporphyrin I is in direct proportion to the amount of hemoglobin produced. Therefore, anything which will increase the synthesis of hemoglobin will at the same time increase the production and consequent excretion of coproporphyrin I. Dobriner[5] confirmed this hypothesis in his experiments with phenylhydrazine hemolysis in dogs when he observed an increase in coproporphyrin excretion occurring during the recovery period when the reticulocyte count was at its maximum.

From the present studies it would not appear that this factor is responsible for the observed increase in coproporphyrin excretion. There has been no correlation between reticulocyte rise and increase in coproporphyrin excretion in the patients who have received total body irradiation. Nor has any other evidence of increased hemoglobin anabolism been observed.

A second possible mechanism for the increased coproporphyrin excretion following irradiation can be seen in the intermediary metabolism of protoporphyrin. Should the reaction:

Coproporphyrin III----------Protoporphyrin IX
decarboxylase

be adversely influenced by ionizing irradiation, the production of hemoglobin would decrease and an excess of coproporphyrin III followed by increased excretion of this porphyrin would result. Moreover, the increase in coproporphyrin excretion would take place during the period of maximal radiation effect rather than during the recovery phase.

It is this second mechanism which is considered to be responsible for the increased coproporphyrin excretion observed in these studies.

3. LIPOPROTEIN STUDIES

REASON FOR STUDY. In rabbits receiving severe whole body irradiation, an early increase in the serum turbidity (caused by chylomicrons) is prognostic of lethal damage. Detailed lipoprotein analyses of such sera have shown that at this time, one to two days after the irradiation, there is a block in the serial conversion of larger to smaller lipoproteins which is a part of the normal fat transport system(7). Since heparin is involved in the "clearing factor" mechanism which is responsible for this conversion, heparin deficiency has been suggested.

Changes in lipid transport following severe whole body irradiation have also been observed in dogs(8). It therefore seemed important to determine the effect of therapeutic doses on the serum lipoproteins of patients.

METHODS. Fasting blood samples are taken before irradiation and one, three, and six days afterward. Additional samples are taken after further intervals of time, whenever it is considered necessary. The serum is checked for turbidity in the spectrophotometer at 65 mu, and stored at 4°C. with the addition of 1:10,000 methiolate for not more than two weeks. For the separation of the lipoproteins 7.05 ml. of serum are mixed with 3.45 ml. of NaCl (density 1.1855) to give a solvent density of 1.063. The solutions are then run in the preparative ultracentrifuge for 16 hours at 40,000 R.P.M. at about 5°C. The lipoproteins of density less than 1.063 which are concentrated in the top layer, are collected with a hypodermic needle and specially designed syringe(9), and transferred to a 5 ml. graduate. The volume is recorded and the lipoprotein content measured from the size of the flotation boundaries obtained in the analytical ultracentrifuge by the Gofman technique. The lipoprotein spectrum is separated into 3 fractions, Sf 2 - 10, Sf 11 - 20, and Sf 21 - 100. Occasionally a fourth component, Sf 100 - 400, is observed. The calculations are made by the procedure described in 1952(10). The total cholesterol is determined(11) in the whole serum and in the lipoproteins of density greater than 1.063, (found in the lower layers of the centrifuge tubes), and the "low-density lipoprotein cholesterol" calculated by difference. The calculated low-density lipoprotein cholesterol levels have usually been fairly close to one-third the total low-density lipoproteins measured in the ultracentrifuge. This agrees with the known chemical composition of these lipoproteins.

It has therefore not seemed necessary to measure the low-density cholesterol directly, since this would necessitate taking considerably larger samples of blood.

RESULTS. The results obtained on the individual patients are shown in Figures 14 through 27. In two patients, (V.M., 120 r, and Y.D., 20 r), neither of whom showed clinical signs of radiation damage, the lipoprotein levels remained constant within the errors of the method. Four patients, (J.N., O.D., B.C. and M.D.), showed early changes indicative of increased clearing factor activity, and two patients, (A.MacA., and C.LeC.), showed decreased activity. No distinction could be made either on the sex of the patient or the nature of his disease.

In five patients, (M.H., J.N., A.MacA., B.C., and M.D.), lipoprotein analyses were made at a time when clinical signs of radiation damage were present. Four had some elevation in clearing activity. There was no correlation with the direction or extent of the lipoprotein changes seen immediately after the initial treatment. This was not to be expected since most of these patients had received additional radiation after day 6 and the development of clinical symptoms.

DISCUSSION. The changes in serum lipoproteins which appear after therapeutic doses of irradiation are of course not as clear cut as the changes seen in animals after lethal doses. In some patients clearing activity appeared to have decreased on the third day following treatment, while in others an apparent increase was seen. Since the circulating lipoproteins represent the balance between lipids added to and removed from the circulation, it would appear that the doses used in these patients can cause this balance to swing

sometimes one way and sometimes the other. In rabbits receiving lethal doses of radiation the lipid transport system is usually inadequate for its load(3). Whether this is the result of a deficiency in the transport or an overloading of the system is still unknown. Atherosclerosis is produced in rabbits by feeding massive doses of cholesterol to these normally herbiverous animals. Also, the plasma lipoproteins of the dog differ greatly from those of the human, the dog having a much larger proportion of the lipid present as alpha lipoprotein. The many lipid and lipoprotein studies now being carried out on animals in connection with studies of atherosclerosis are therefore frequently criticized as not being applicable to human physiology. Since the same criticism might be leveled at studies of irradiation effects on the lipoprotein metabolism of animals, one valuable result of the present study is the observation that the lipoprotein changes found in humans soon after irradiation may be similar to those seen in animals.

The second observation of importance is that almost every patient who showed hemorrhagic tendencies and a thrombocytopenia had an increase in clearing factor activity <u>at that time</u>. All patients who had bleeding tendencies had thrombocytopenia of sufficient severity to account for the hemorrhagic manifestations observed. If the increased clearing factor activity seen in these patients is a result of a hyperheparinemia, it reinforces the suggestion that post-irradiation hemorrhage may be the result of an increase in circulating heparin in addition to, or associated with, the thrombocytopenia.

4. ELECTROLYTE STUDIES. Electrolyte transfers across the lumen of the bowel have been measured for various levels of the dog bowel.

EXPERIMENTAL METHODS. Chronic Thiry-Vella loops[12] of the appropriate segment of bowel were surgically prepared. Jejunal and ileal segments were 30 cm. in length and were taken from the mid-portion of the respective regions. Colon loops were about 15 cm. in length. The length was determined by selecting a segment which was drained by one major vessel. The ends of the loop were then anastomosed to the skin as fistula sites. The continuity of the remaining bowel was established by end to end anastomosis.

All experiments were performed under intravenous pentothal anesthesia, 5 mg./kg. of body wt. In all experiments #24 Foley catheters were introduced into each end of the isolated loop and the balloons sufficiently inflated so as to prevent leakage of any fluids introduced into the lumen of the gut, but not to a degree which might compromise the blood supply of the segment. With this arrangement solutions could be introduced at one end and withdrawn or collected at the other end in a controlled manner.

Sodium concentration in the collected samples was determined by means of a standard flame photometer[13] or on a NaI scintillation counter when Na^{24} was used. Chloride determinations were according to Van Slyke's modification of the Sendroy method[14] and CO_2 was measured in a Van Slyke apparatus[15].

Na^{24} and K^{42} were utilized to measure directional shift of sodium or potassium into the perfusate from the plasma or the disappearance of the ion from the perfusate. Solutions used for perfusion were isotonic $MgSO_4$, Polyvynalpyrolidon (PVP), or mixtures of electrolytes of varying concentrations maintained at isotonicity by $MgSO_4$ and $MgCl_2$.

Irradiations were done using 250 kv x-rays (HVL 2.0 mm cu.). Loops were localized with barium perfusion and lateral and anterior-posterior diagnostic

films. Each irradiation was so designed as to include the entire isolated loop but to keep the irradiation to the normal bowel at a minimum. This was done so that there would be no radiation-induced vomiting or diarrhea to alter the plasma electrolyte balance.

RESULTS AND DISCUSSION. The mechanisms of transport of electrolytes in the jejunum and ileum are qualitatively the same though quantitatively different, and hence results of the experiments will be discussed together.

If isotonic or hypertonic $MgSO_4$ is introduced into the lumen and is sampled as a function of time the electrolyte concentrations are found to increase reaching a constant and characteristic level. In the jejunum this level is between 130 and 140 meq/L and is essentially equal to the plasma concentrations. Chloride parallels the sodium and exceeds plasma levels of chloride. Equilibrium is reached in about 90 minutes as is shown in Figure 23. A similar rise is seen in the ileum with Na levels reaching approximately 120 meq/L and the Cl rising to only one-half the sodium value. Bicarbonate is the other cation present.

The exponential form would suggest that the rate of Na shiit into the bowel is constant and that the transfer from the lumen to the plasma is proportional to the concentration of sodium in the lumen[16,17,18]. Such an analysis gives rise to the equation:

$$Na = \frac{K_1}{K_2} (1 - e - K_2 t)$$

where: Na = Sodium concentration in the lumen at any time, t.

K_1 = rate of Na shift into the lumen.

K_2 = rate of Na shift out of the lumen.

The value of K_1 can be obtained from the slope at the origin and the value of K_2 then calculated from the equilibrium concentration. The theoretical curve obtained from such a calculation is also shown in Figure 27.

To verify the dynamic nature of this equilibrium, a mixture of electrolytes of composition identical with the equilibrium mixture was introduced into the lumen. The rate of appearance of Na^{24} was measured and the meq. of plasma sodium which this activity represented was calculated from the plasma specific activity. Results of a typical jejunal and ileal experiment are shown in Figures 28 and 29. Also shown in Figure 29 is the calculated curve and the slope of the origin is indicated.

The isolated loop may be perfused with $MgSO_4$ or other solutions of salt mixtures at varying perfusion rates. In both jejunum (Fig. 30) and ileum (Fig. 31) the concentration of electrolytes in the collected perfusate decreases with increasing perfusion rate. If the concentration is plotted as a function of the reciprocal of the perfusion rate (min./cc) a linearly increasing relation is obtained indicating that the concentration of electrolytes in the collected perfusate is directly proportional to the time which the perfusate is in contact with the bowel wall.

Dogs were given Na^{24} twenty-four hours before experiments were performed to permit equilibration of the tracer with the extracellular sodium. Loops were then perfused at a constant rate (1.58 cc/min.) with solutions containing varying concentrations of sodium. The tonicity was kept constant with the appropriate amount of $MgSO_4$. Total sodium (Na_i) put into the lumen could be calculated from the concentration, as measured on the flame photometer, times the volume perfused. Similarly the total sodium in the collected perfusate

(Na_o) may be calculated from the flame photometer measurement of the concentration. The total plasma sodium shifted into the lumen (Na_{P-L}) may be obtained from the CPM in the collected perfusate divided by the plasma specific activity.

Clearly, the total sodium in the collected perfusate is equal to the total sodium perfused plus the amount shifted into the lumen from the plasma minus whatever sodium was shifted back into the plasma from the lumen (Na_{L-P}). Hence, we obtain the equation:

$$Na_o = Na_i - Na_{L-P} \quad Na_{P-L}$$

where:

Na_o = total sodium in the collected perfusate

Na_i = total sodium in perfusing solution

Na_{L-P} = total sodium shifted from lumen to the plasma

Na_{P-L} = total sodium shifted from plasma to the lumen

All quantities in this equation can be measured directly except for Na_{L-P}. The equation may be solved for this quantity, and hence the shifts of sodium in and out of the lumen can be measured. Figures 32 and 33 show that in both jejunum and ileum the transfer of sodium from the lumen to the plasma is directly proportional to the concentration of sodium within the lumen, and the shift of sodium from body stores into the lumen is independent of the concentration within the bowel.

Electrolyte handling in the colon is markedly different, in that concentration gradients can be maintained across the colonic mucosa[19]. The most striking feature of the colon is the large water absorption when isotonic solutions are introduced.

Isotonic solutions containing varying concentrations of potassium tagged with K^{42} were introduced into the isolated loop and sampled as a function of time. The counts per minute of any sample when divided by the initial specific activity are a measure of the meq/L of initial potassium remaining. The total potassium could be measured on the flame photometer. In all experiments the total potassium rose slightly regardless of the concentration which was introduced and the potassium which was introduced fell slightly (Fig. 34). Using calculations similar to those outlined above, the total potassium shift can be measured. In all cases the total potassium shifting from the colon into the plasma was found to be proportional to the concentration of potassium instilled. The techniques do not permit any statement to be made about the plasma to lumen transfer. Because of the marked water absorption, better methods of measuring electrolyte transfer must be worked out.

Because of the extensive work which was required to develop techniques for the study of the normal physiology of the intestine, only preliminary radiation studies have been done. Isolated loops of jejunum and ileum receiving doses of 500 and 1000 r have shown no qualitative changes in electrolyte transfer mechanisms. Quantitative differences do exist. No definitive statement can be made at this time as to the magnitude of these changes. Moreover at higher doses, where extensive damage is done to the gut epithelium, breakdown of transport mechanism may occur.

SUMMARY AND CONCLUSIONS

1. Thirteen patients have been studied following total body irradiation with doses ranging from 20 -150 r using alterations in the formed elements of the blood, coproporphyrin excretion in the urine, and changes in the serum lipoprotein levels as objective criteria.

2. There was a decrease in the formed elements of the blood with the time of fall being in the order of lymphocytes, platelets, and granulocytes. The maximum depression occurred at about the fourth week after irradiation in contrast to the experimental animal data which show a maximal depression in the formed elements of the blood at two weeks. The degree of depression was more severe in patients who had received previous total body irradiation or "radiomimetic" drugs. It is felt that this is of considerable importance in the military setting and that persons who have received one exposure in the range of 100 r should not be re-exposed within at least 6-8 weeks. This is not a conservative estimate and further investigation along this line will be pursued.

3. Increased coproporphyrin excretion in the urine has not been a systematic finding. When it does occur the increase occurs within the first few days and in some instances within the first few hours. In general, even though the level of excretion in any 24 hour period did not exceed the upper limit of normal, the integral excretion post-irradiation was increased. It is felt that radiation damage blocks the decarboxylation of coproporphyrin III to protoporphyrin IX with a subsequent increase in the excretion of coproporphyrin III.

4. It is probable that the observations on serum lipoprotein levels in animals following total body irradiation may be carried over to man though the time scales may be different. Almost every patient who developed a thrombocytopenia associated with a bleeding tendency showed an increase in clearing factor at that time.

5. The rate of transport of electrolytes into the lumen of the dog bowel is constant and independent of the concentration within the lumen. The rate of absorption from the lumen is proportional to the concentration within the lumen in the jejunum and ileum. Maximal activity in the colon is associated with water absorption. Preliminary studies would suggest that irradiation alters these mechanisms qualitatively but not quantitatively.

REFERENCES

1) Cronkite, E.P., Shulman, N.R., Sandhaus, C.A., Sharp, R.A., and Bond, V.P.
Rad. Res. Soc. May, 1955 (Abstract)

2) Bond, V.P., Cronkite, E.P., Farr, R.S., and Hechter, H.H.
Rad. Res. Soc. May, 1955 (Abstract)

3) Koch, H.J., et al
J. Clin. Endocr. 10, 1950

4) Fisher, H., and Stern, A.
Die Chemie Des Pyrrols., Ann Arbor, 1943

5) Lemberg, R.
Biochem. J., 29, 1322, 1935

6) Dobriner, K., and Rhoads, C.P.
Physiol. Rev., 20, 1940

7) Hewitt, J.E., Hayes, T.L., Gofman, J.W., Jones, H.B., and Pierce, F.T.
Am. J. Physiol. 172:579, 1953

8) Goldwater, W.H., and Entenmann, C.
Am. Chemical Society, March, 1953.

9) Barclay, M., Cogin, G.E., Escher, G.C., Kaufman, R.J., Kidder, E.D. and Petermann, M.L.
Cancer 8:253, 1955

10) A Symposium. Technical Group, Committee on Lipoproteins and Atherosclerosis
National Advisory Heart Council, 1952

11) Abell, L.D., Levy, B.B., Brodie, B.B. and Kendall, F.E.
J. Biol. Chem. 195:357, 1952

12) Vella, L.
Bull. d Sc. Med. Bologna, 7:441, 1881

13) Overman, R.R. and Davis, A.K.
J. Biol. Chem., 168:641, 1947

14) Peters, J. and Van Slyke, D.D.
Quantative Clinical Chemistry, Baltimore, Vol. 2, 1943, p. 839

REFERENCES (concluded)

15) Van Slyke, D.D. and Neill, J.M.
J. Biol. Chem., 61:523, 1924

16) Visscher, M.B., Vanco, R.H., Can, C.W., Dean, R.B. and Erickson, D.
Am. J. Physiol. 142:550, 1944

17) Visscher, M.B., Fetcher, E.S., Can, C.W., Gregor, H.P., Bushey, M.S., and Barker, D.E.
Am. J. Physiol. 142:550, 1944

18) Visscher, M.B.
Ann. N. Y. Acad. Sci., 57:291, 1953

19) D'Agostino, A., Leadbetter, W.F. and Schwartz, W.B.
J.C.I., 32:444,1953

Figure 1

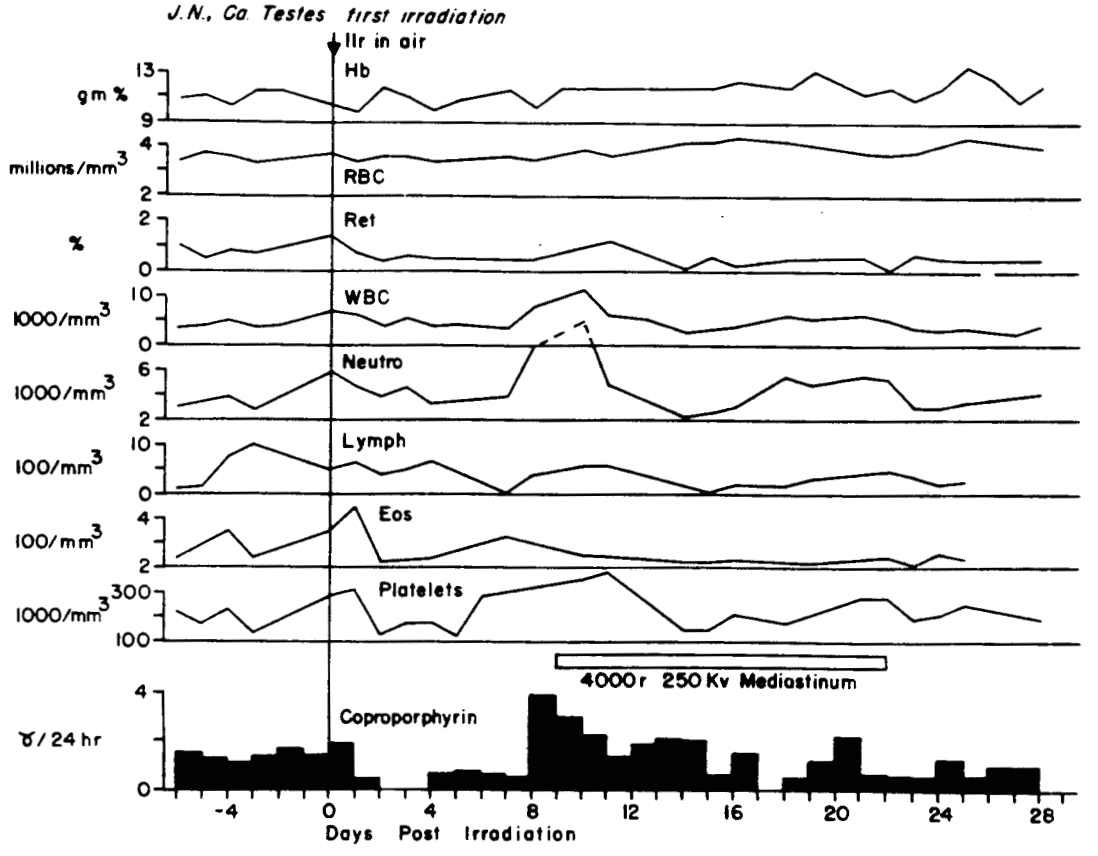
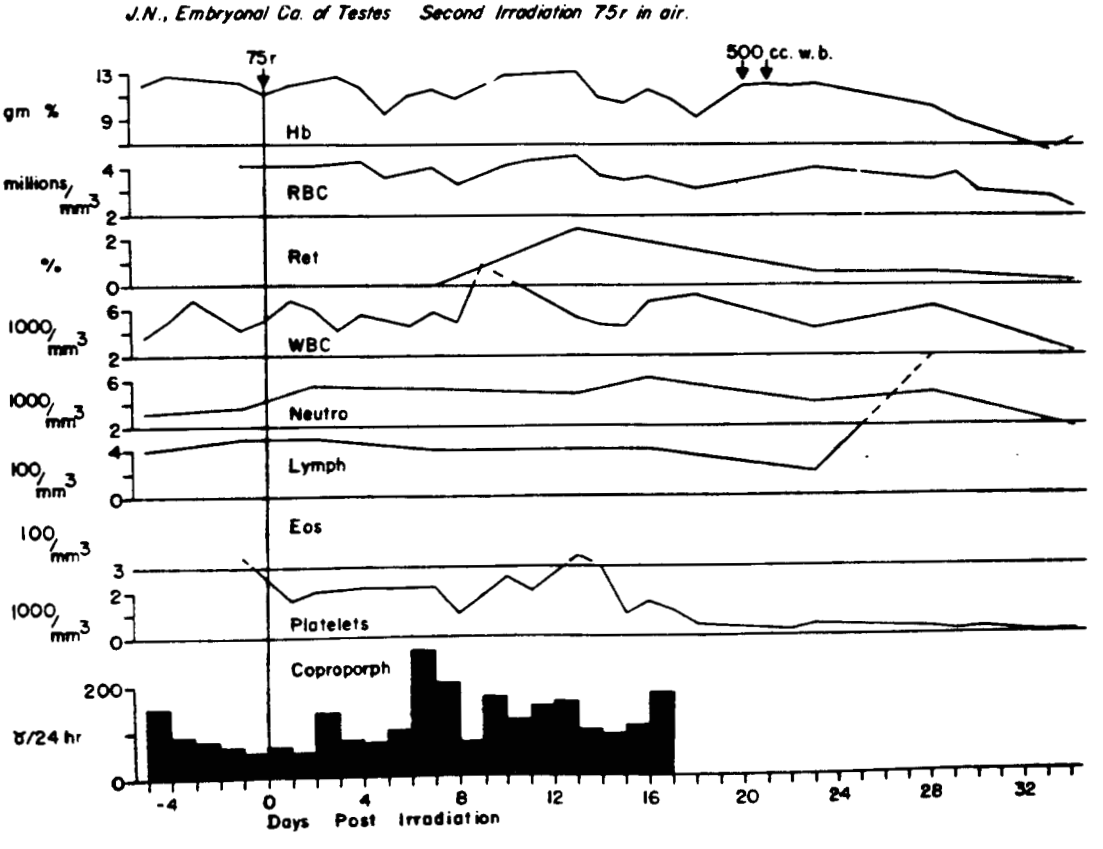
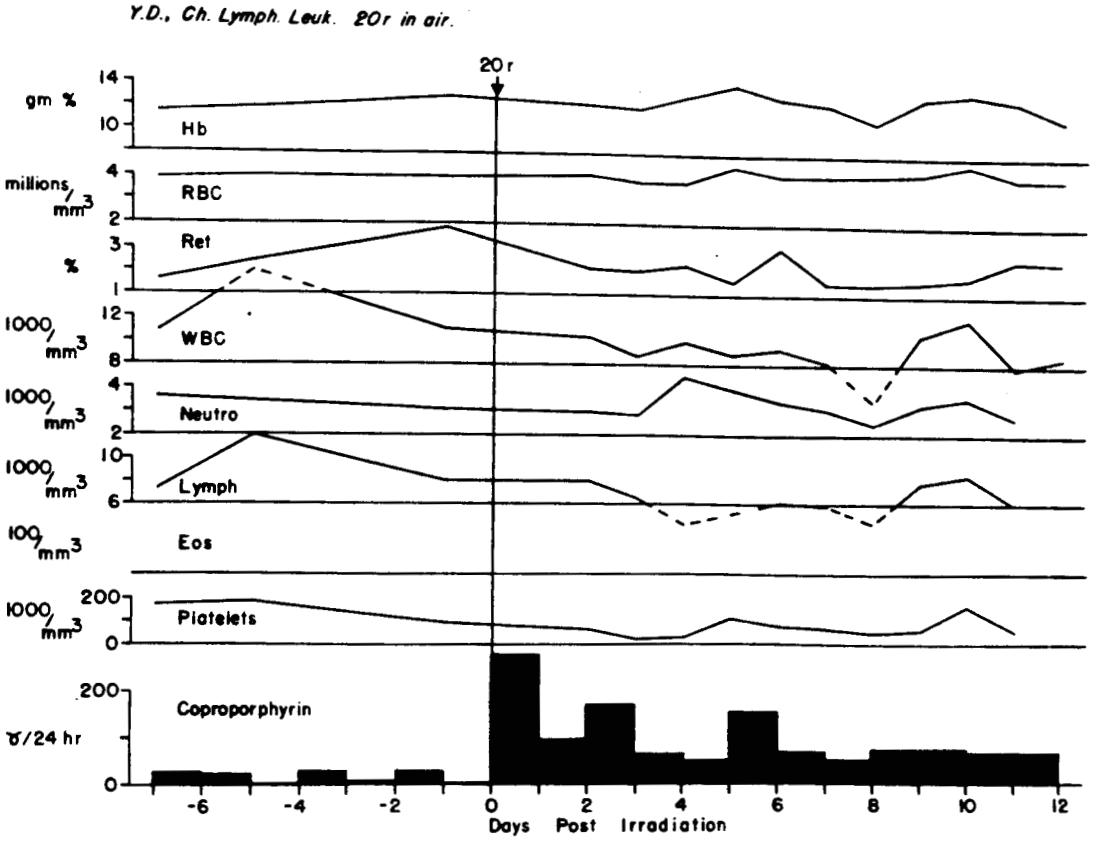


Figure 2

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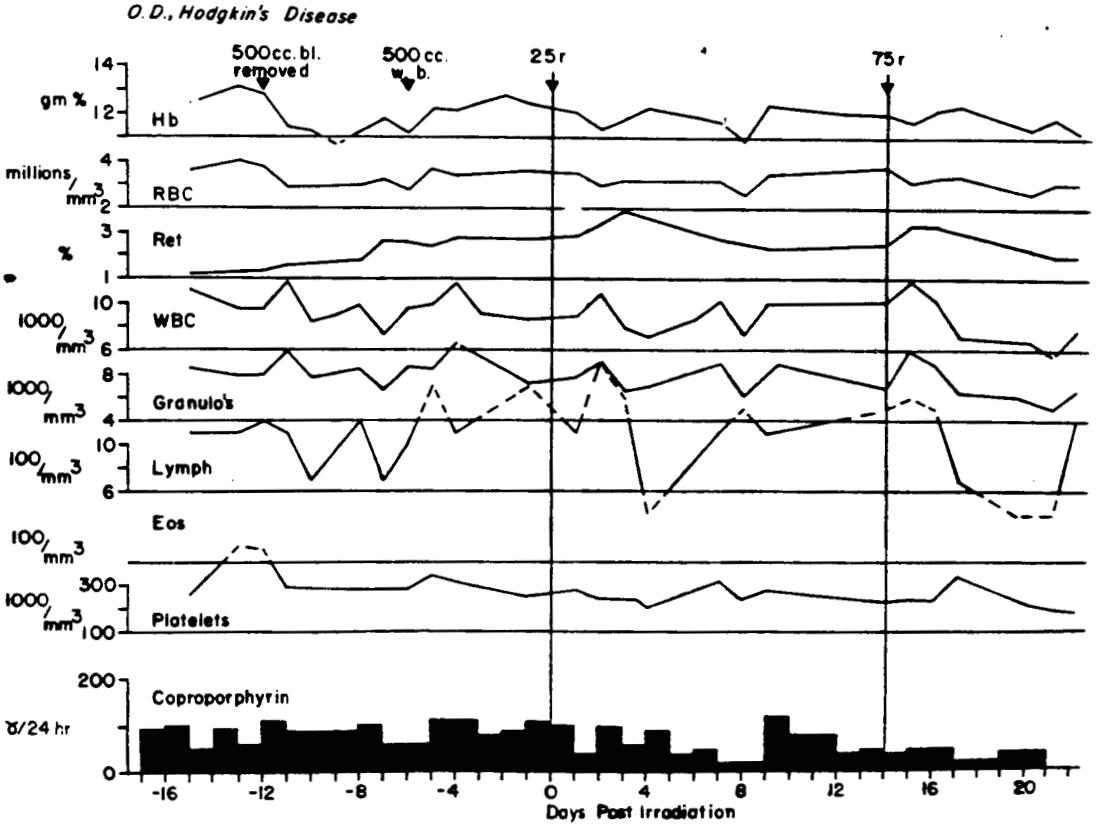
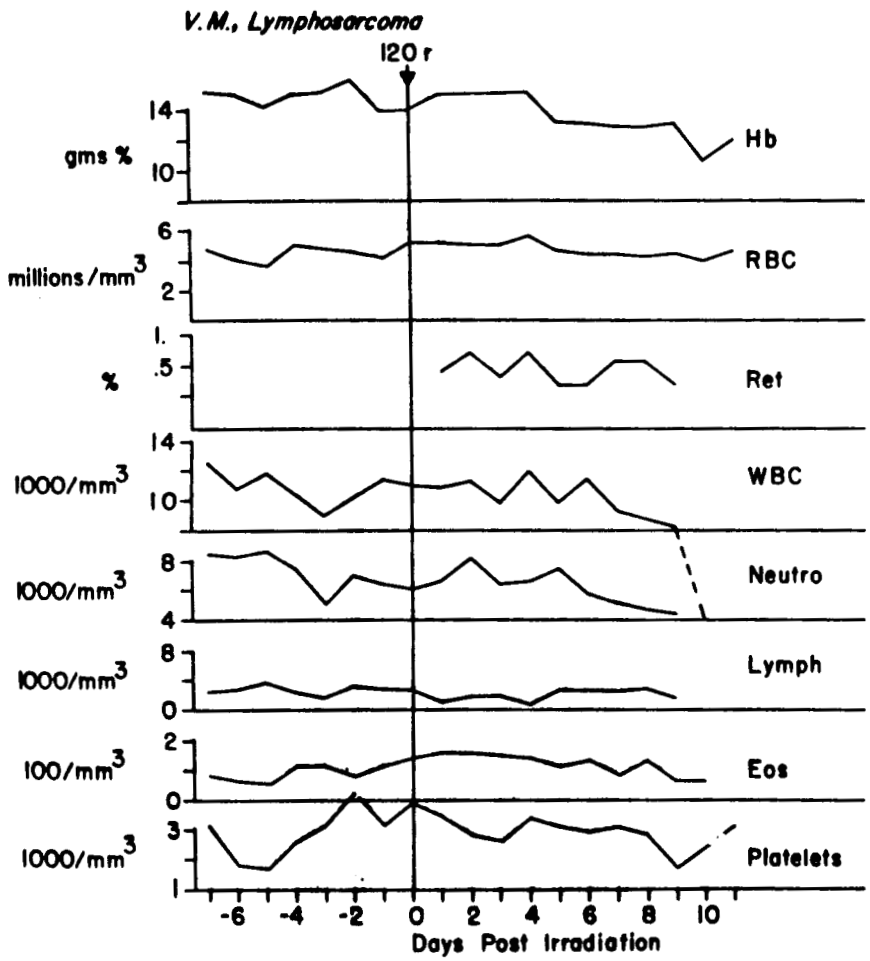
20r
gm %
Hb
millions/mm3
RBC
%
Ret
1000/mm3
WBC
1000/mm3
Neutro
1000/mm3
Lymph
100/mm3
Eos
1000/mm3
Platelets
γ/24 hr
Coproporphyrin
Days Post Irradiation

Figure 4


O.D., Hodgkin's Disease
500cc. bl. removed
500cc. w.b.
25r
75r
gm %
Hb
millions/mm3
RBC
%
Ret
1000/mm3
WBC
1000/mm3
Granulo's
100/mm3
Lymph
100/mm3
Eos
1000/mm3
Platelets
γ/24 hr
Coproporphyrin
Days Post Irradiation


V.M., Lymphosarcoma
120 r
gms %
14
10
Hb
millions/mm3
6
2
RBC
%
1.
.5
Ret
1000/mm3
14
10
WBC
1000/mm3
8
4
Neutro
1000/mm3
8
0
Lymph
100/mm3
2
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Eos
1000/mm3
3
1
Platelets
-6 -4 -2 0 2 4 6 8 10
Days Post Irradiation

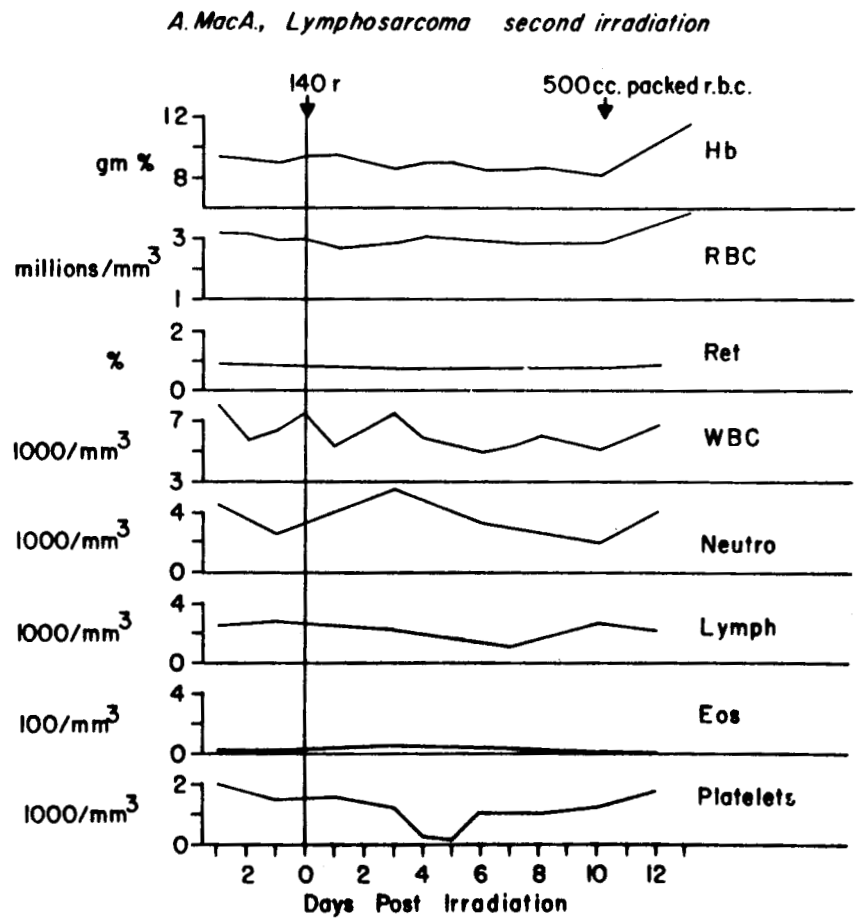

A. MacA., Lymphosarcoma second irradiation
140 r
500cc. packed r.b.c.
gm %
12
8
Hb
millions/mm3
3
1
RBC
%
2
0
Ret
1000/mm3
7
3
WBC
1000/mm3
4
0
Neutro
1000/mm3
4
0
Lymph
100/mm3
4
0
Eos
1000/mm3
2
0
Platelets
2 0 2 4 6 8 10 12
Days Post Irradiation

Figure 6

Figure 7

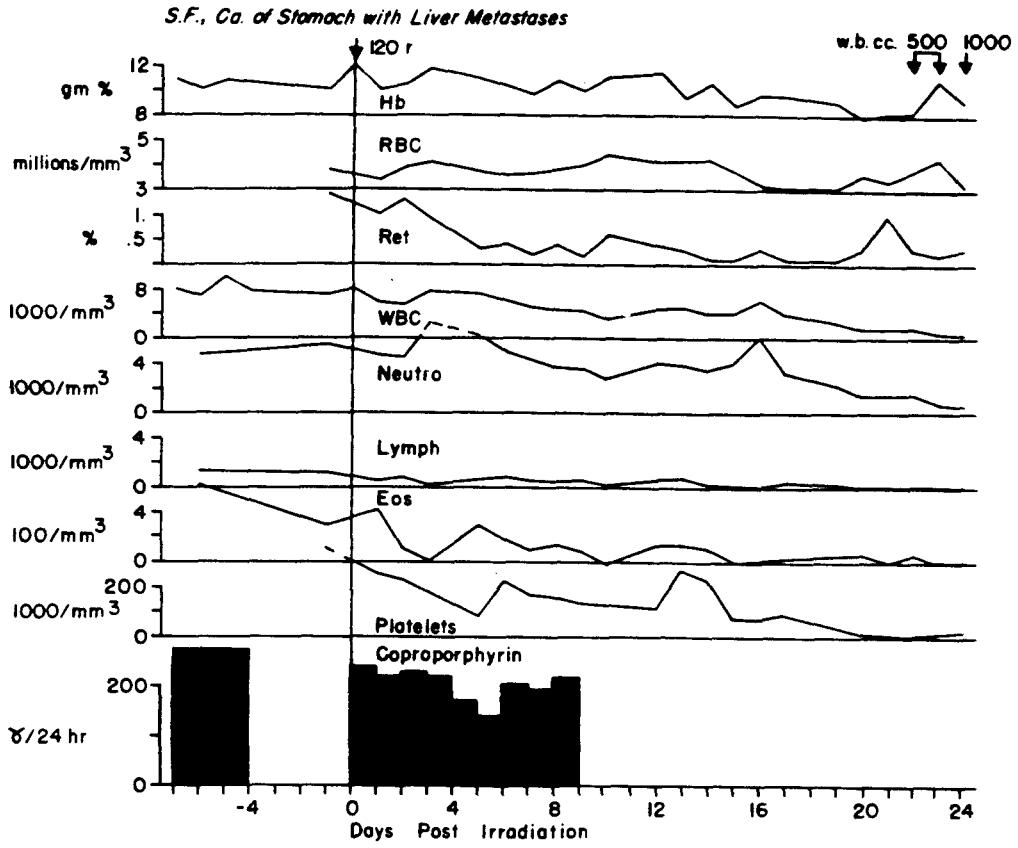


Figure 8

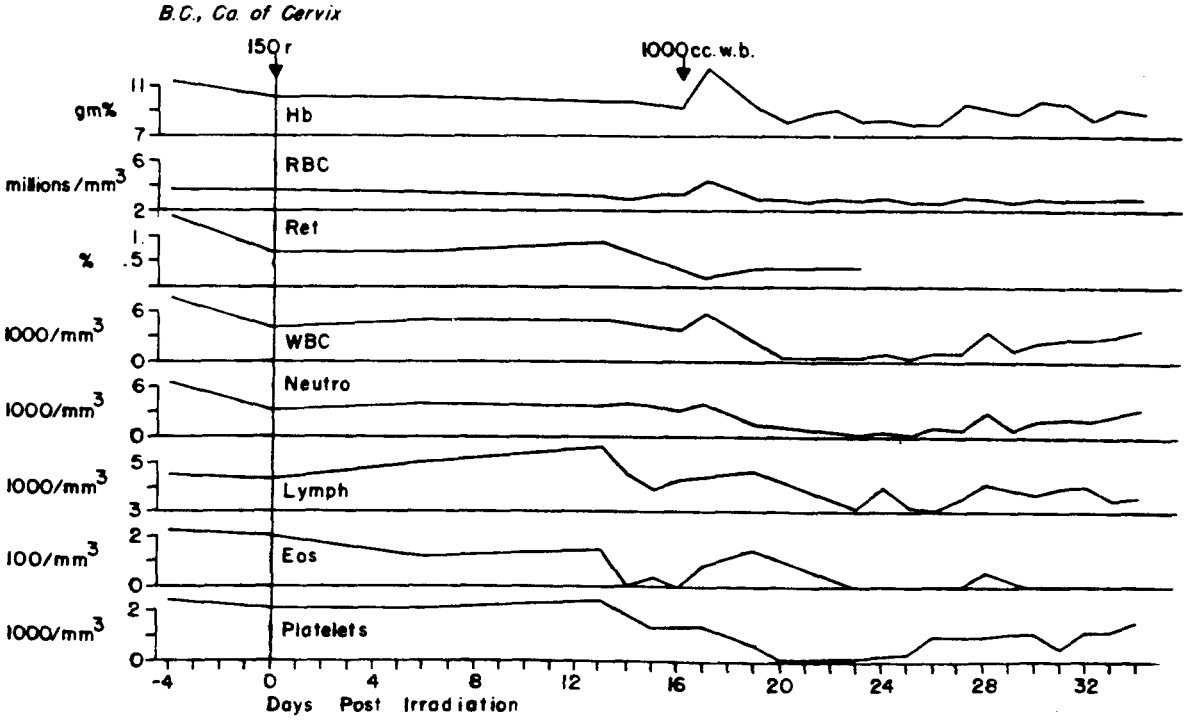


Figure 9

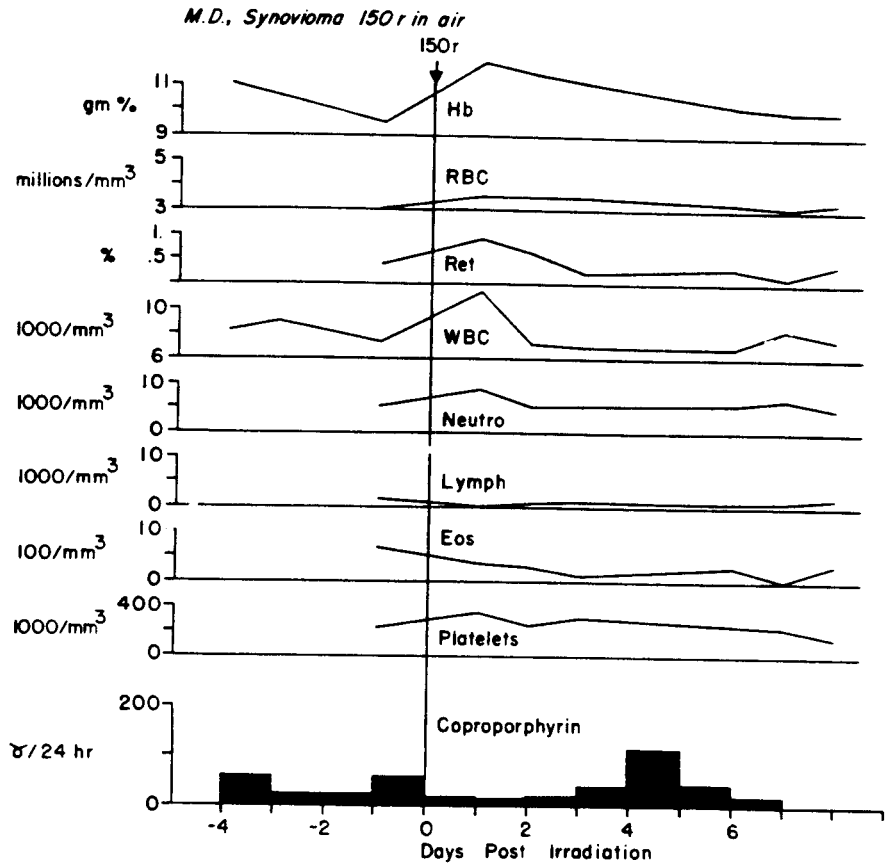
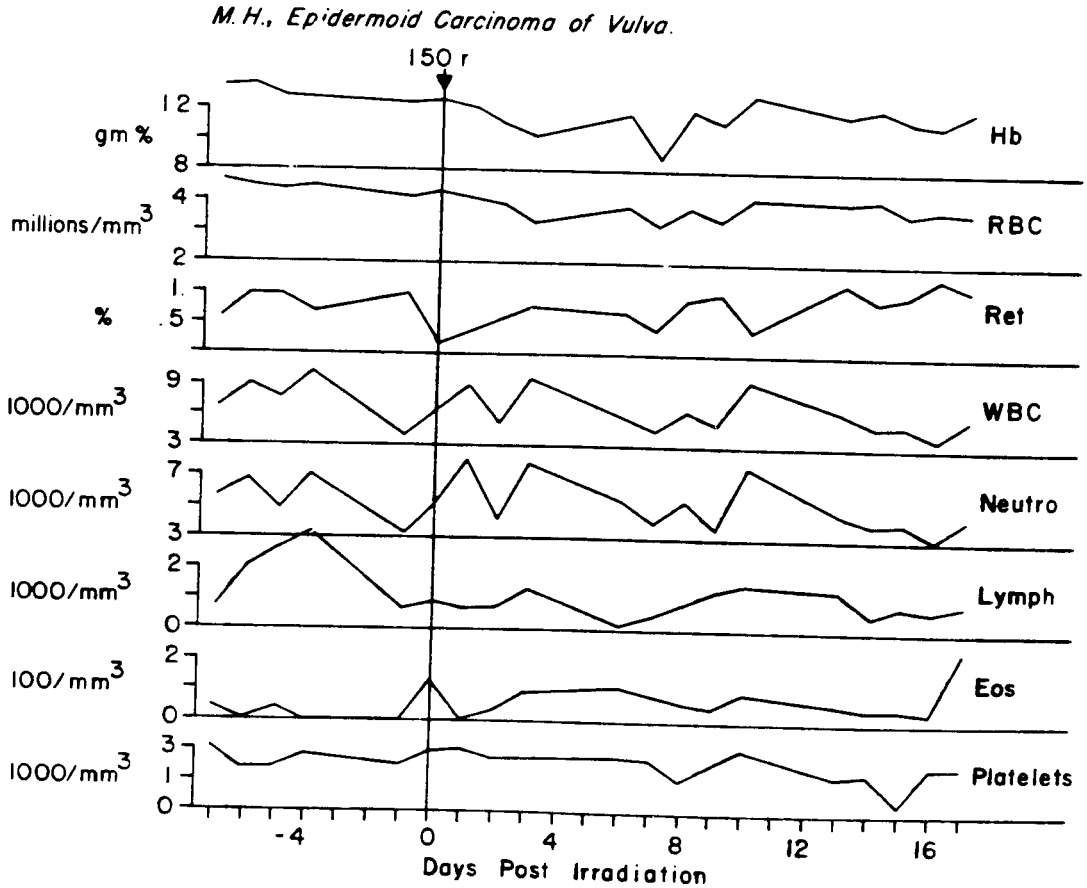


Figure 10



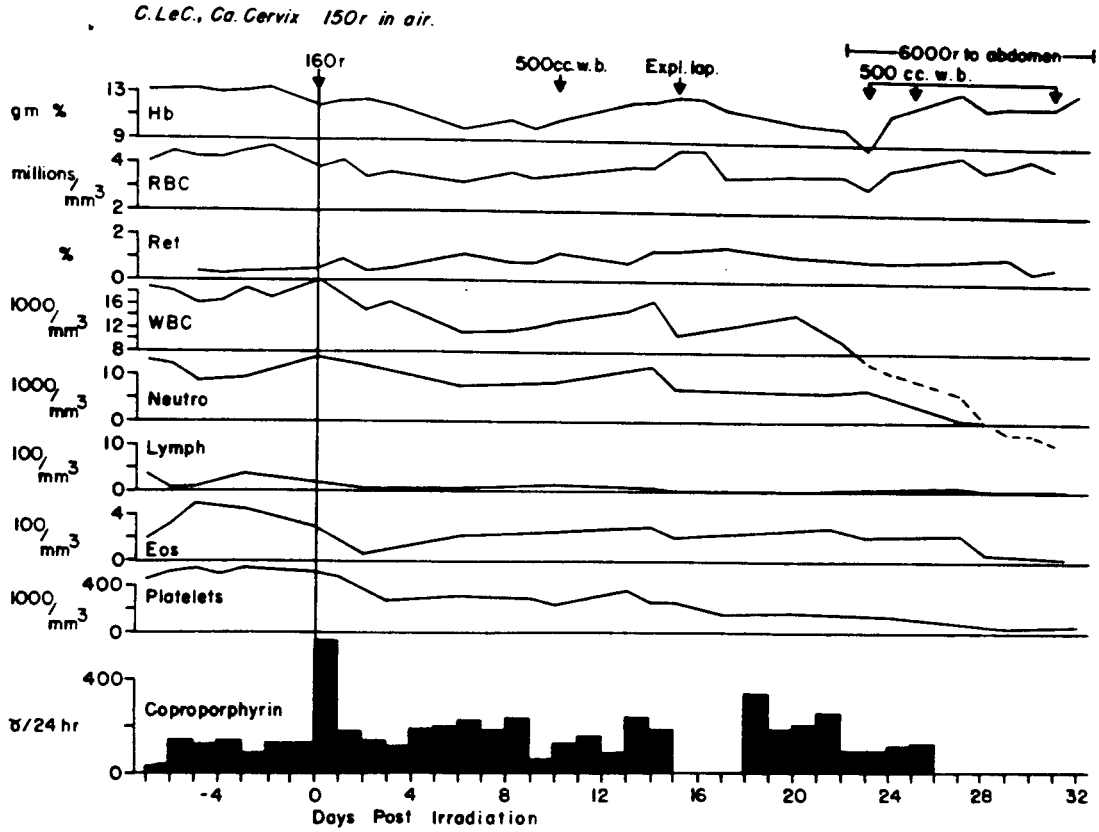
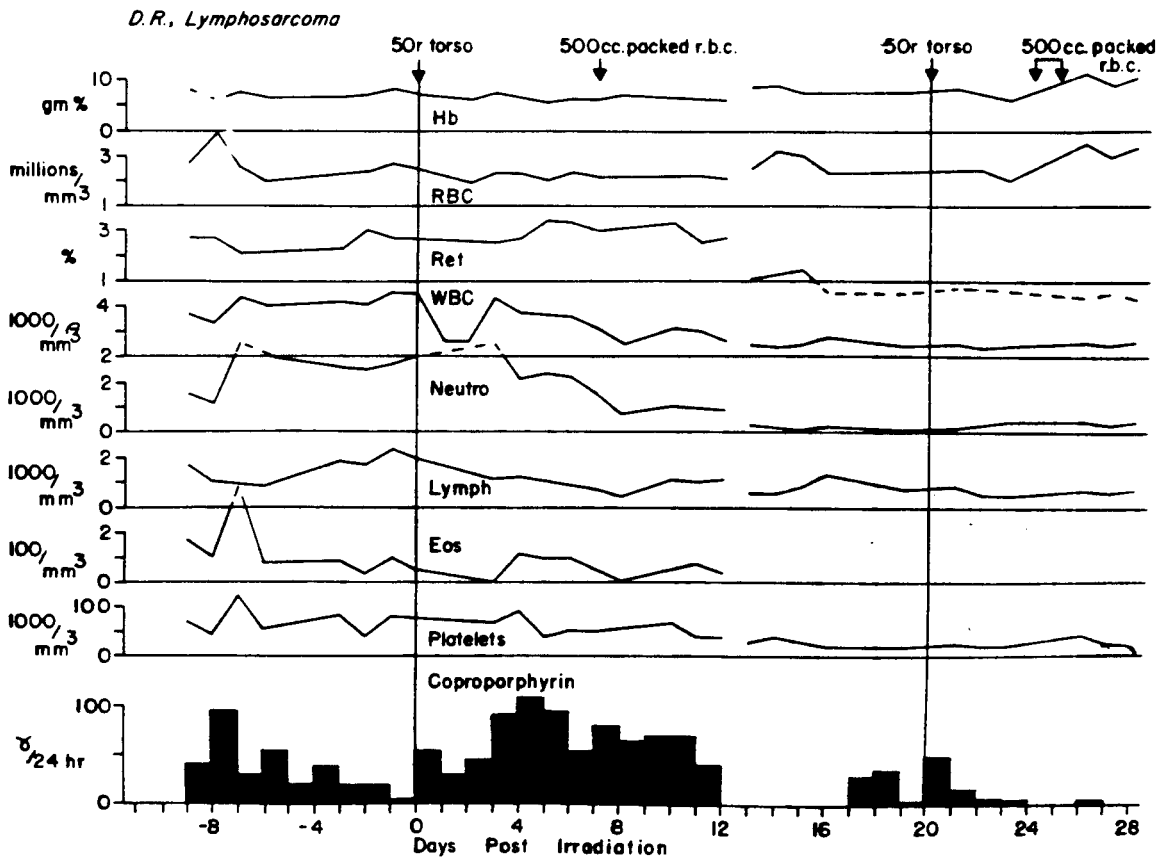

C.LeC., Ca. Cervix 150r in air.
160r
500cc.w.b.
Expl. lap.
6000r to abdomen
500 cc. w.b.
gm %
Hb
millions/mm3
RBC
%
Ret
1000/mm3
WBC
Neutro
Lymph
100/mm3
Eos
Platelets
Coproporphyrin
γ/24 hr
Days Post Irradiation

Figure II


D.R., Lymphosarcoma
50r torso
500cc.packed r.b.c.
50r torso
500cc.packed r.b.c.
gm %
Hb
millions/mm3
RBC
%
Ret
WBC
Neutro
Lymph
Eos
Platelets
Coproporphyrin
γ/24 hr
Days Post Irradiation

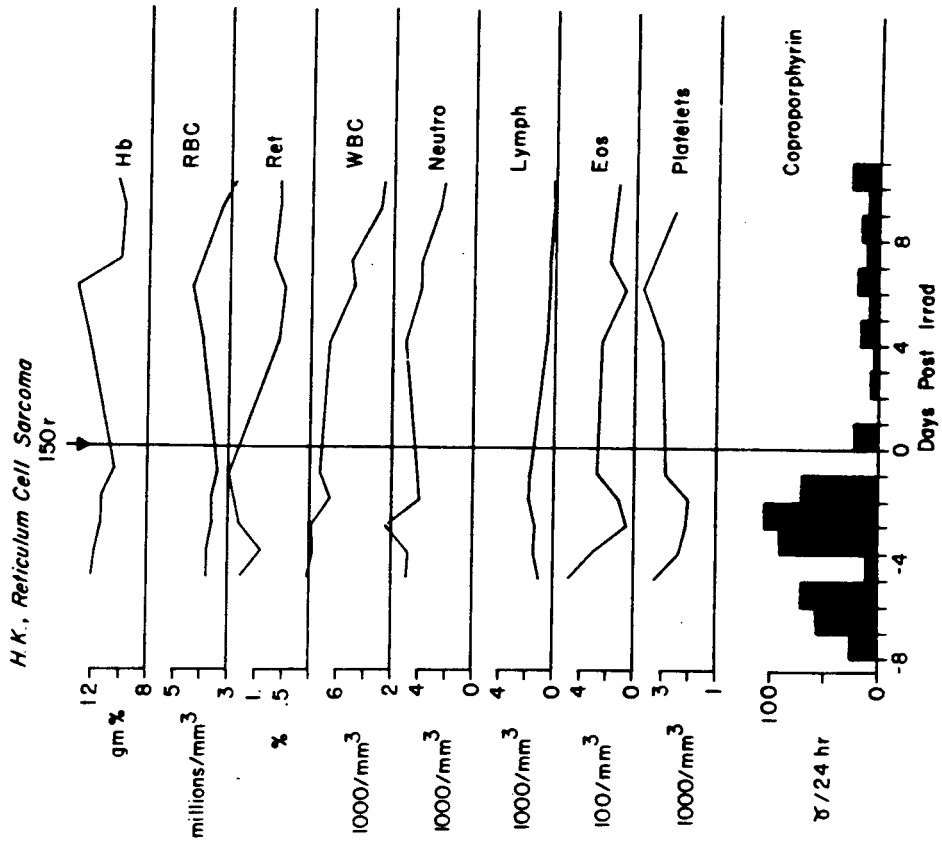
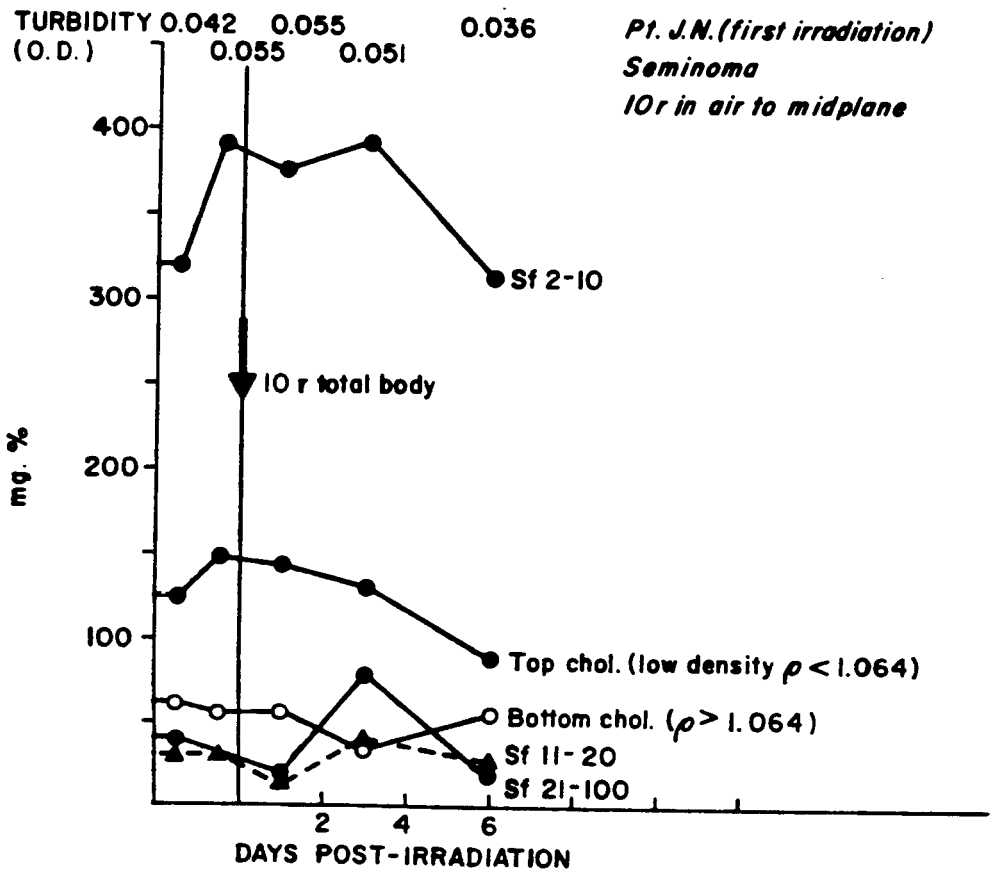

H K., Reticulum Cell Sarcoma
150 r
Hb
RBC
Ret
WBC
Neutro
Lymph
Eos
Platelets
Coproporphyrin
gm %
millions/mm3
%
1000/mm3
1000/mm3
1000/mm3
100/mm3
1000/mm3
γ/24 hr
Days Post Irrad

Figure 11


TURBIDITY (O.D.)
0.042
0.055
0.055
0.051
0.036
Pt. J.N. (first irradiation)
Seminoma
10 r in air to midplane
mg. %
400
300
200
100
Sf 2-10
10 r total body
Top chol. (low density ρ < 1.064)
Bottom chol. (ρ > 1.064)
Sf 11-20
Sf 21-100
2
4
6
DAYS POST-IRRADIATION

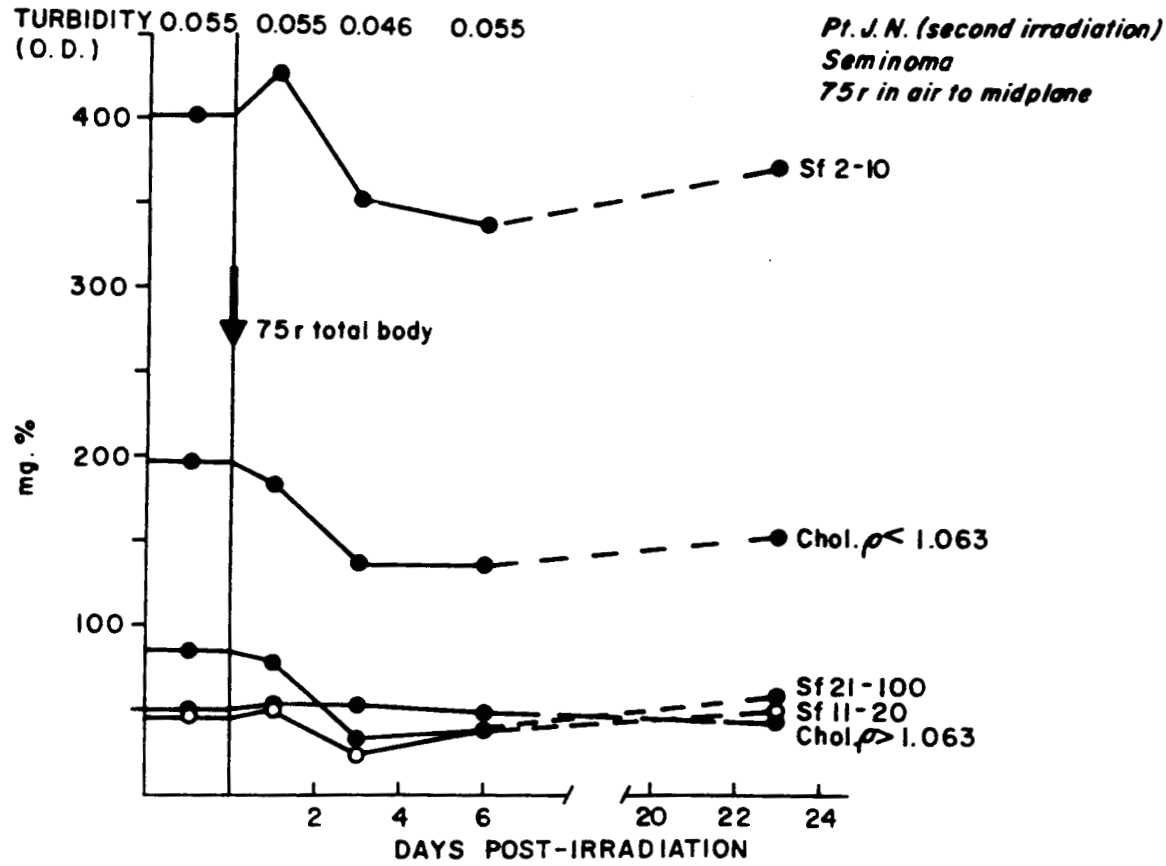
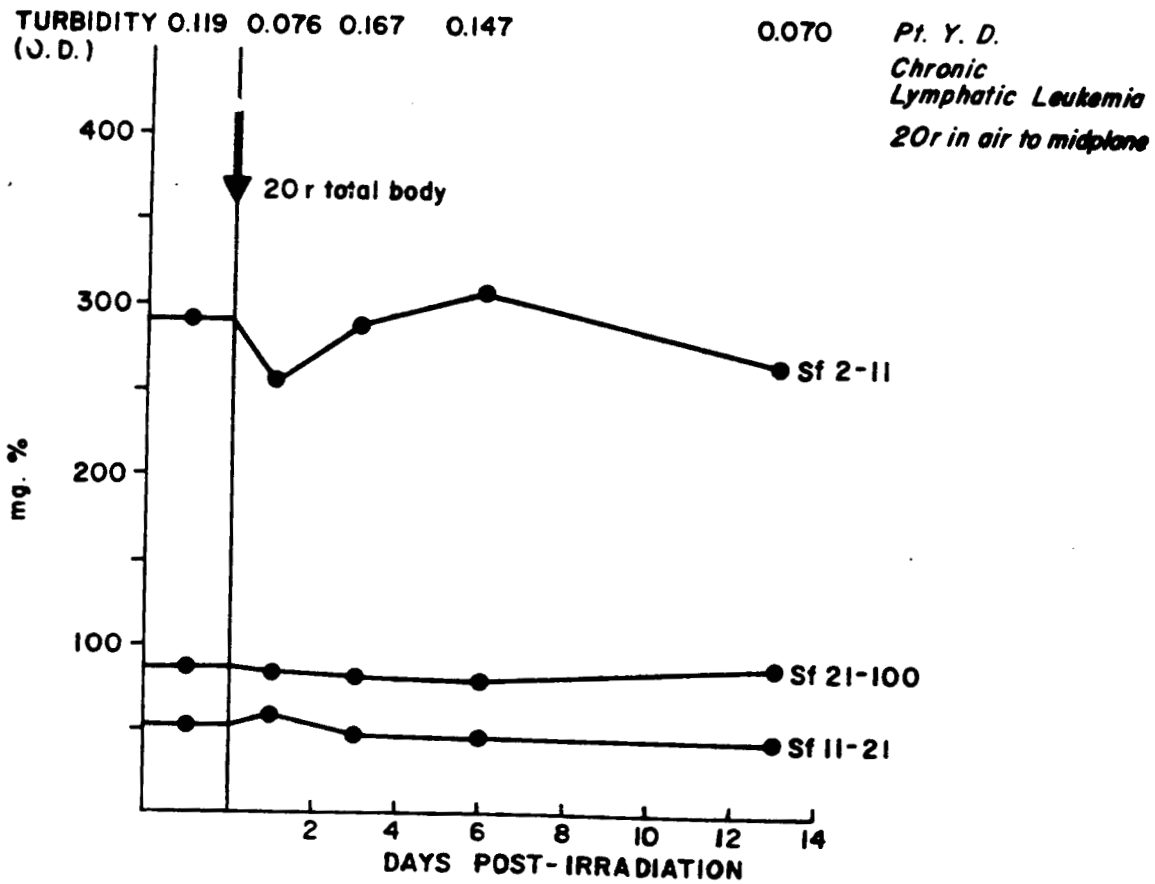
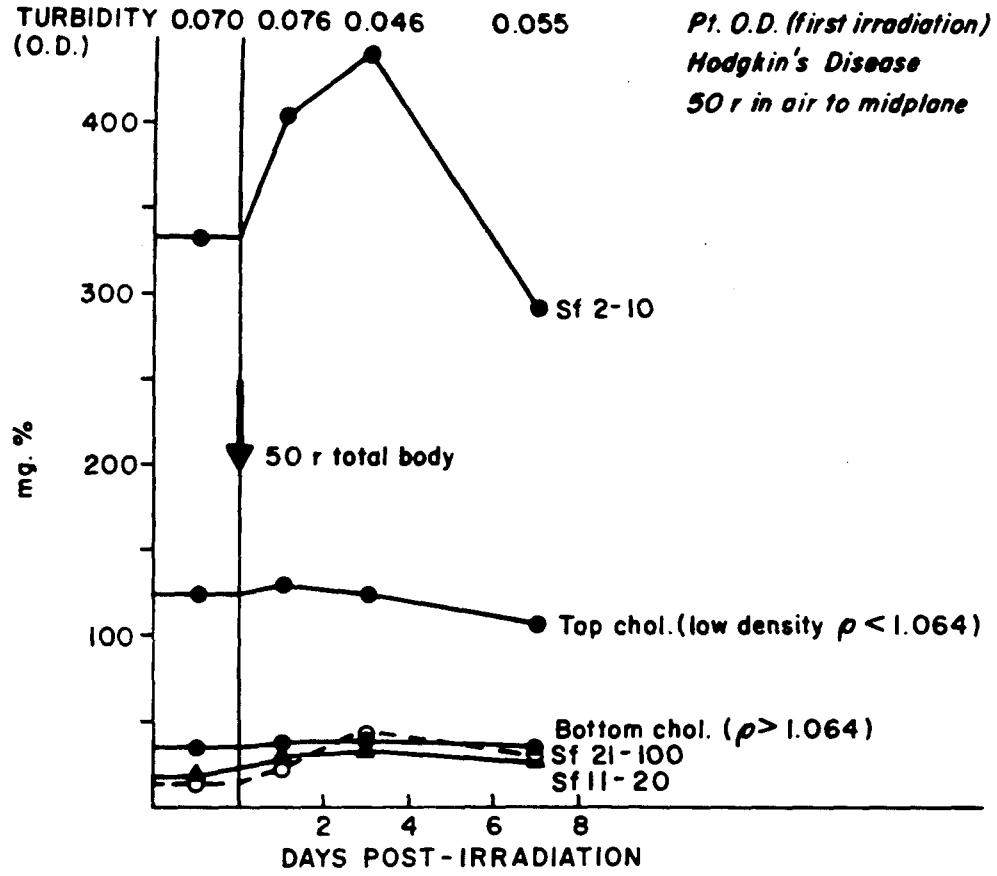
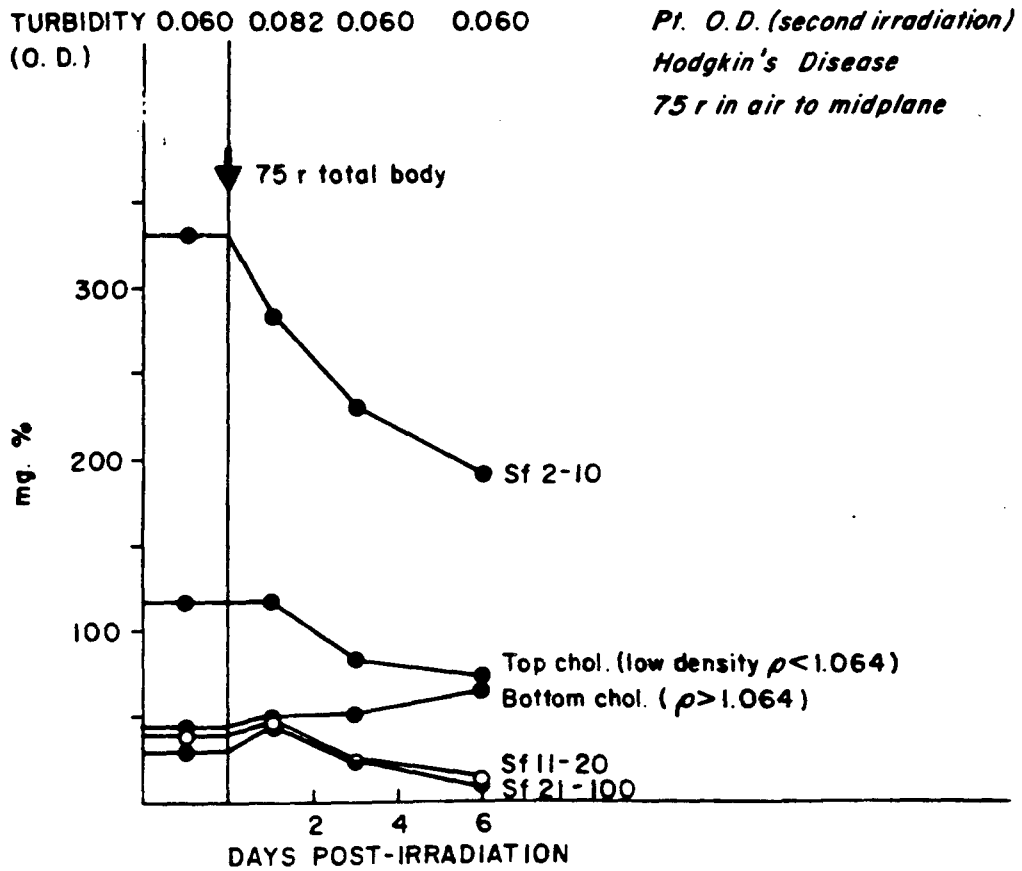

TURBIDITY (O.D.)
0.055 0.055 0.046 0.055
Pt. J. N. (second irradiation)
Seminoma
75r in air to midplane
400
300
200
100
mg. %
75r total body
Sf 2-10
Chol. ρ< 1.063
Sf 21-100
Sf 11-20
Chol. ρ> 1.063
2 4 6 20 22 24
DAYS POST-IRRADIATION

Figure 16


TURBIDITY (O.D.)
0.119 0.076 0.167 0.147 0.070
Pt. Y. D.
Chronic
Lymphatic Leukemia
20r in air to midplane
400
300
200
100
mg. %
20 r total body
Sf 2-11
Sf 21-100
Sf 11-21
2 4 6 8 10 12 14
DAYS POST-IRRADIATION


TURBIDITY (O.D.) 0.070 0.076 0.046 0.055
Pt. O.D. (first irradiation)
Hodgkin's Disease
50 r in air to midplane
400
300
200
100
mg. %
50 r total body
Sf 2-10
Top chol. (low density ρ < 1.064)
Bottom chol. (ρ > 1.064)
Sf 21-100
Sf 11-20
2
4
6
8
DAYS POST-IRRADIATION


TURBIDITY (O. D.) 0.060 0.082 0.060 0.060
Pt. O. D. (second irradiation)
Hodgkin's Disease
75 r in air to midplane
75 r total body
300
200
100
mg. %
Sf 2-10
Top chol. (low density ρ < 1.064)
Bottom chol. (ρ > 1.064)
Sf 11-20
Sf 21-100
2
4
6
DAYS POST-IRRADIATION

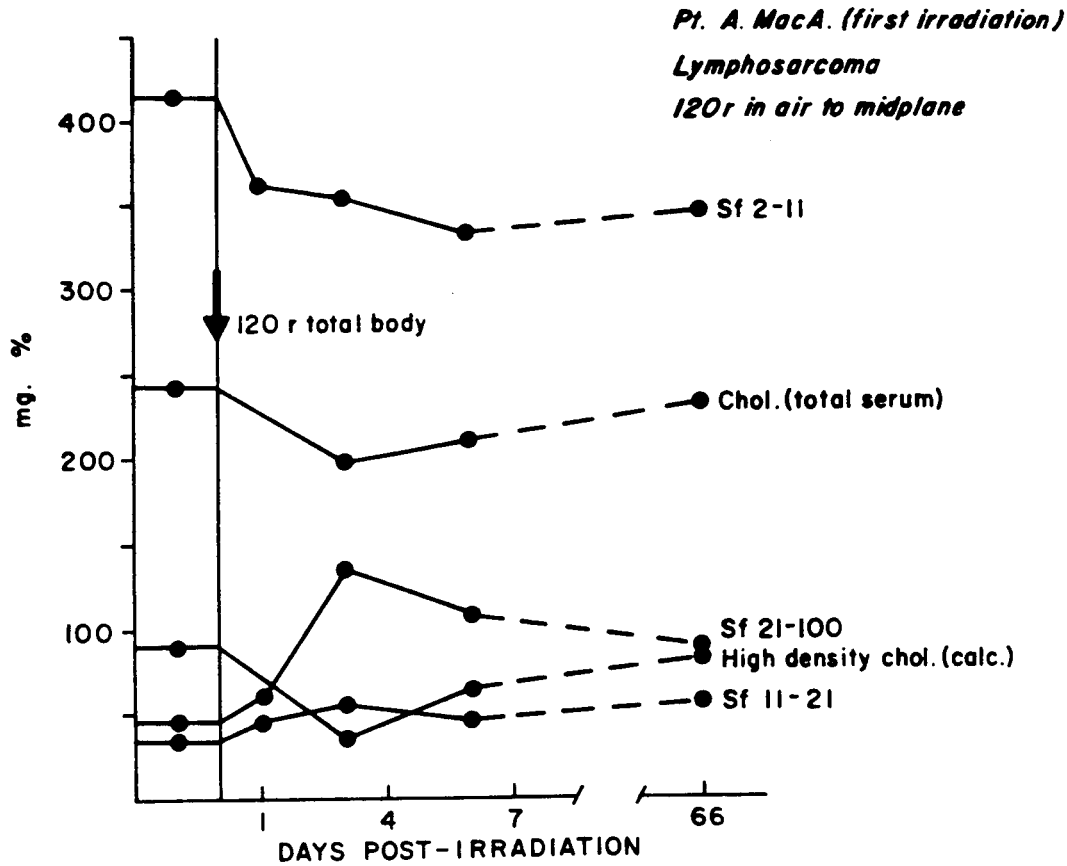
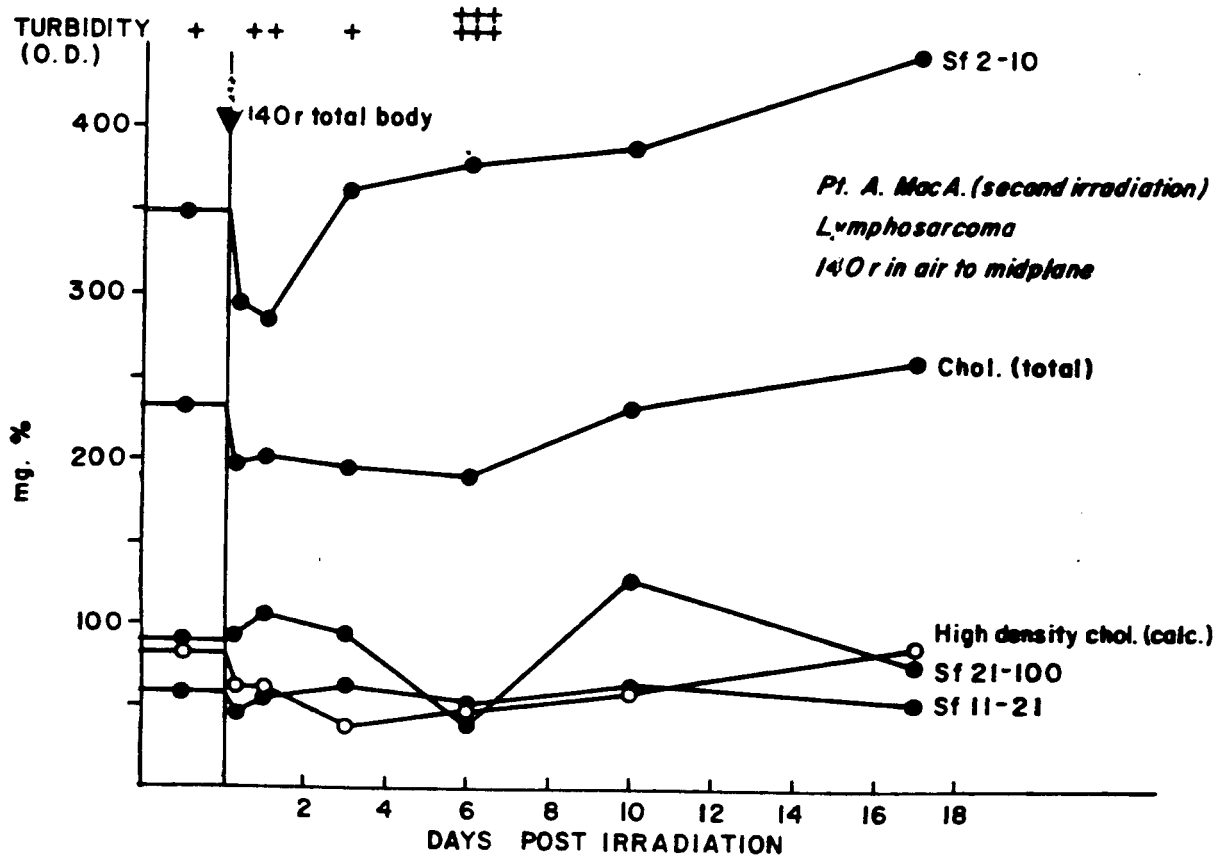
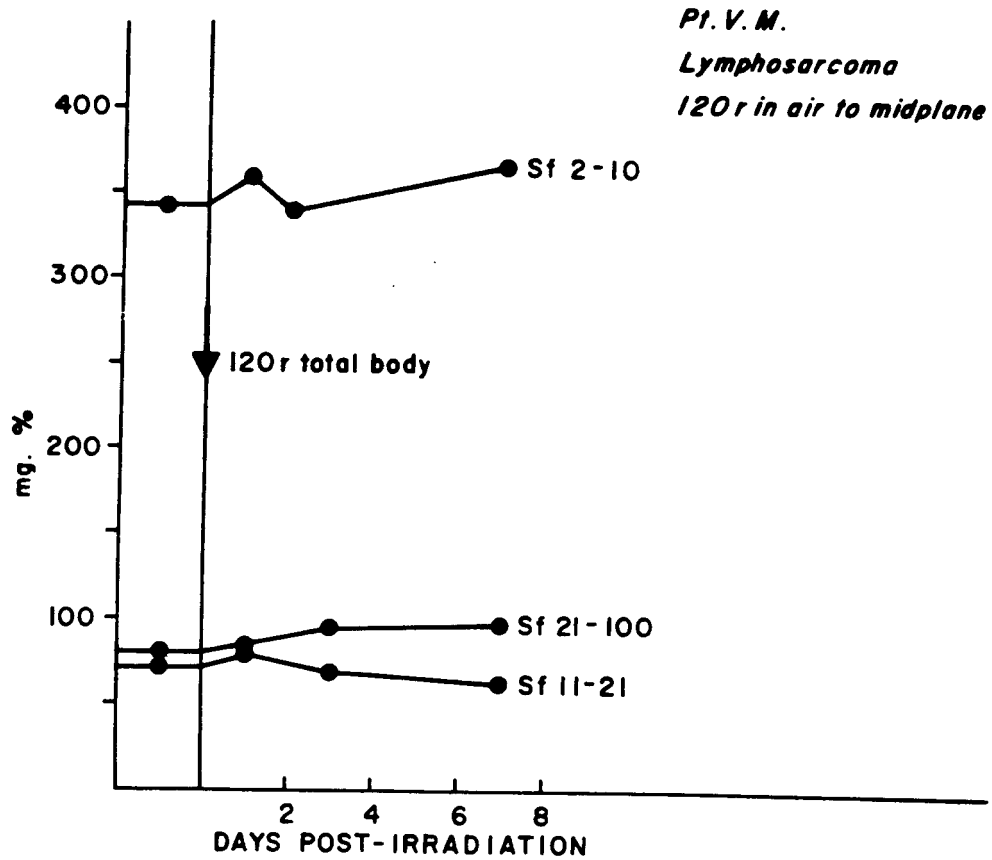
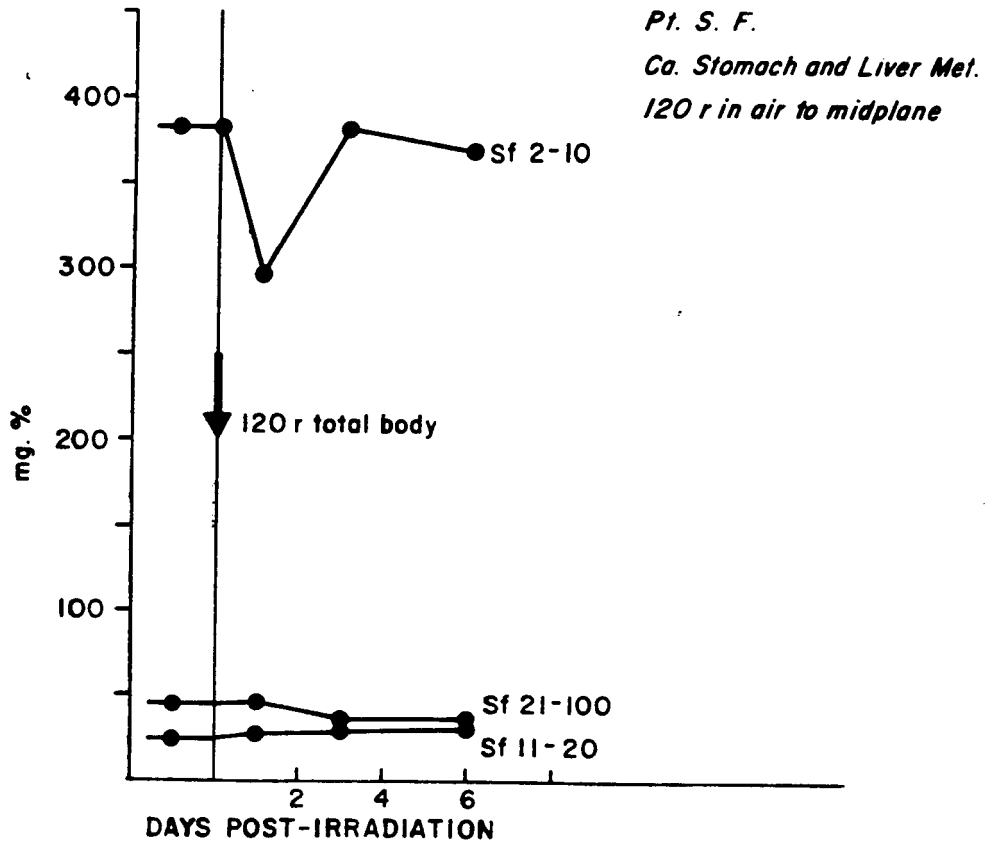
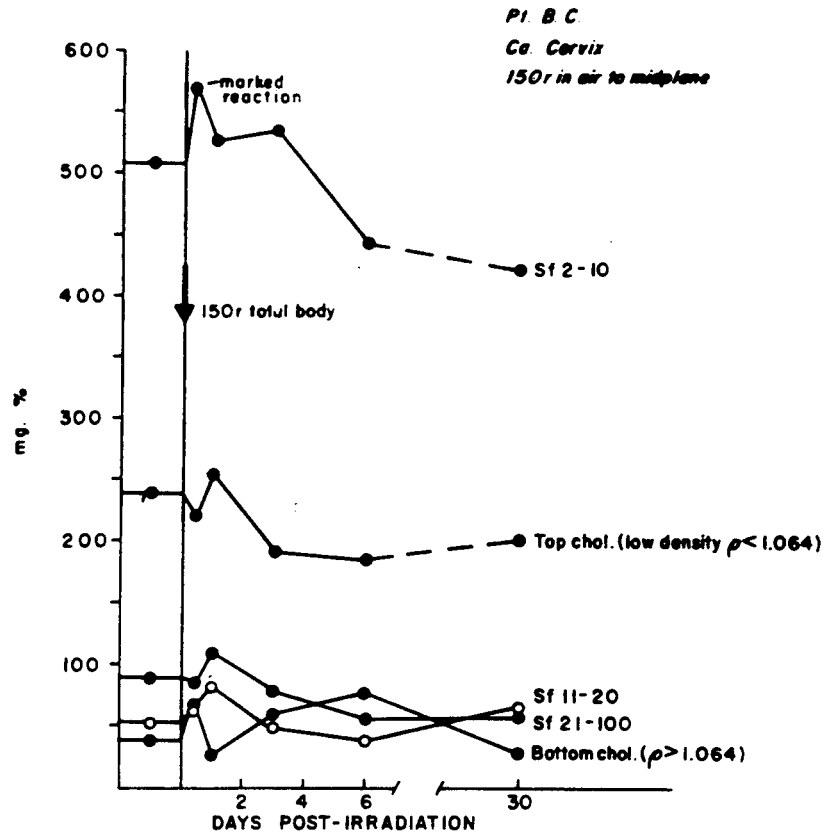

Pt. A. MacA. (first irradiation)
Lymphosarcoma
120r in air to midplane
400
300
200
100
mg. %
120 r total body
Sf 2-11
Chol. (total serum)
Sf 21-100
High density chol. (calc.)
Sf 11-21
1
4
7
66
DAYS POST-IRRADIATION

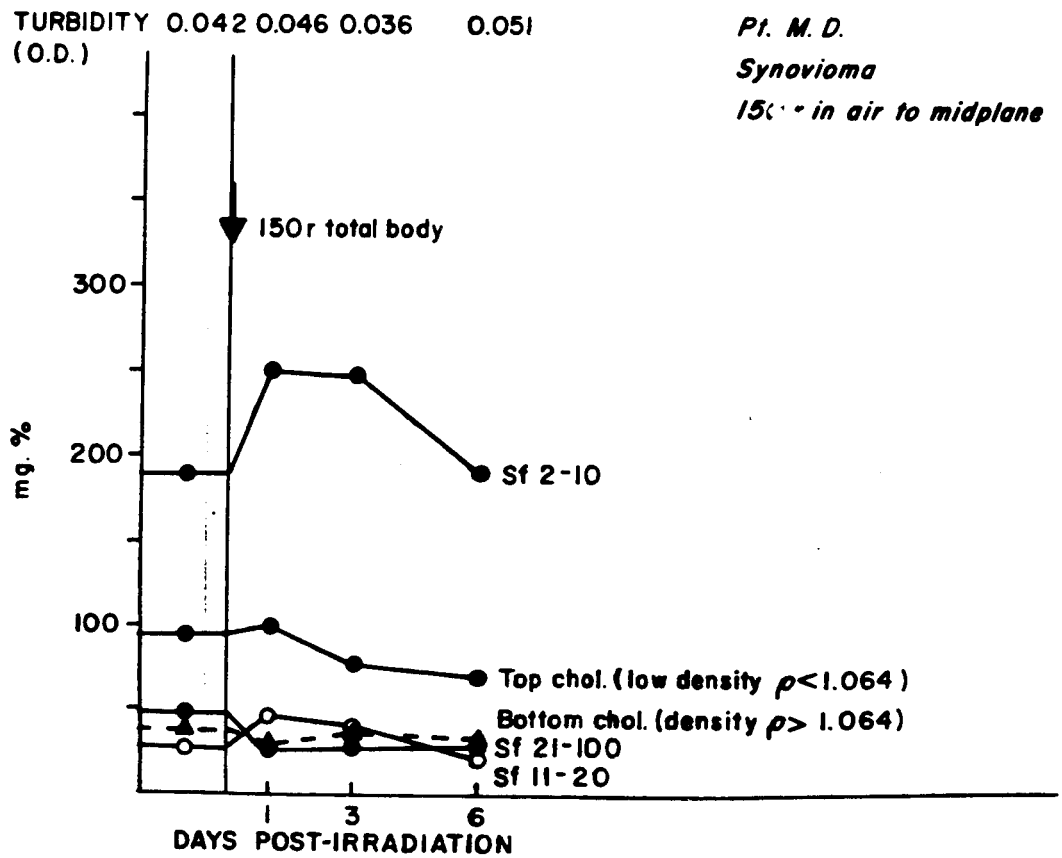
Figure 20

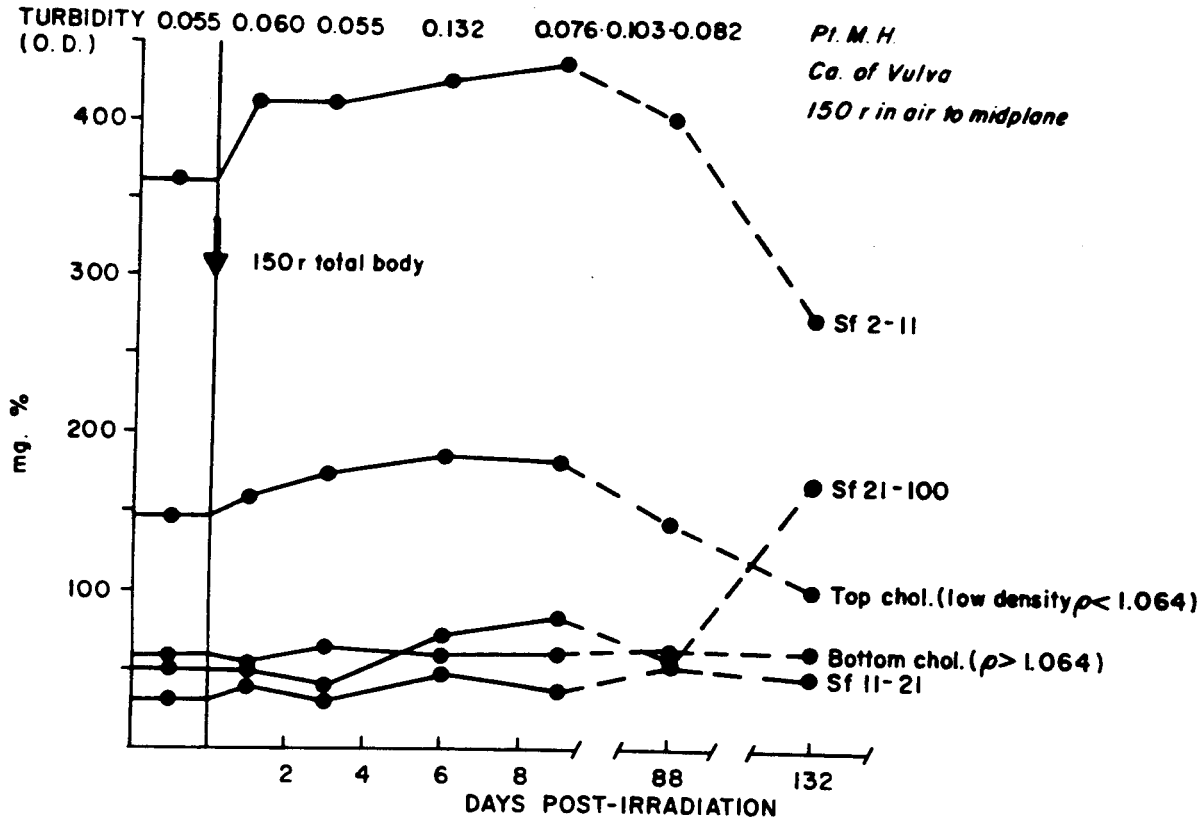

TURBIDITY (O.D.)
+
++
+
###
140r total body
Sf 2-10
Pt. A. MacA. (second irradiation)
Lymphosarcoma
140r in air to midplane
Chol. (total)
High density chol. (calc.)
Sf 21-100
Sf 11-21
400
300
200
100
mg. %
2
4
6
8
10
12
14
16
18
DAYS POST IRRADIATION

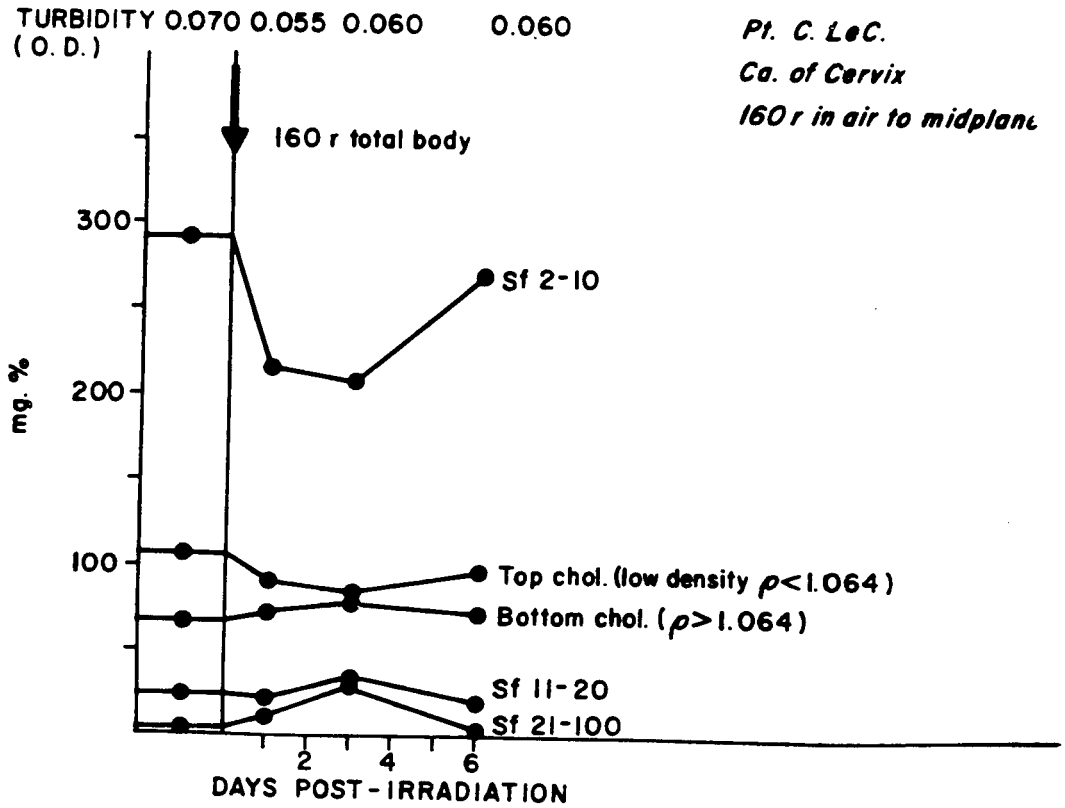

Pt. V. M.
Lymphosarcoma
120 r in air to midplane
400
300
200
100
mg. %
Sf 2-10
120 r total body
Sf 21-100
Sf 11-21
2
4
6
8
DAYS POST-IRRADIATION


Pt. S. F.
Ca. Stomach and Liver Met.
120 r in air to midplane
400
300
200
100
mg. %
Sf 2-10
120 r total body
Sf 21-100
Sf 11-20
2
4
6
DAYS POST-IRRADIATION

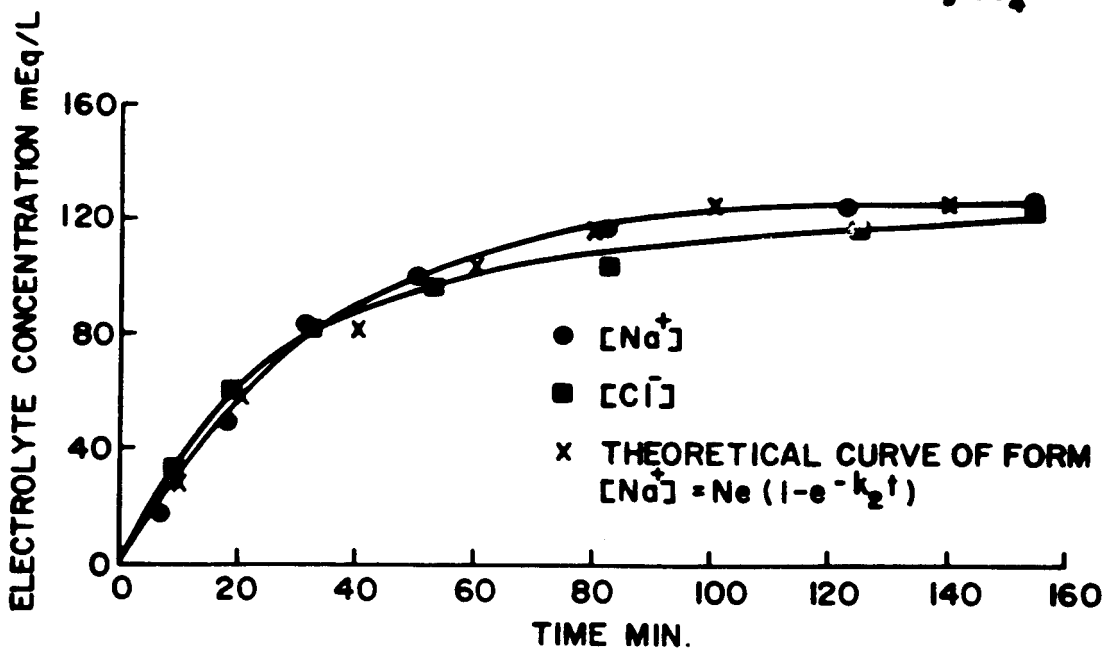

Pt. B.C.
Ca. Cervix
150r in air to midplane
marked reaction
150r total body
Sf 2-10
Top chol. (low density ρ< 1.064)
Sf 11-20
Sf 21-100
Bottom chol. (ρ> 1.064)
mg. %
600
500
400
300
200
100
2
4
6
30
DAYS POST-IRRADIATION


TURBIDITY (O.D.) 0.042 0.046 0.036 0.051
Pt. M. D.
Synovioma
150r in air to midplane
150r total body
Sf 2-10
Top chol. (low density ρ<1.064)
Bottom chol. (density ρ> 1.064)
Sf 21-100
Sf 11-20
mg. %
300
200
100
1
3
6
DAYS POST-IRRADIATION

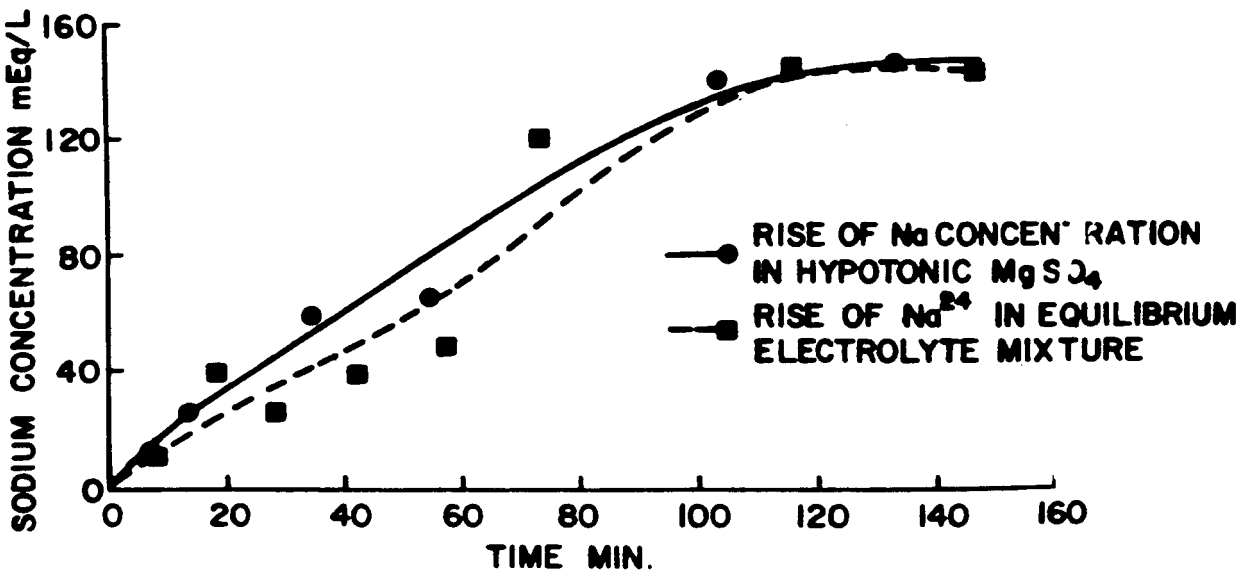

TURBIDITY (O. D.)
0.055 0.060 0.055 0.132 0.076 0.103 0.082
Pt. M. H.
Ca. of Vulva
150 r in air to midplane
150 r total body
Sf 2-11
Sf 21-100
Top chol. (low density ρ< 1.064)
Bottom chol. (ρ> 1.064)
Sf 11-21
mg. %
400
300
200
100
2
4
6
8
88
132
DAYS POST-IRRADIATION


TURBIDITY (O. D.)
0.070 0.055 0.060 0.060
Pt. C. LeC.
Ca. of Cervix
160 r in air to midplane
160 r total body
Sf 2-10
Top chol. (low density ρ<1.064)
Bottom chol. (ρ>1.064)
Sf 11-20
Sf 21-100
mg. %
300
200
100
2
4
6
DAYS POST-IRRADIATION

ELECTROLYTE CONCENTRATION VS TIME. DOG NO. 77
(JEJUNUM), SOLUTION: 5 X ISOTONIC $MgSO_4$



CHANGE IN SODIUM CONCENTRATION WITH TIME IN HYPOTONIC $MgSO_4$ AND IN ELECTROLYTE MIXTURE CHARACTERISTIC OF THE BOWEL SEGMENT. DOG NO. 74, (JEJUNUM)



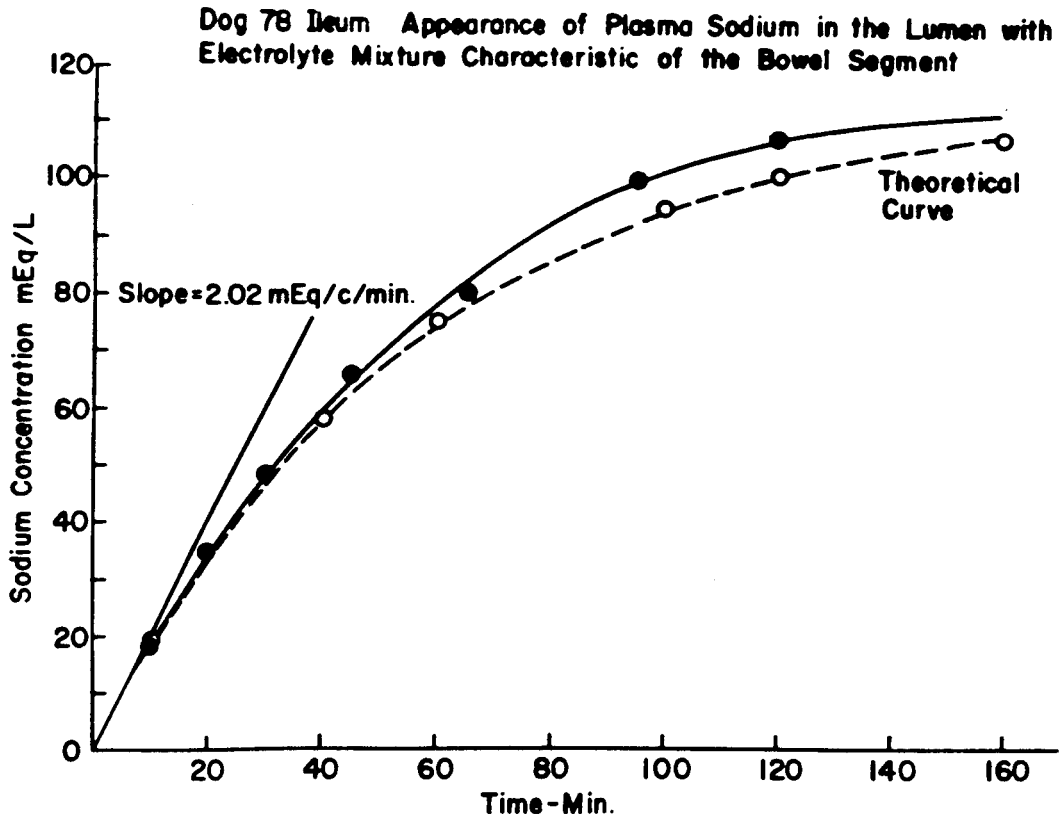

Dog 78 Ileum Appearance of Plasma Sodium in the Lumen with Electrolyte Mixture Characteristic of the Bowel Segment
Sodium Concentration mEq/L
Slope=2.02 mEq/c/min.
Theoretical Curve
Time-Min.
0
20
40
60
80
100
120
140
160

Figure 30

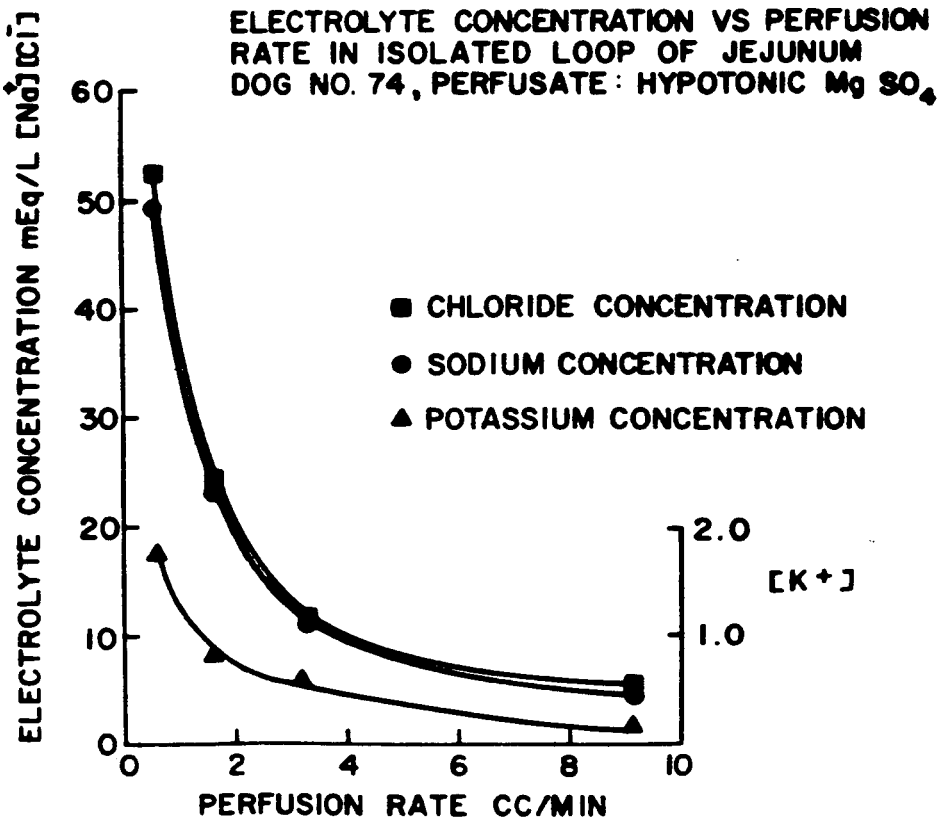

ELECTROLYTE CONCENTRATION VS PERFUSION RATE IN ISOLATED LOOP OF JEJUNUM
DOG NO. 74, PERFUSATE: HYPOTONIC Mg SO4
ELECTROLYTE CONCENTRATION mEq/L [Na+][Cl-]
CHLORIDE CONCENTRATION
SODIUM CONCENTRATION
POTASSIUM CONCENTRATION
[K+]
2.0
1.0
PERFUSION RATE CC/MIN
0
2
4
6
8
10
20
30
40
50
60

Figure 31

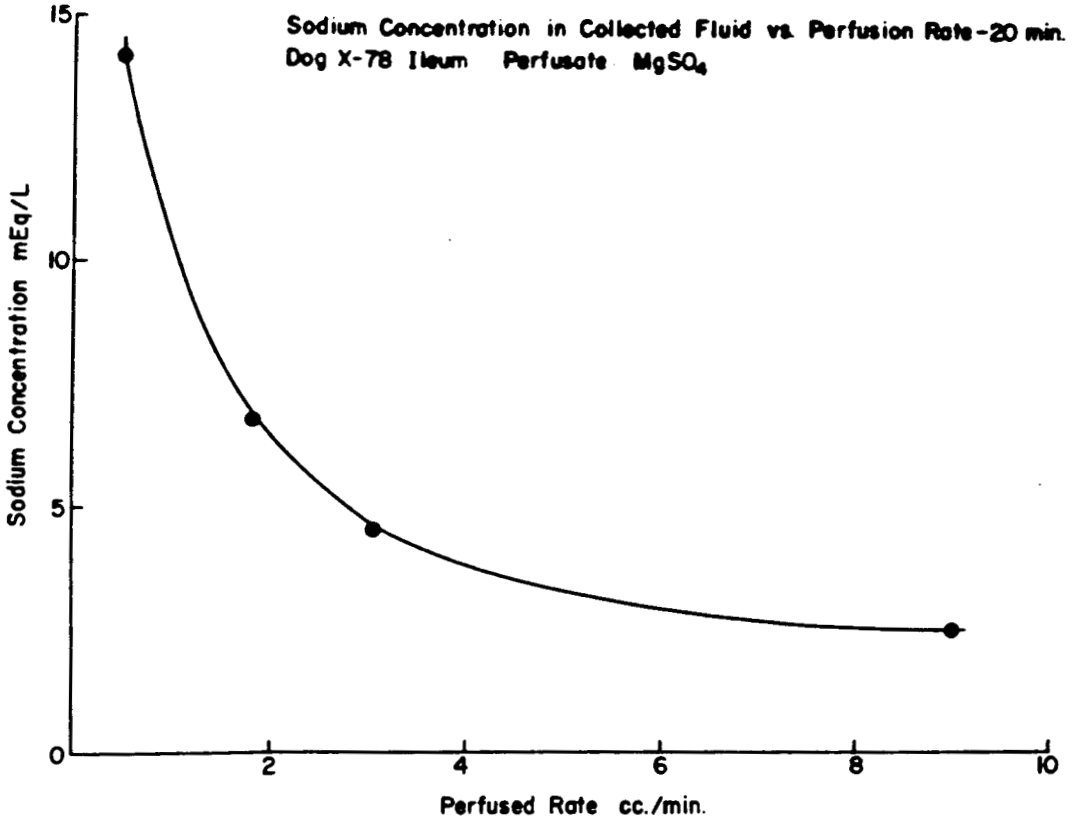
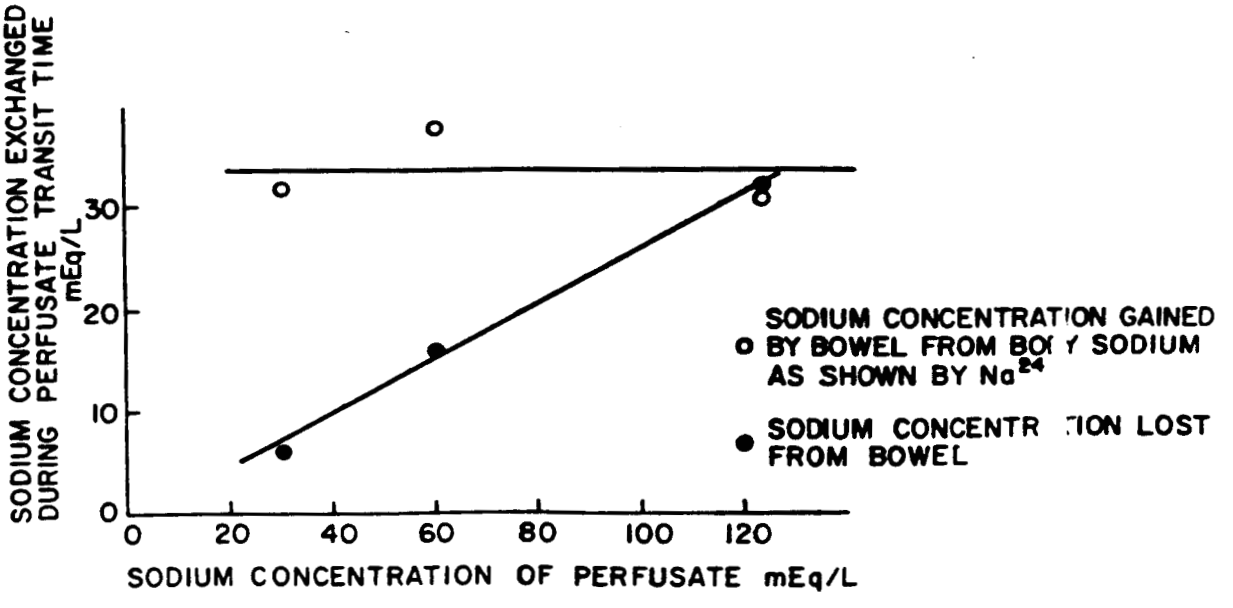


Figure 32

SODIUM CONCENTRATION EXCHANGED DURING PERFUSATE TRANSIT TIME VS PERFUSATE SODIUM CONCENTRATION

DOG NO. 77 (UPPER JEJUNUM), PERFUSATE: ISOTONIC IN NaCl AND $MgSO_4$ PERFUSION RATE: 1.54 CC/MIN.



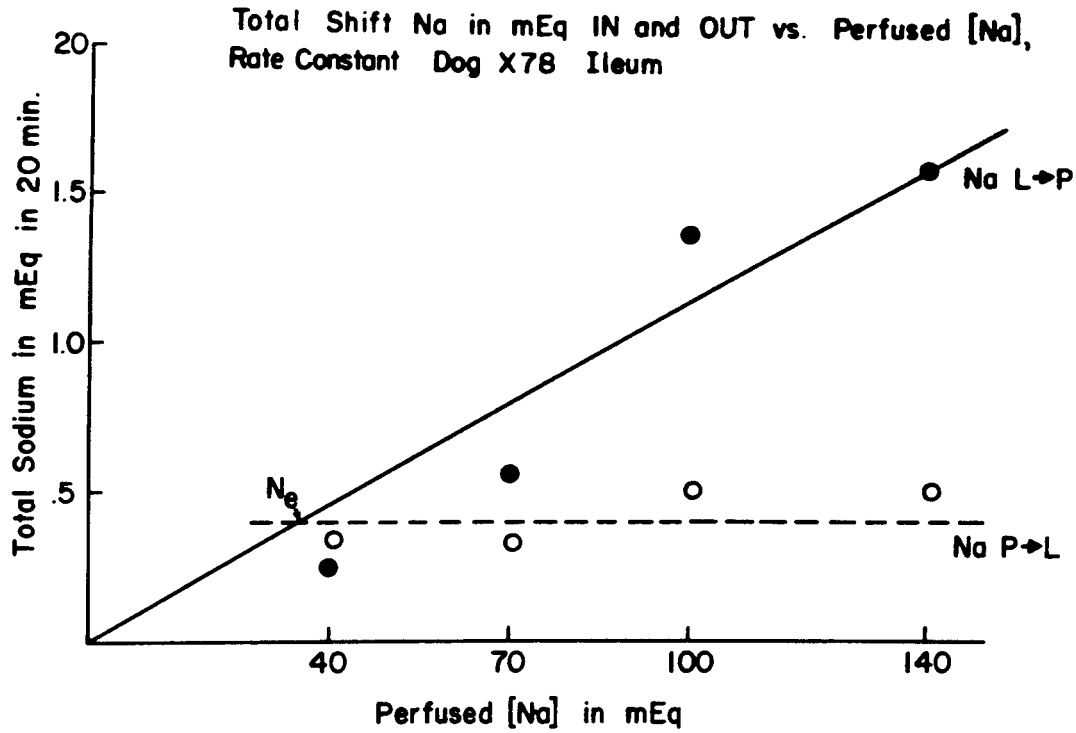
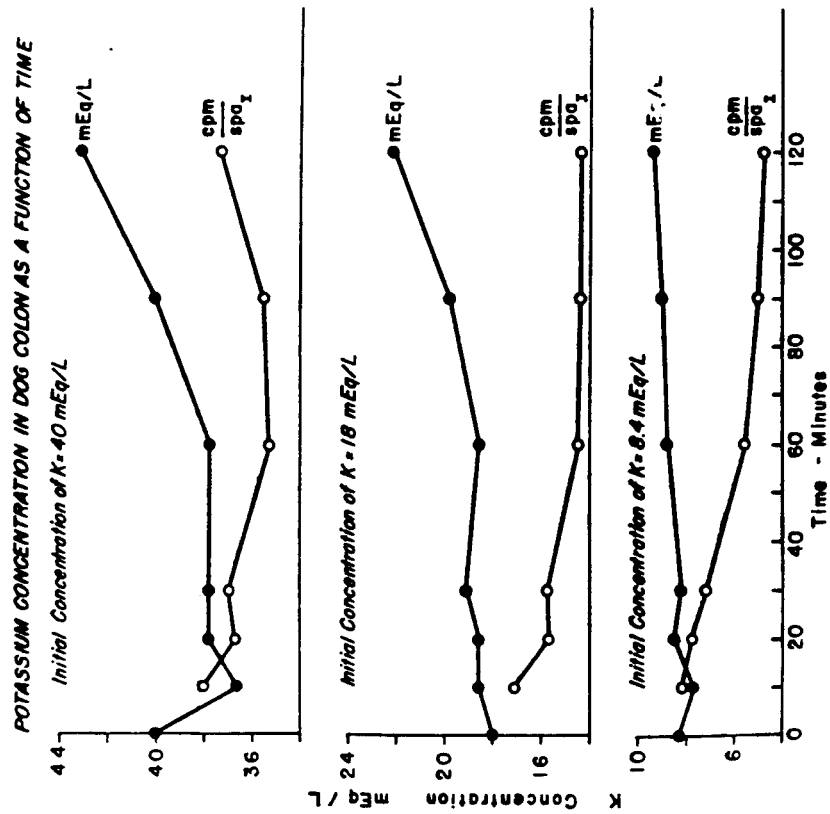

Total Shift Na in mEq IN and OUT vs. Perfused [Na],
Rate Constant Dog X78 Ileum
Total Sodium in mEq in 20 min.
20
1.5
1.0
.5
Na L→P
Na P→L
40
70
100
140
Perfused [Na] in mEq

Figure 34


POTASSIUM CONCENTRATION IN DOG COLON AS A FUNCTION OF TIME
Initial Concentration of K = 40 mEq/L
Initial Concentration of K = 18 mEq/L
Initial Concentration of K = 8.4 mEq/L
mEq/L
cpm/spa_I
K Concentration mEq/L
Time - Minutes

A.F.S.W.P. - 743

PROGRESS REPORT NO. 3

STUDY OF THE POST-IRRADIATION SYNDROME IN HUMANS

Department of the Army
Contract No. DA-49-007-MD-533

Period: April 1, 1954 - March 31, 1955

James J. Nickson, M.D.
Henry J. Koch, Jr., M.D.

Sloan-Kettering Institute for Cancer Research
410 East 68th Street, New York 21, New York

Washington National Record Center
Office of the Army Surgeon General
Record Group 112
Accession #: 64A 2161
Box #: 123
File: Drs. Nickson and Koch MD533 Sloan-Kettering Institute

INTRODUCTION

To date twelve persons have received total body irradiation. Three of these patients have received two courses. One patient is included who received total torso irradiation. Radiation was done with the 1 Mev General Electric generator, HVL=3. 3mm Pb, and was delivered through four ports, upper and lower half of the body, anterior and posterior. The target skin distance in all cases was about 290 cm and the dose rate was between 5 and 7 r/min. In general, coproporphyrin excretion, serum lipoprotein levels, and formed elements of the peripheral blood were measured. In a few instances one of the major studies was not performed.

All persons receiving total body irradiation were patients at the Memorial Center, with widespread cancer. However, no person showing biochemical evidence of renal or hepatic dysfunction was included in the investigative group nor was any person included who showed evidence of bone marrow impairment.

No person included in the series had received x-ray therapy or chemotherapy for at least six weeks prior to the present investigation. Patients were selected who had no complaints referable to their disease process.

The total dose delivered ranged from 20 r to 150 r; all doses were measured in air at the mid-plane of the body. Calculations of volume doses are not yet completed and at best will be approximations because of the varying anterior-posterior diameter from head to foot. Such calculations will be completed before publication of this work.

For purposes of organization, this report will be divided into four parts: 1. Clinical observations. 2. Hematological and Coproporphyrin studies. 3. Lipoprotein studies. 4. Electrolyte studies.

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1. CLINICAL OBSERVATIONS

Patients receiving more than 50 r total body irradiation complained of weakness one to two hours following irradiation. This complaint persisted for the first twenty-four hours to thirty-six hours. It is difficult to know what role the physical and psychic rigors of the irradiation played in the production of this complaint. It is felt that this is not the only reason, because those receiving lower doses did not complain of weakness.

Only three persons experienced any nausea and vomiting. Onset of nausea was within one hour following completion of radiation. Vomiting usually started two to three hours thereafter. In one patient, (C.LeC.), the vomiting subsided spontaneously within eight hours but mild nausea and anorexia persisted for 24 hours. One patient, (M.D.), had rather severe nausea and vomiting for the first 48 to 72 hours post-irradiation. This subsided without medication. The patient again experienced severe nausea and vomiting on the 6th, 7th and 8th days; however, this followed the oral administration of tracer amounts of C^{14} labeled Trytophane, which had produced vomiting on a previous administration. The third patient, (B.C.), had such severe nausea and vomiting that on the third day it was deemed necessary to give Thorazine, 25 mgm, i.m. t.i.d. and, hence, nothing can be said of the subsequent symptomatology.

Six patients developed severe thrombocytopenia. The maximum effect occurring about 28 days post-irradiation (more detailed discussion will be found in the following section). Of these six patients, four developed hemorrhagic tendencies, (J.N., M.H.,* B.C.,* A.MacA.,* M.D., and W.J.*#). It is important to note that the first three of these had subsequent radiation therapy to large ports between the time they received total body irradiation and the time they showed a thrombocytopenia. The latter three patients had received either

*Hemorrhagic tendencies.
#Not otherwise included in this report, having been irradiated so recently that complete data are not available.

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total body irradiation (A.MacA.) or nitrogen mustard therapy two months or more before total body irradiation (M.D. and W.J.). The latter three patients all had normal peripheral blood counts prior to receiving total body irradiation. With the exception of J.N., who received 75 r total body irradiation, all were given 120 r or more.

The incidence of symptomatology (1) and thrombocytopenia (2) in this series is less than that reported amongst those exposed in the Marshall Islands, even though the doses as reported are about comparable. These differences may be due to a real difference in volume dose, the time over which the dose was delivered, or the bone marrow depression from the isotope ingestion. The severe nausea and vomiting reported in this group could conceivably be due to, or be augmented by, the dust ingested.

Two cases of lymphosarcoma (A.MacA. and D.R.) had dramatic regression of their diseases. These were the only cases that showed any change in the neoplastic process.

2. HEMATOLOGY AND COPROPORPHYRIN STUDIES

INTRODUCTION. The effect of ionizing radiation on the formed elements of the blood has been the subject of investigation for many years and the results of studies in this area have formed the principal criteria for radiation damage. From the studies cited in the literature and from our own observations, the earliest evidence of radiation damage would appear to be a decrease in the number of circulating lymphocytes. The blood platelets are next in the order of sensitivity, followed by a decrease in the number of circulating granulocytes. Finally, the red blood cells decrease as the pancytopenic picture develops.

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It would appear that the fall in the numbers of circulating mature cells is secondary to damage to the reticulo-endothelial system and to the bone marrow. Conversely, there is little evidence to support the hypothesis that the decrease is caused by a direct effect on the circulating elements themselves.

RESULTS. From the experiments conducted in this investigation, it is apparent that the time lag between the administration of total body radiation and the fall in the circulating cells is greater in humans than it is in experimental animals (2). The decrease in the white cell count is seldom apparent before the third week post-irradiation. In most cases it does not reach maximal depression before the fourth or fifth week.

The lag in platelet depression is even greater than that seen in the white cells. When it occurs, however, its duration is longer, usually lasting weeks rather than days. Detailed graphs of the hematologic alterations in the thirteen patients studied are presented in Figures 1 to 13. It should be noted that ordinate scales are different on the various graphs.

The long life span of the mature red blood cell is undoubtedly responsible for the very late occurrence of anemia following ionizing irradiation. Unless total aplasia of the red cell precursors takes place, there must be a lapse of one red cell half-life (60 days) before obvious clinical anemia could be expected to occur.

Previous radiation insult to the bone marrow, even in the presence of a normal hematological picture, is a real hazard to the individual exposed to total body radiation. Several of the patients studied in this group had received local x-ray therapy or had been exposed to so-called "radiomimetic agents" such as nitrogen mustard or triethylene melamine prior to total body

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irradiation. Although all of them had a normal peripheral blood and bone marrow picture prior to exposure, their response to the dosage administered was definitely exaggerated both in duration and extent.

This danger cannot be too strongly emphasized since we have no objective criterion to date by means of which we can predict the degree of hematological response in patients who have received previous irradiation.

INTRODUCTION. In an effort to find a more sensitive index of radiation effect than the formed elements of the blood, a study of the response of the precursors of hemoglobin to ionizing radiation was begun in our laboratory several years ago. During the course of this study it was found that the excretion of coproporphyrin in the urine and in the feces was increased following therapeutic dose levels of isotopic or local x-radiation(3). This increase occurred at dosage levels which did not produce a change in the formed elements of the blood.

The present study was begun to evaluate this observation more critically and to quantitate, if possible, the radiation dosage in terms of coproporphyrin excretion.

It is not the intention of this report to give a detailed description of the metabolism of the porphyrins. However, a short discussion of the synthesis of protoporphyrin IX is essential to the understanding of the possible mechanisms by which the observed increase in coproporphyrin excretion is produced.

From *in vitro* and *in vivo* experiments it has been shown that the synthesis of the porphyrin ring and the introduction of the prosthetic groups which characterize the specific porphyrins follow immutable and irreversable lines.

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In the process of synthesizing heme Fisher[4] found that he could remove carboxyl groups from uroporphyrin to produce coproporphyrin but that the reverse reaction of recarboxylation was not possible. Similarly, to produce heme, two carboxyl groups are removed from the coproporphyrin ring before the introduction of iron. The reverse of this process has been an attractive hypothesis for the explanation of the presence of the coproporphyrin stereoisomer III during the degradation of hemoglobin after hemolysis. The work of Lemberg[5] has conclusively shown, however, that the porphyrin ring of the hemoglobin molecule is opened in the alpha position before it loses its iron. Thus, an increase in coproporphyrin excretion following hemolysis cannot be explained in this way.

RESULTS. Two independent and physiologically dissimilar hypotheses may be considered as possible etiological agents in the increased coproporphyrin excretion which follows ionizing irradiation.

The first is the so-called dual hypothesis of Fisher[4] which states that for every moiety of porphyrin stereoisomer III formed, a small but definite quantity of stereoisomer I is concomitantly synthesized. According to this theory, the excretion of coproporphyrin I is in direct proportion to the amount of hemoglobin produced. Therefore, anything which will increase the synthesis of hemoglobin will at the same time increase the production and consequent excretion of coproporphyrin I. Dobriner[5] confirmed this hypothesis in his experiments with phenylhydrazine hemolysis in dogs when he observed an increase in coproporphyrin excretion occurring during the recovery period when the reticulocyte count was at its maximum.

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From the present studies it would not appear that this factor is responsible for the observed increase in coproporphyrin excretion. There has been no correlation between reticulocyte rise and increase in coproporphyrin excretion in the patients who have received total body irradiation. Nor has any other evidence of increased hemoglobin anabolism been observed.

A second possible mechanism for the increased coproporphyrin excretion following irradiation can be seen in the intermediary metabolism of protoporphyrin. Should the reaction:

Coproporphyrin III----------Protoporphyrin IX
decarboxylase

be adversely influenced by ionizing irradiation, the production of hemoglobin would decrease and an excess of coproporphyrin III followed by increased excretion of this porphyrin would result. Moreover, the increase in coproporphyrin excretion would take place during the period of maximal radiation effect rather than during the recovery phase.

It is this second mechanism which is considered to be responsible for the increased coproporphyrin excretion observed in these studies.

3. LIPOPROTEIN STUDIES

REASON FOR STUDY. In rabbits receiving severe whole body irradiation, an early increase in the serum turbidity (caused by chylomicrons) is prognostic of lethal damage. Detailed lipoprotein analyses of such sera have shown that at this time, one to two days after the irradiation, there is a block in the serial conversion of larger to smaller lipoproteins which is a part of the normal fat transport system(7). Since heparin is involved in the "clearing factor" mechanism which is responsible for this conversion, heparin deficiency has been suggested.

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Changes in lipid transport following severe whole body irradiation have also been observed in dogs(8). It therefore seemed important to determine the effect of therapeutic doses on the serum lipoproteins of patients.

METHODS. Fasting blood samples are taken before irradiation and one, three, and six days afterward. Additional samples are taken after further intervals of time, whenever it is considered necessary. The serum is checked for turbidity in the spectrophotometer at 65 mu, and stored at 4°C. with the addition of 1:10,000 methiolate for not more than two weeks. For the separation of the lipoproteins 7.05 ml. of serum are mixed with 3.45 ml. of NaCl (density 1.1855) to give a solvent density of 1.063. The solutions are then run in the preparative ultracentrifuge for 16 hours at 40,000 R.P.M. at about 5°C. The lipoproteins of density less than 1.063 which are concentrated in the top layer, are collected with a hypodermic needle and specially designed syringe(9), and transferred to a 5 ml. graduate. The volume is recorded and the lipoprotein content measured from the size of the flotation boundaries obtained in the analytical ultracentrifuge by the Gofman technique. The lipoprotein spectrum is separated into 3 fractions, Sf 2 - 10, Sf 11 - 20, and Sf 21 - 100. Occasionally a fourth component, Sf 100 - 400, is observed. The calculations are made by the procedure described in 1952(10). The total cholesterol is determined(11) in the whole serum and in the lipoproteins of density greater than 1.063, (found in the lower layers of the centrifuge tubes), and the "low-density lipoprotein cholesterol" calculated by difference. The calculated low-density lipoprotein cholesterol levels have usually been fairly close to one-third the total low-density lipoproteins measured in the ultracentrifuge. This agrees with the known chemical composition of these lipoproteins.

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It has therefore not seemed necessary to measure the low-density cholesterol directly, since this would necessitate taking considerably larger samples of blood.

RESULTS. The results obtained on the individual patients are shown in Figures 14 through 27. In two patients, (V.M., 120 r, and Y.D., 20 r), neither of whom showed clinical signs of radiation damage, the lipoprotein levels remained constant within the errors of the method. Four patients, (J.N., O.D., B.C. and M.D.), showed early changes indicative of increased clearing factor activity, and two patients, (A.MacA., and C.LeC.), showed decreased activity. No distinction could be made either on the sex of the patient or the nature of his disease.

In five patients, (M.H., J.N., A.MacA., B.C., and M.D.), lipoprotein analyses were made at a time when clinical signs of radiation damage were present. Four had some elevation in clearing activity. There was no correlation with the direction or extent of the lipoprotein changes seen immediately after the initial treatment. This was not to be expected, since most of these patients had received additional radiation after day 6 and the development of clinical symptoms.

DISCUSSION. The changes in serum lipoproteins which appear after therapeutic doses of irradiation are of course not as clear cut as the changes seen in animals after lethal doses. In some patients clearing activity appeared to have decreased on the third day following treatment, while in others an apparent increase was seen. Since the circulating lipoproteins represent the balance between lipids added to and removed from the circulation, it would appear that the doses used in these patients can cause this balance to swing

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sometimes one way and sometimes the other. In rabbits receiving lethal doses of radiation the lipid transport system is usually inadequate for its load[3]. Whether this is the result of a deficiency in the transport or an overloading of the system is still unknown. Atherosclerosis is produced in rabbits by feeding massive doses of cholesterol to these normally herbiverous animals. Also, the plasma lipoproteins of the dog differ greatly from those of the human, the dog having a much larger proportion of the lipid present as alpha lipoprotein. The many lipid and lipoprotein studies now being carried out on animals in connection with studies of atherosclerosis are therefore frequently criticized as not being applicable to human physiology. Since the same criticism might be leveled at studies of irradiation effects on the lipoprotein metabolism of animals, one valuable result of the present study is the observation that the lipoprotein changes found in humans soon after irradiation may be similar to those seen in animals.

The second observation of importance is that almost every patient who showed hemorrhagic tendencies and a thrombocytopenia had an increase in clearing factor activity at that time. All patients who had bleeding tendencies had thrombocytopenia of sufficient severity to account for the hemorrhagic manifestations observed. If the increased clearing factor activity seen in these patients is a result of a hyperheparinemia, it reinforces the suggestion that post-irradiation hemorrhage may be the result of an increase in circulating heparin in addition to, or associated with, the thrombocytopenia.

4. ELECTROLYTE STUDIES. Electrolyte transfers across the lumen of the bowel have been measured for various levels of the dog bowel.

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EXPERIMENTAL METHODS. Chronic Thiry-Vella loops[12] of the appropriate segment of bowel were surgically prepared. Jejunal and ileal segments were 30 cm. in length and were taken from the mid-portion of the respective regions. Colon loops were about 15 cm. in length. The length was determined by selecting a segment which was drained by one major vessel. The ends of the loop were then anastomosed to the skin as fistula sites. The continuity of the remaining bowel was established by end to end anastomosis.

All experiments were performed under intravenous pentothal anesthesia, 5 mg./kg. of body wt. In all experiments #24 Foley catheters were introduced into each end of the isolated loop and the balloons sufficiently inflated so as to prevent leakage of any fluids introduced into the lumen of the gut, but not to a degree which might compromise the blood supply of the segment. With this arrangement solutions could be introduced at one end and withdrawn or collected at the other end in a controlled manner.

Sodium concentration in the collected samples was determined by means of a standard flame photometer[13] or on a NaI scintillation counter when Na^{24} was used. Chloride determinations were according to Van Slyke's modification of the Sendroy method[14] and CO_2 was measured in a Van Slyke apparatus[15].

Na^{24} and K^{42} were utilized to measure directional shift of sodium or potassium into the perfusate from the plasma or the disappearance of the ion from the perfusate. Solutions used for perfusion were isotonic $MgSO_4$, Polyvynalpyrolidon (PVP), or mixtures of electrolytes of varying concentrations maintained at isotonicity by $MgSO_4$ and $MgCl_2$.

Irradiations were done using 250 kv x-rays (HVL 2.0 mm cu.). Loops were localized with barium perfusion and lateral and anterior-posterior diagnostic

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films. Each irradiation was so designed as to include the entire isolated loop but to keep the irradiation to the normal bowel at a minimum. This was done so that there would be no radiation-induced vomiting or diarrhea to alter the plasma electrolyte balance.

RESULTS AND DISCUSSION. The mechanisms of transport of electrolytes in the jejunum and ileum are qualitatively the same though quantitatively different, and hence results of the experiments will be discussed together.

If isotonic or hypertonic $MgSO_4$ is introduced into the lumen and is sampled as a function of time the electrolyte concentrations are found to increase reaching a constant and characteristic level. In the jejunum this level is between 130 and 140 meq/L and is essentially equal to the plasma concentrations. Chloride parallels the sodium and exceeds plasma levels of chloride. Equilibrium is reached in about 90 minutes as is shown in Figure 23. A similar rise is seen in the ileum with Na levels reaching approximately 120 meq/L and the Cl rising to only one-half the sodium value. Bicarbonate is the other cation present.

The exponential form would suggest that the rate of Na shift into the bowel is constant and that the transfer from the lumen to the plasma is proportional to the concentration of sodium in the lumen[16,17,18]. Such an analysis gives rise to the equation:

$$Na = \frac{K_1}{K_2} (1 - e - K_2 t)$$

where: Na = Sodium concentration in the lumen at any time, t.

K_1 = rate of Na shift into the lumen.

K_2 = rate of Na shift out of the lumen.

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The value of K_1 can be obtained from the slope at the origin and the value of K_2 then calculated from the equilibrium concentration. The theoretical curve obtained from such a calculation is also shown in Figure 27.

To verify the dynamic nature of this equilibrium, a mixture of electrolytes of composition identical with the equilibrium mixture was introduced into the lumen. The rate of appearance of Na^{24} was measured and the meq. of plasma sodium which this activity represented was calculated from the plasma specific activity. Results of a typical jejunal and ileal experiment are shown in Figures 28 and 29. Also shown in Figure 29 is the calculated curve and the slope of the origin is indicated.

The isolated loop may be perfused with $MgSO_4$ or other solutions of salt mixtures at varying perfusion rates. In both jejunum (Fig. 30) and ileum (Fig. 31) the concentration of electrolytes in the collected perfusate decreases with increasing perfusion rate. If the concentration is plotted as a function of the reciprocal of the perfusion rate (min./cc) a linearly increasing relation is obtained indicating that the concentration of electrolytes in the collected perfusate is directly proportional to the time which the perfusate is in contact with the bowel wall.

Dogs were given Na^{24} twenty-four hours before experiments were performed to permit equilibration of the tracer with the extracellular sodium. Loops were then perfused at a constant rate (1.58 cc/min.) with solutions containing varying concentrations of sodium. The tonicity was kept constant with the appropriate amount of $MgSO_4$. Total sodium (Na_i) put into the lumen could be calculated from the concentration, as measured on the flame photometer, times the volume perfused. Similarly the total sodium in the collected perfusate

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(Na_o) may be calculated from the flame photometer measurement of the concentration. The total plasma sodium shifted into the lumen (Na_{P-L}) may be obtained from the CPM in the collected perfusate divided by the plasma specific activity.

Clearly, the total sodium in the collected perfusate is equal to the total sodium perfused plus the amount shifted into the lumen from the plasma minus whatever sodium was shifted back into the plasma from the lumen (Na_{L-P}). Hence, we obtain the equation:

$$Na_o = Na_i - Na_{L-P} \quad Na_{P-L}$$

where:

Na_o = total sodium in the collected perfusate

Na_i = total sodium in perfusing solution

Na_{L-P} = total sodium shifted from lumen to the plasma

Na_{P-L} = total sodium shifted from plasma to the lumen

All quantities in this equation can be measured directly except for Na_{L-P}. The equation may be solved for this quantity, and hence the shifts of sodium in and out of the lumen can be measured. Figures 32 and 33 show that in both jejunum and ileum the transfer of sodium from the lumen to the plasma is directly proportional to the concentration of sodium within the lumen, and the shift of sodium from body stores into the lumen is independent of the concentration within the bowel.

Electrolyte handling in the colon is markedly different, in that concentration gradients can be maintained across the colonic mucosa[19]. The most striking feature of the colon is the large water absorption when isotonic solutions are introduced.

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Isotonic solutions containing varying concentrations of potassium tagged with K^{42} were introduced into the isolated loop and sampled as a function of time. The counts per minute of any sample when divided by the initial specific activity are a measure of the meq/L of initial potassium remaining. The total potassium could be measured on the flame photometer. In all experiments the total potassium rose slightly regardless of the concentration which was introduced and the potassium which was introduced fell slightly (Fig. 34). Using calculations similar to those outlined above, the total potassium shift can be measured. In all cases the total potassium shifting from the colon into the plasma was found to be proportional to the concentration of potassium instilled. The techniques do not permit any statement to be made about the plasma to lumen transfer. Because of the marked water absorption, better methods of measuring electrolyte transfer must be worked out.

Because of the extensive work which was required to develop techniques for the study of the normal physiology of the intestine, only preliminary radiation studies have been done. Isolated loops of jejunum and ileum receiving doses of 500 and 1000 r have shown no qualitative changes in electrolyte transfer mechanisms. Quantitative differences do exist. No definitive statement can be made at this time as to the magnitude of these changes. Moreover at higher doses, where extensive damage is done to the gut epithelium, breakdown of transport mechanism may occur.

SUMMARY AND CONCLUSIONS

1. Thirteen patients have been studied following total body irradiation with doses ranging from 20 -150 r using alterations in the formed elements of the blood, coproporphyrin excretion in the urine, and changes in the serum lipoprotein levels as objective criteria.

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2. There was a decrease in the formed elements of the blood with the time of fall being in the order of lymphocytes, platelets, and granulocytes. The maximum depression occurred at about the fourth week after irradiation in contrast to the experimental animal data which show a maximal depression in the formed elements of the blood at two weeks. The degree of depression was more severe in patients who had received previous total body irradiation or "radiomimetic" drugs. It is felt that this is of considerable importance in the military setting and that persons who have received one exposure in the range of 100 r should not be re-exposed within at least 6-8 weeks. This is not a conservative estimate and further investigation along this line will be pursued.

3. Increased coproporphyrin excretion in the urine has not been a systematic finding. When it does occur the increase occurs within the first few days and in some instances within the first few hours. In general, even though the level of excretion in any 24 hour period did not exceed the upper limit of normal, the integral excretion post-irradiation was increased. It is felt that radiation damage blocks the decarboxylation of coproporphyrin III to protoporphyrin IX with a subsequent increase in the excretion of coproporphyrin III.

4. It is probable that the observations on serum lipoprotein levels in animals following total body irradiation may be carried over to man though the time scales may be different. Almost every patient who developed a thrombocytopenia associated with a bleeding tendency showed an increase in clearing factor at that time.

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5. The rate of transport of electrolytes into the lumen of the dog bowel is constant and independent of the concentration within the lumen. The rate of absorption from the lumen is proportional to the concentration within the lumen in the jejunum and ileum. Maximal activity in the colon is associated with water absorption. Preliminary studies would suggest that irradiation alters these mechanisms qualitatively but not quantitatively.

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REFERENCES

1) Cronkite, E.P., Shulman, N.R., Sandhaus, C.A., Sharp, R.A., and Bond, V.P.
Rad. Res. Soc. May, 1955 (Abstract)

2) Bond, V.P., Cronkite, E.P., Farr, R.S., and Hechter, H.H.
Rad. Res. Soc. May, 1955 (Abstract)

3) Koch, H.J., et al
J. Clin. Endocr. 10, 1950

4) Fisher, H., and Stern, A.
Die Chemie Des Pyrrols., Ann Arbor, 1943

5) Lemberg, R.
Biochem. J., 29, 1322, 1935

6) Dobriner, K., and Rhoads, C.P.
Physiol. Rev., 20, 1940

7) Hewitt, J.E., Hayes, T.L., Gofman, J.W., Jones, H.B., and Pierce, F.T.
Am. J. Physiol. 172:579, 1953

8) Goldwater, W.H., and Entenmann, C.
Am. Chemical Society, March, 1953.

9) Barclay, M., Cogin, G.E., Escher, G.C., Kaufman, R.J., Kidder, E.D. and Petermann, M.L.
Cancer 8:253, 1955

10) A Symposium. Technical Group, Committee on Lipoproteins and Atherosclerosis
National Advisory Heart Council, 1952

11) Abell, L.D., Levy, B.B., Brodie, B.B. and Kendall, F.E.
J. Biol. Chem. 195:357, 1952

12) Vella, L.
Bull. d Sc. Med. Bologna, 7:441, 1881

13) Overman, R.R. and Davis, A.K.
J. Biol. Chem., 168:641, 1947

14) Peters, J. and Van Slyke, D.D.
Quantative Clinical Chemistry, Baltimore, Vol. 2, 1943, p. 839

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Accession #: 64A 2161
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REFERENCES (concluded)

15) Van Slyke, D.D. and Neill, J.M.
J. Biol. Chem., 61:523, 1924

16) Visscher, M.B., Vanco, R.H., Can, C.W., Dean, R.B. and Erickson, D.
Am. J. Physiol. 142:550, 1944

17) Visscher, M.B., Fetcher, E.S., Can, C.W., Gregor, H.P., Bushey, M.S., and Barker, D.E.
Am. J. Physiol. 142:550, 1944

18) Visscher, M.B.
Ann. N. Y. Acad. Sci., 57:291, 1953

19) D'Agostino, A., Leadbetter, W.F. and Schwartz, W.B.
J.C.I., 32:444,1953

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Figure 1

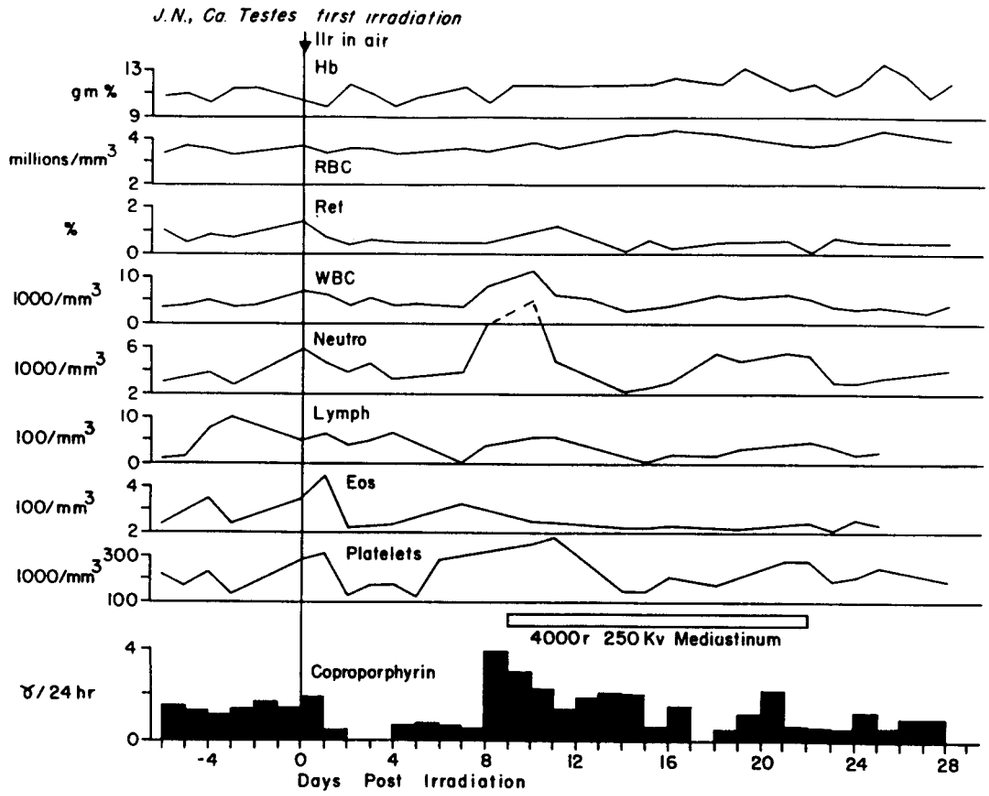

Figure 2

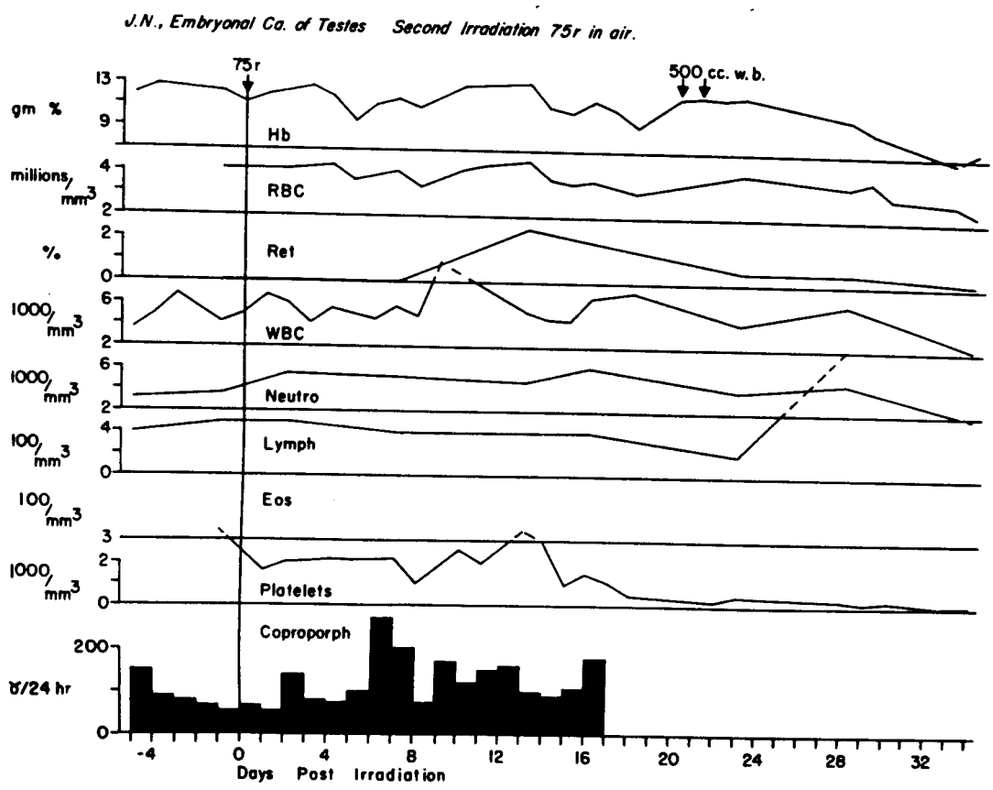

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Figure 3

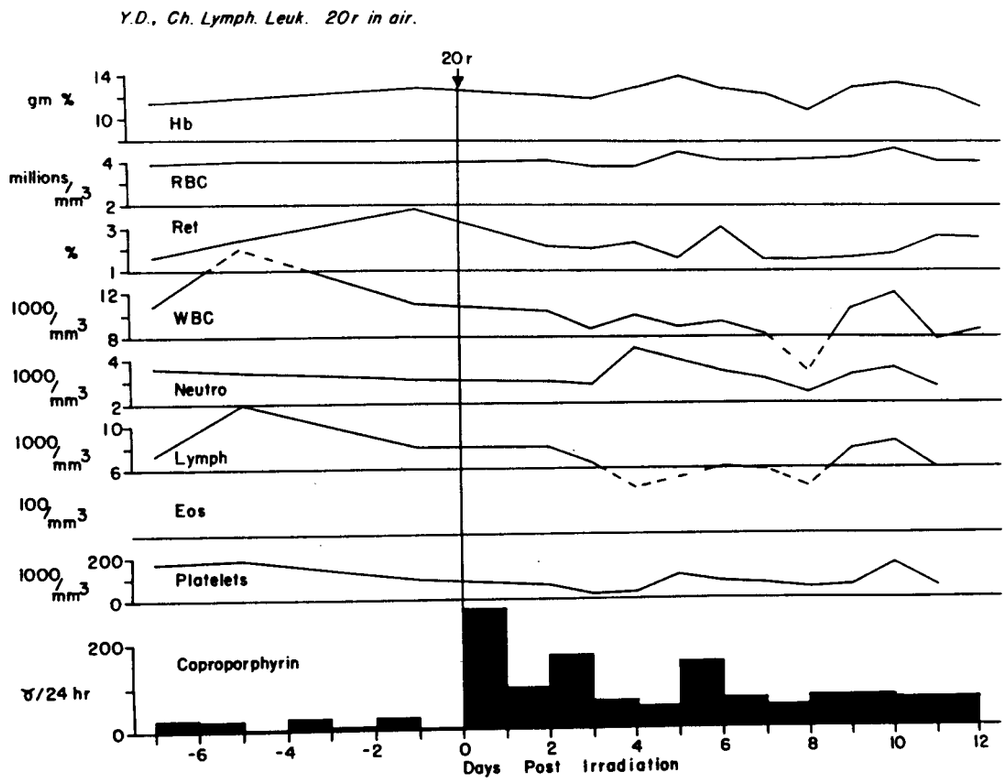

Figure 4

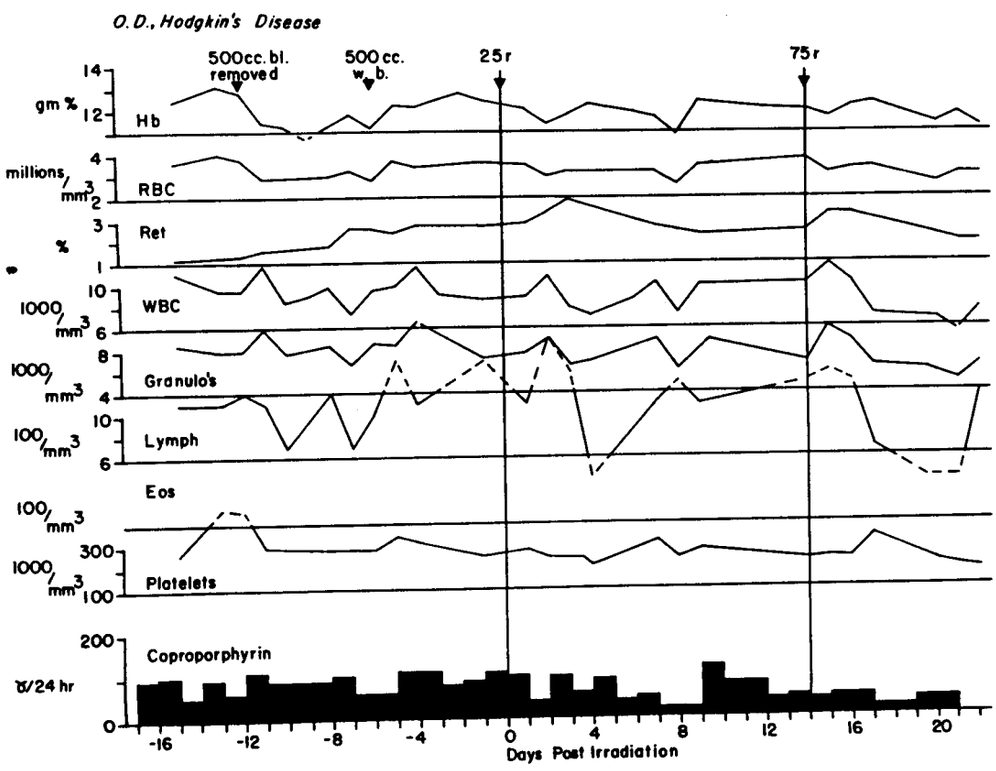

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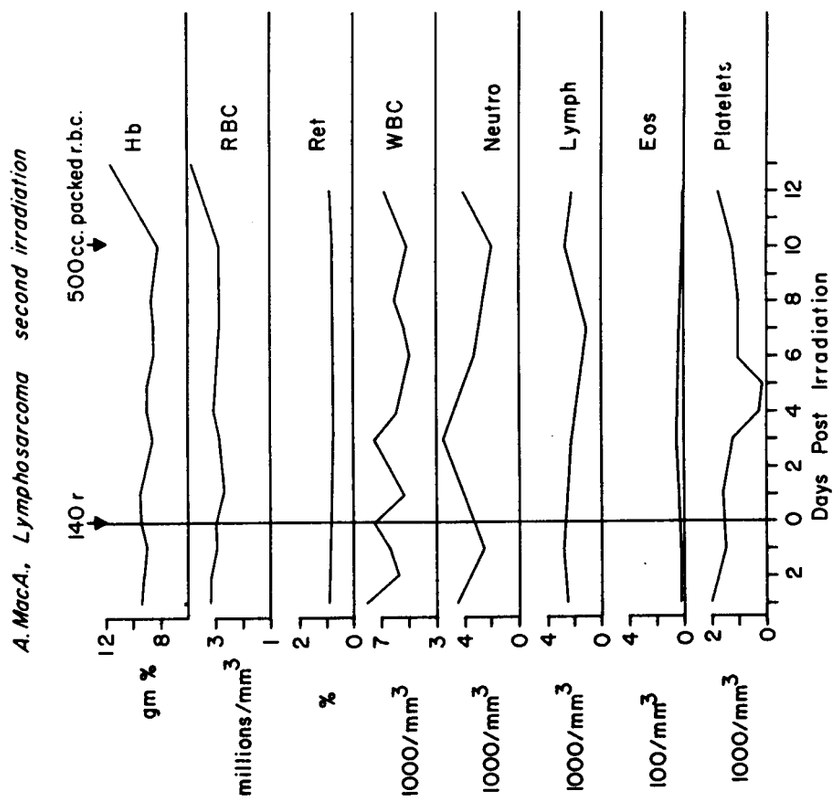
Figure 5



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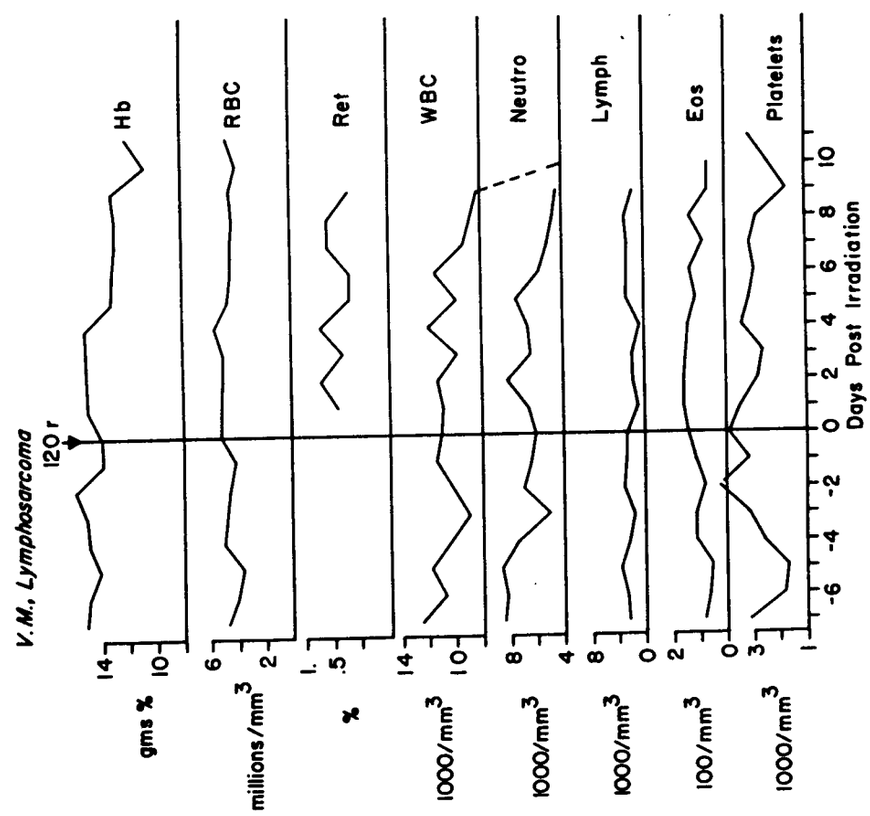

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Figure 7

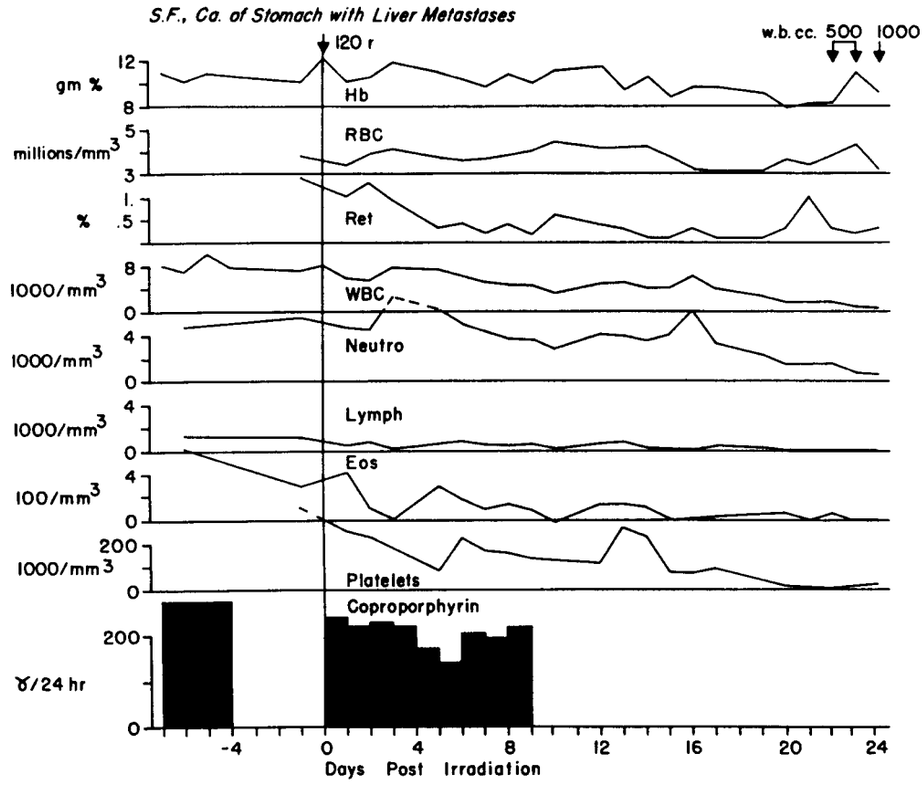

Figure 8

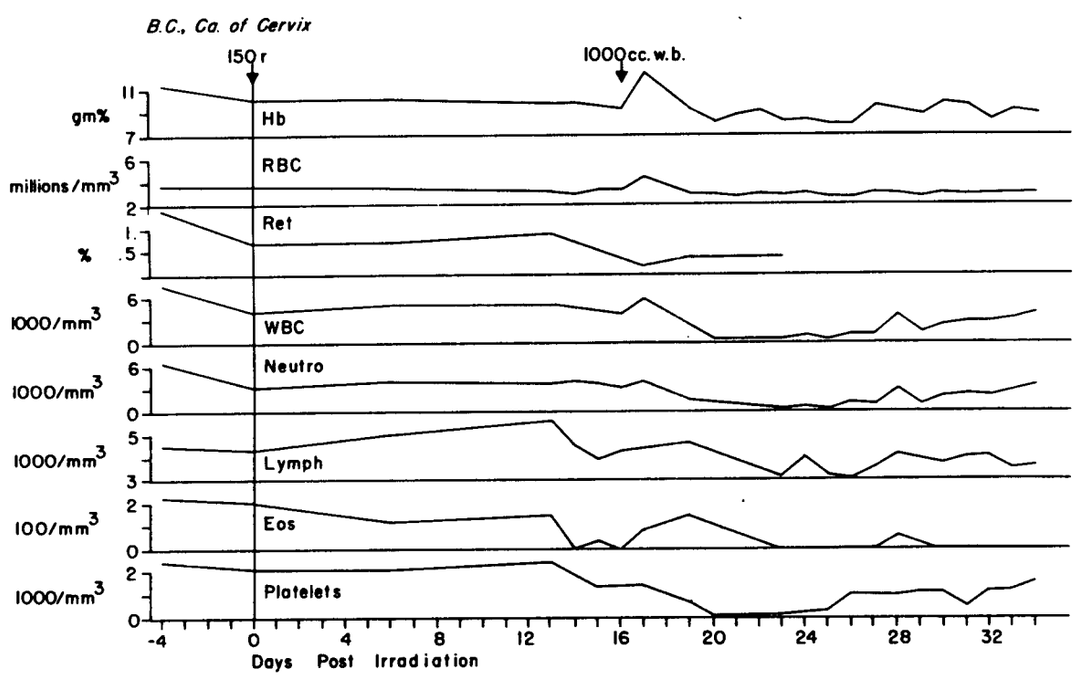

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Figure 9

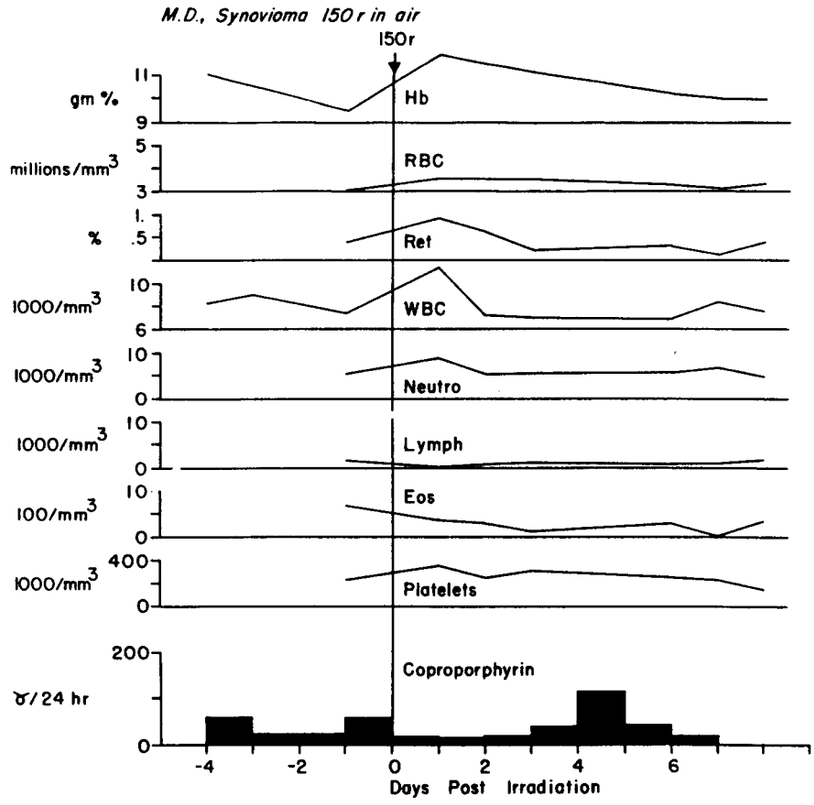

Figure 10

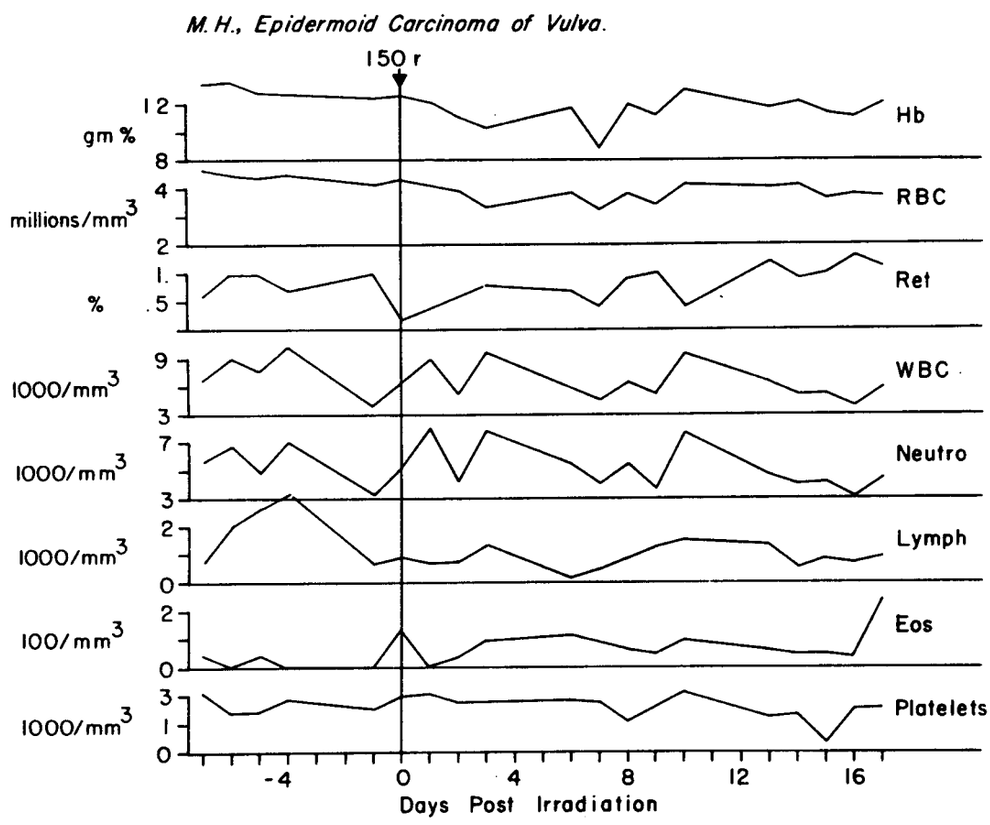

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Figure 11

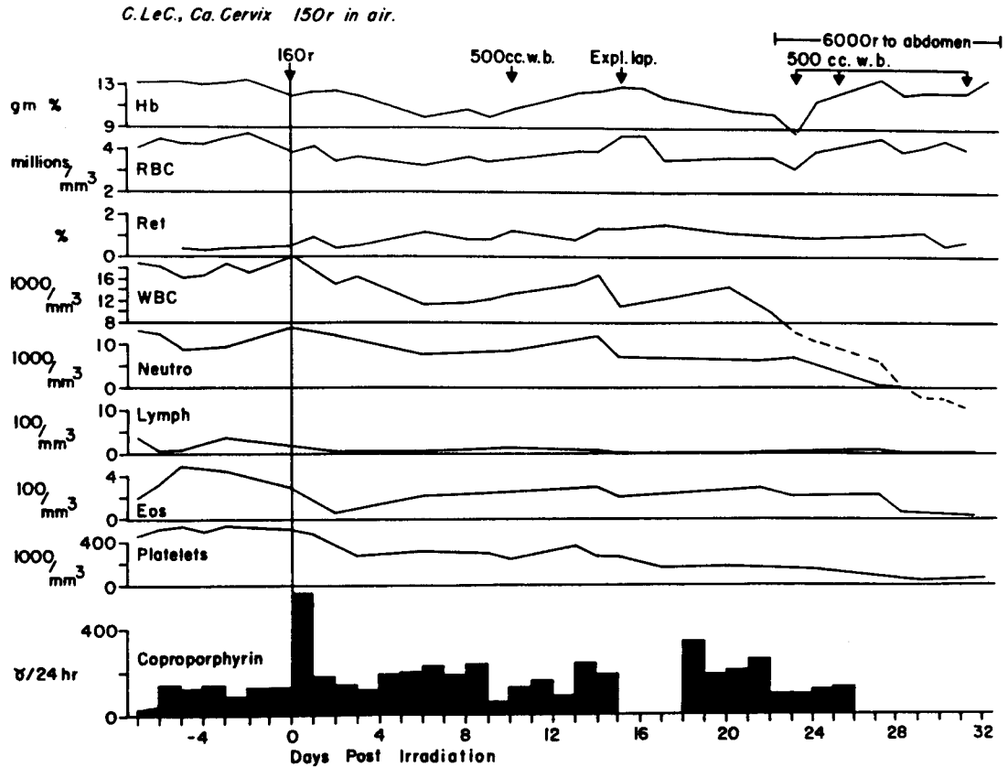

Figure 12

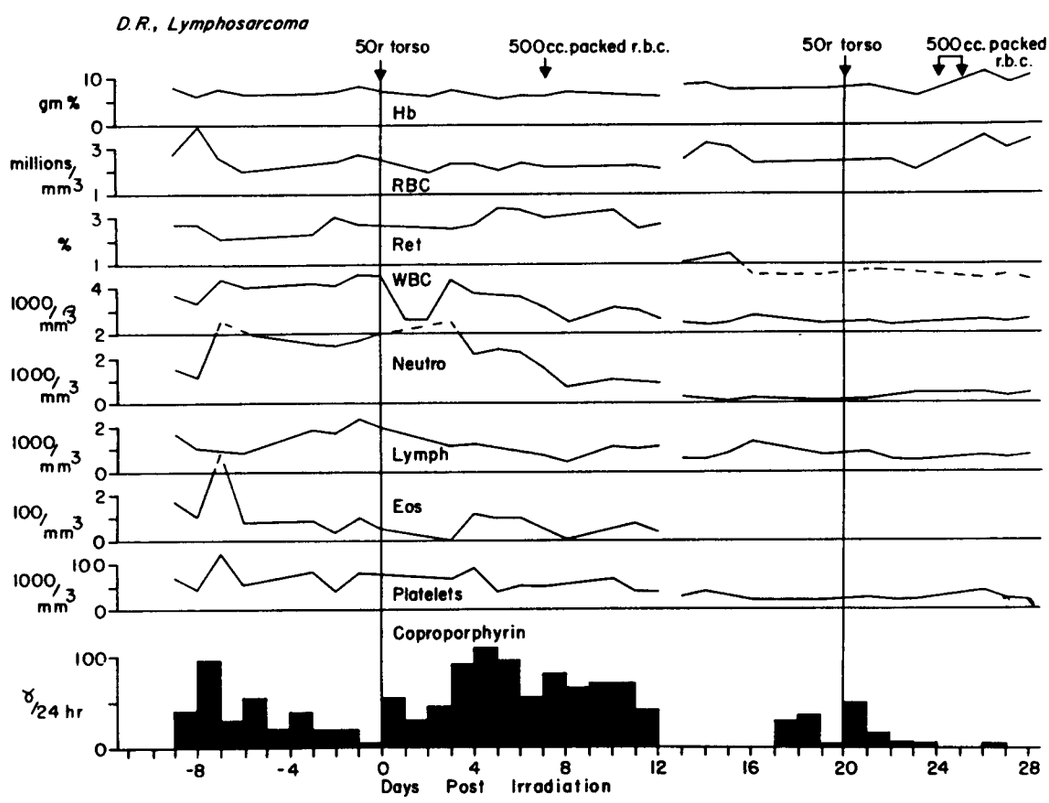

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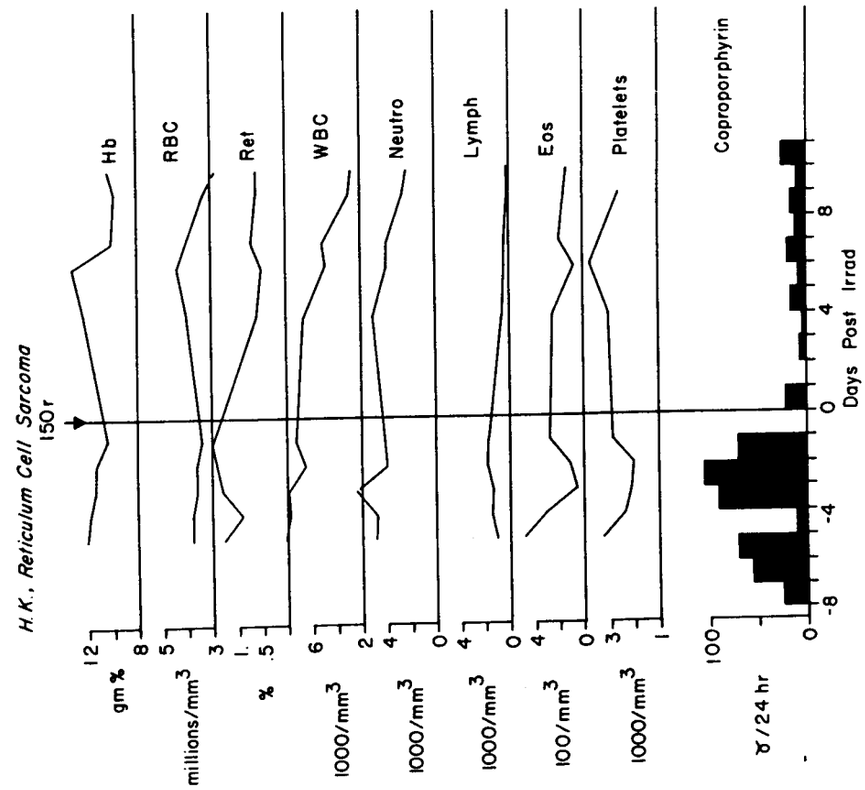
Figure 13



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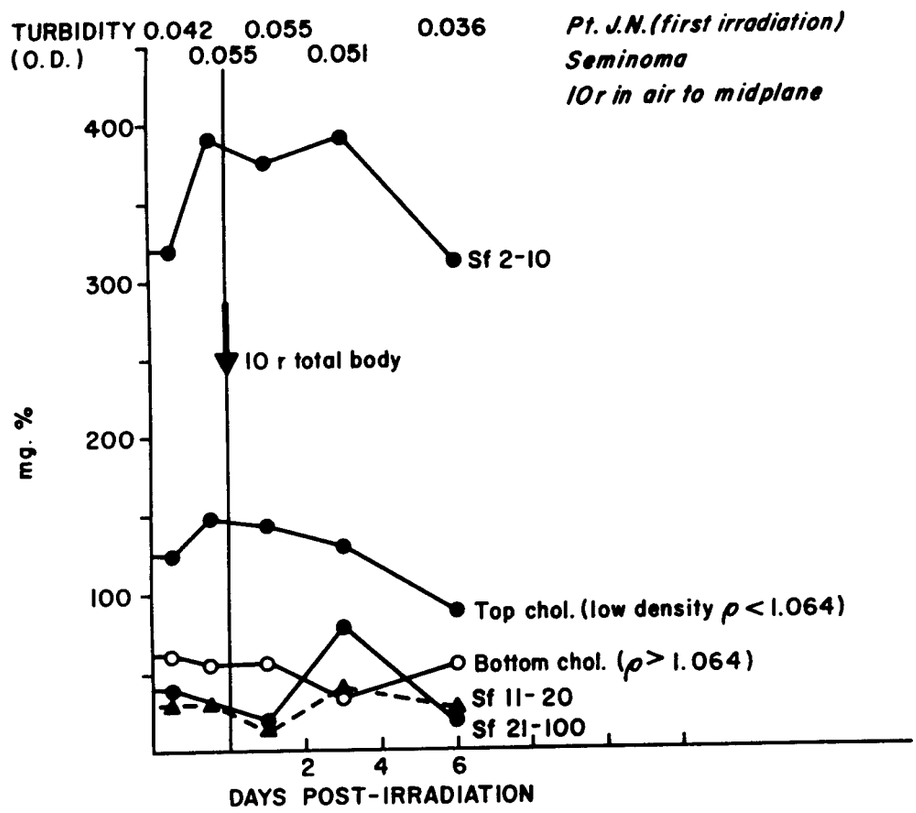

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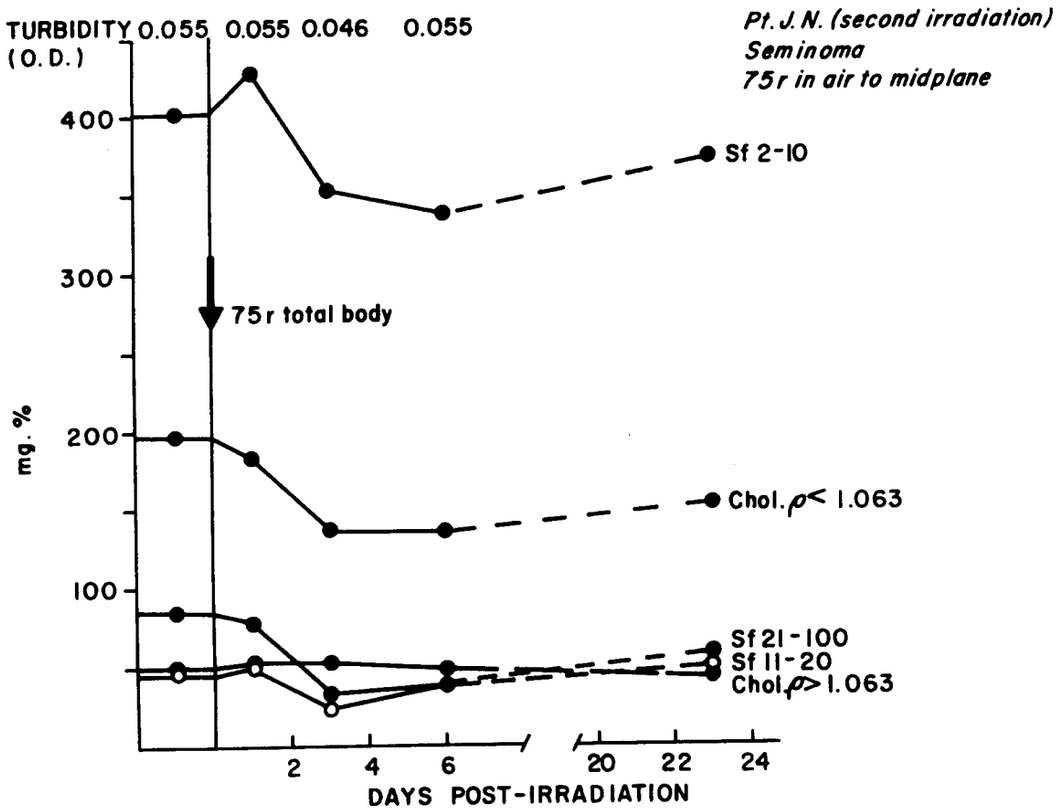
Figure 15



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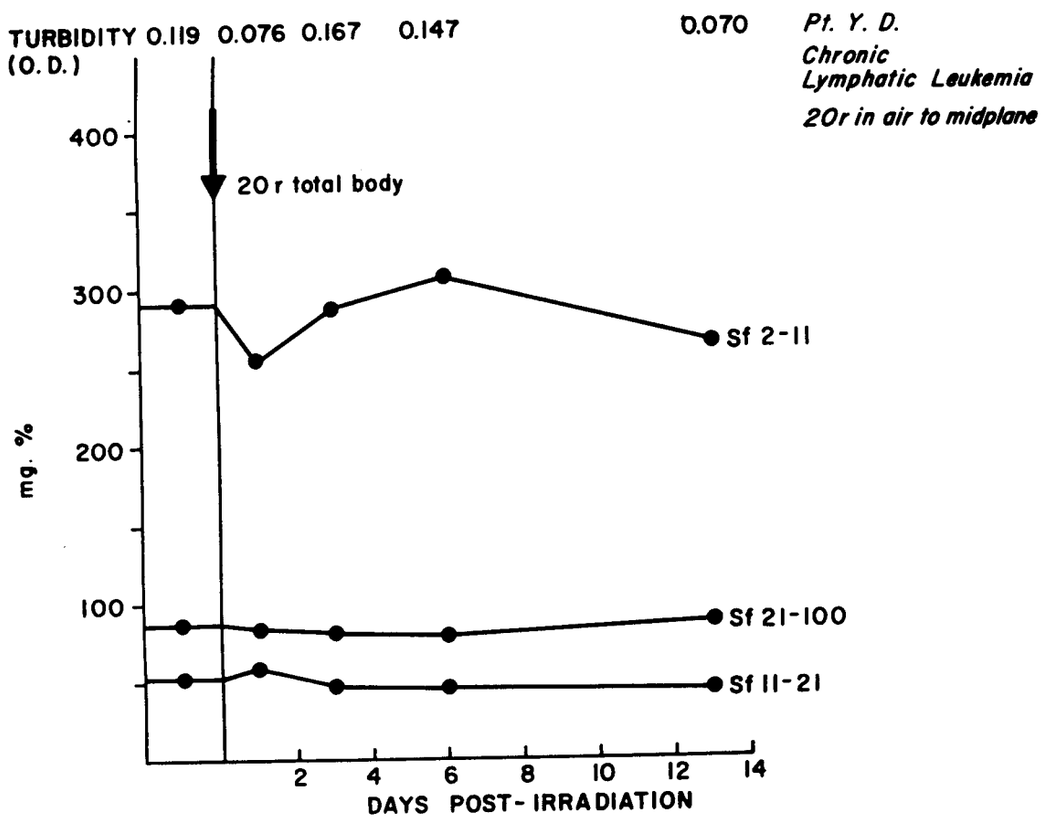

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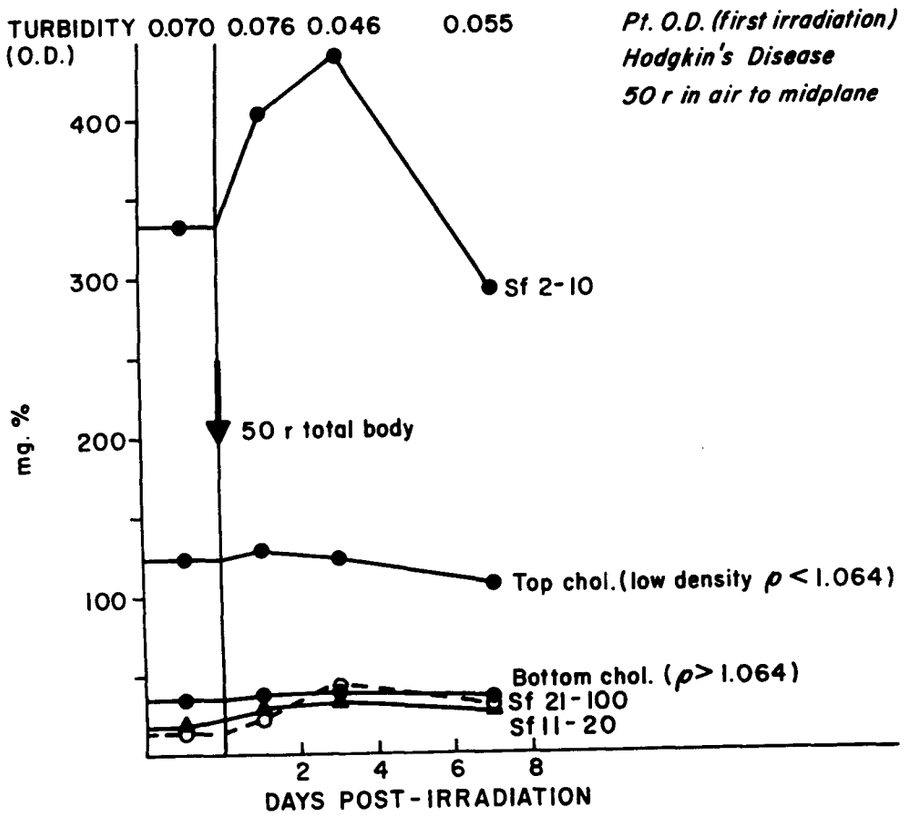
Figure 17



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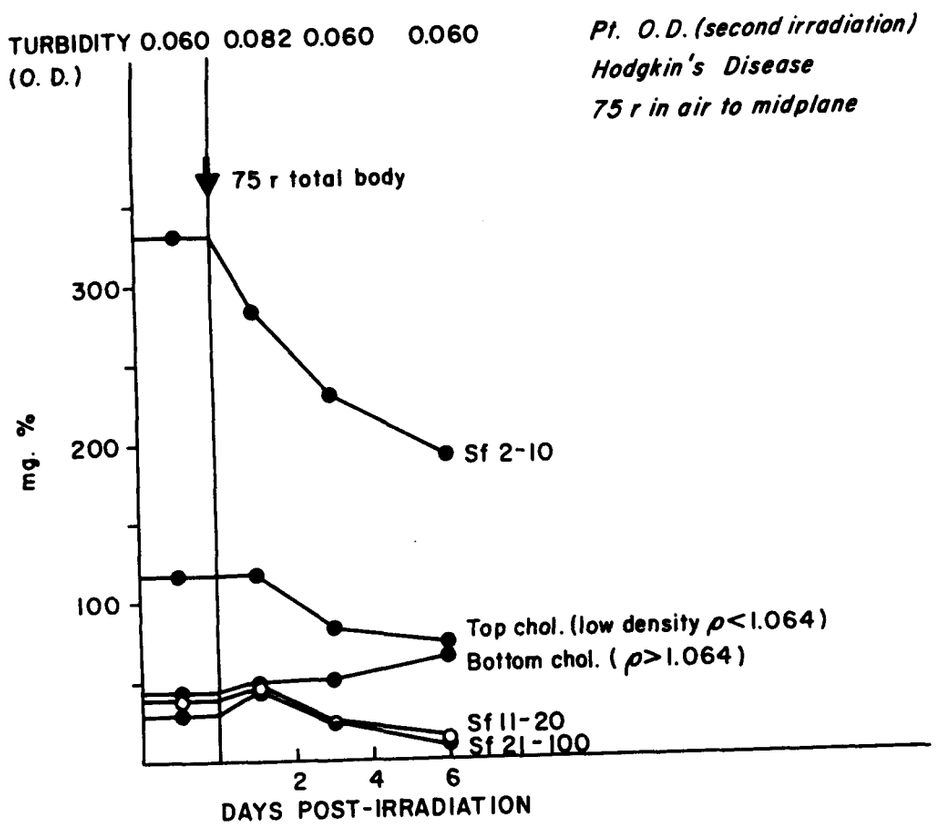

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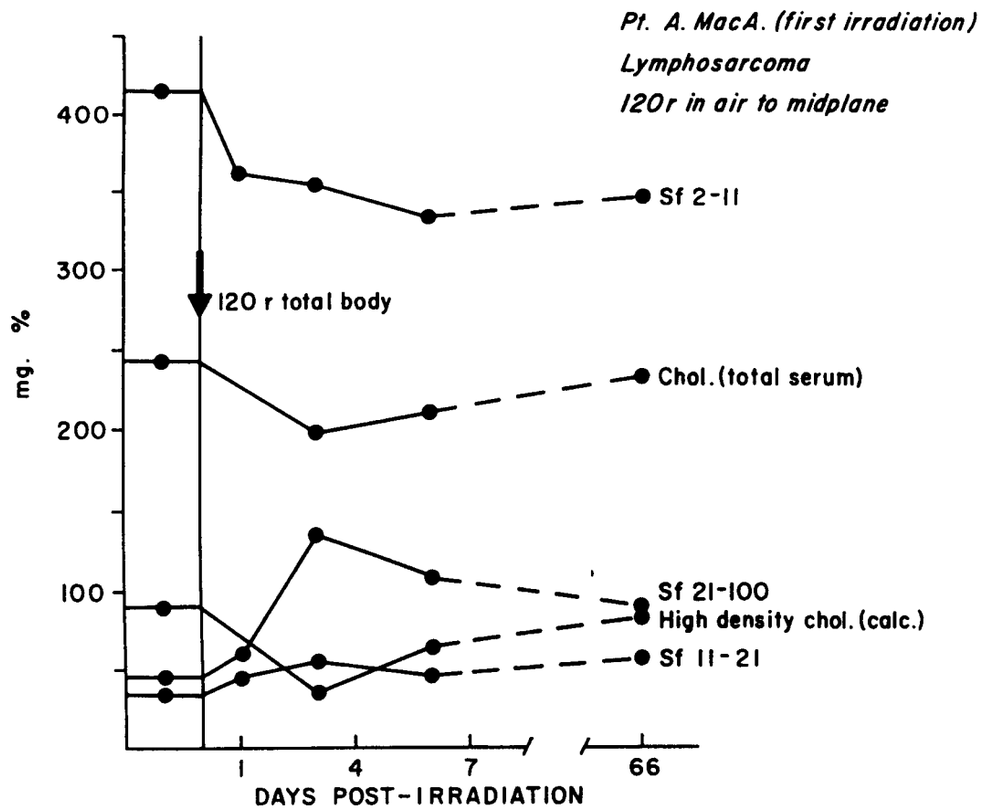
Figure 19



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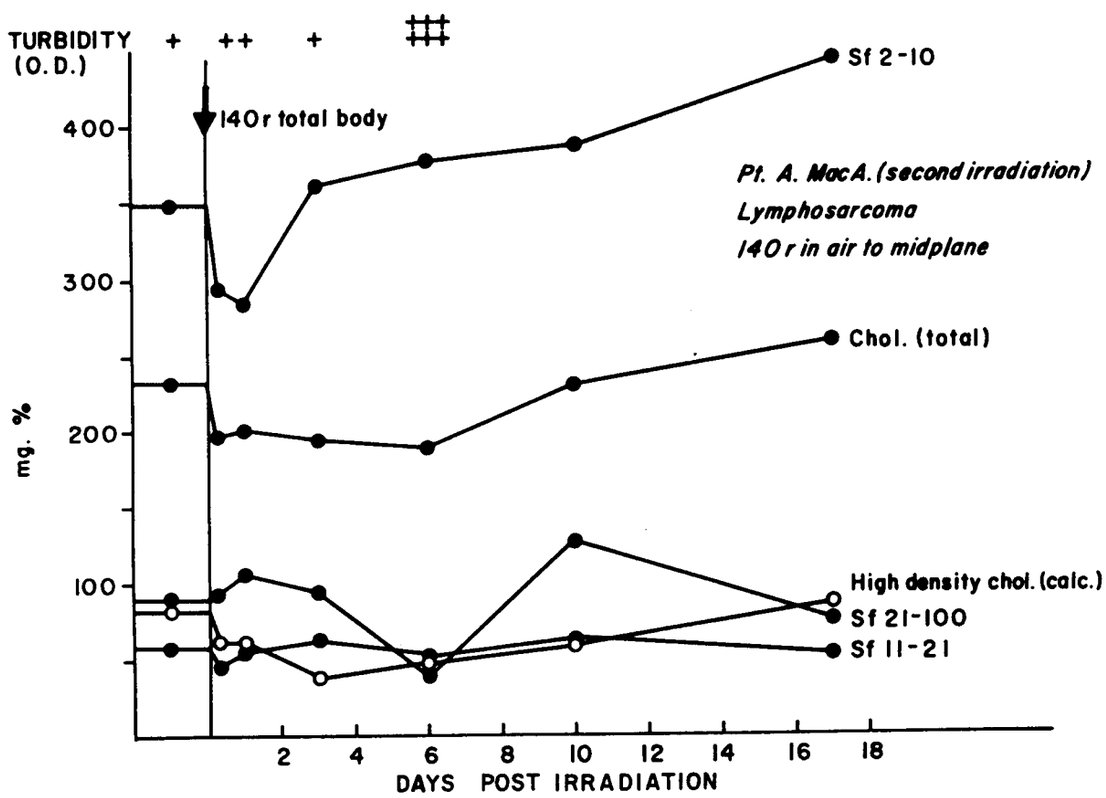

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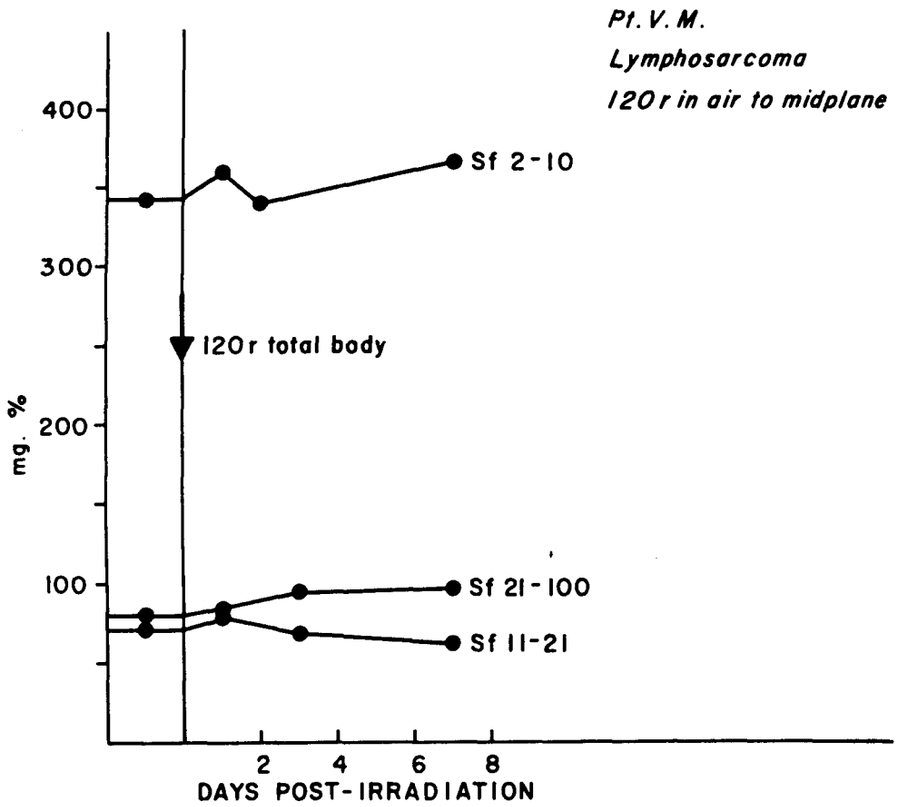
Figure 21



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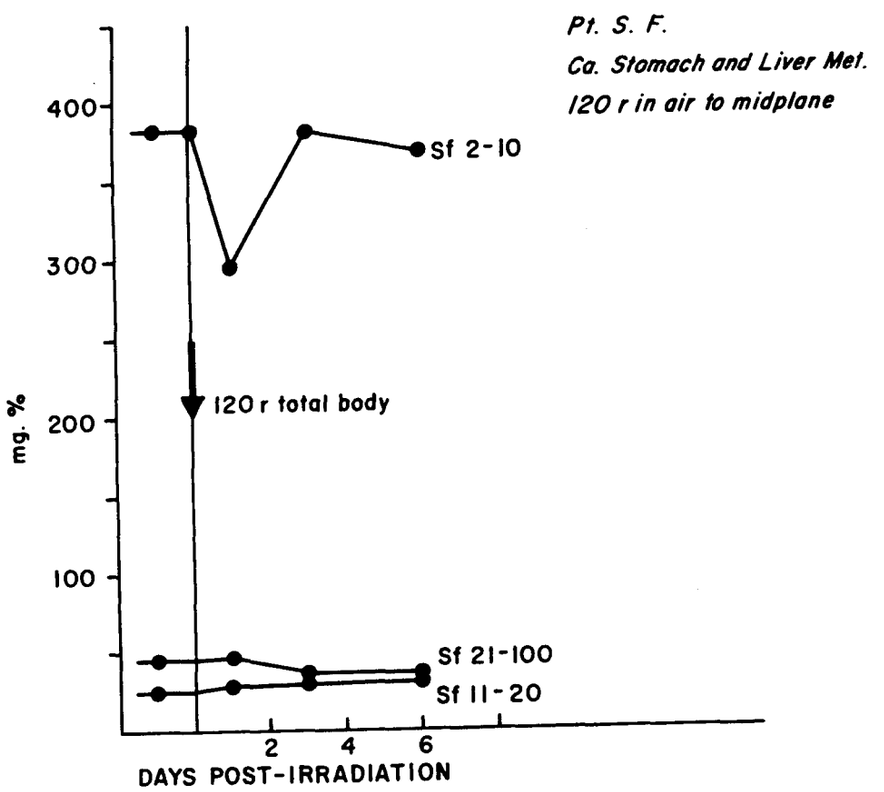

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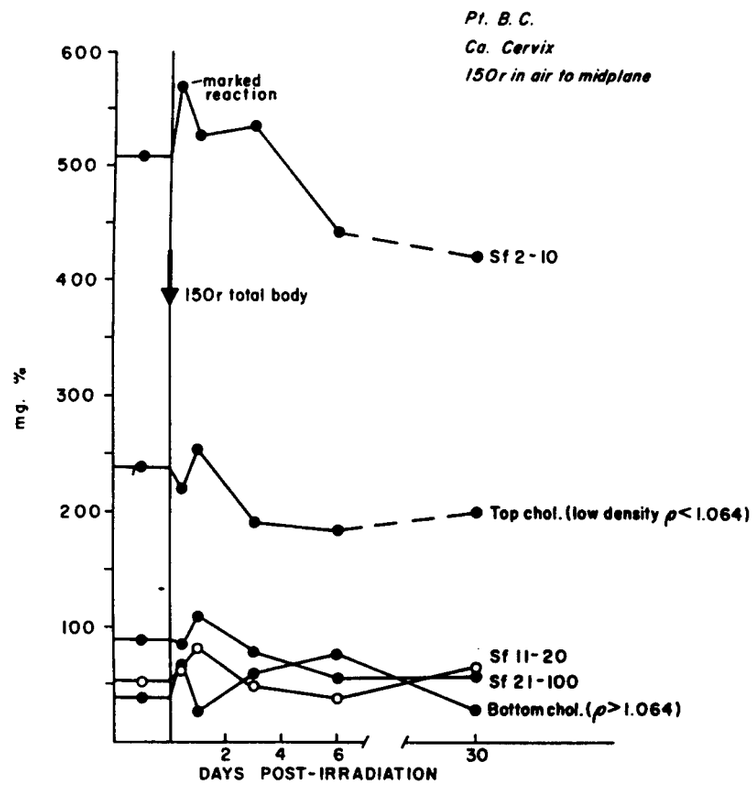
Figure 23



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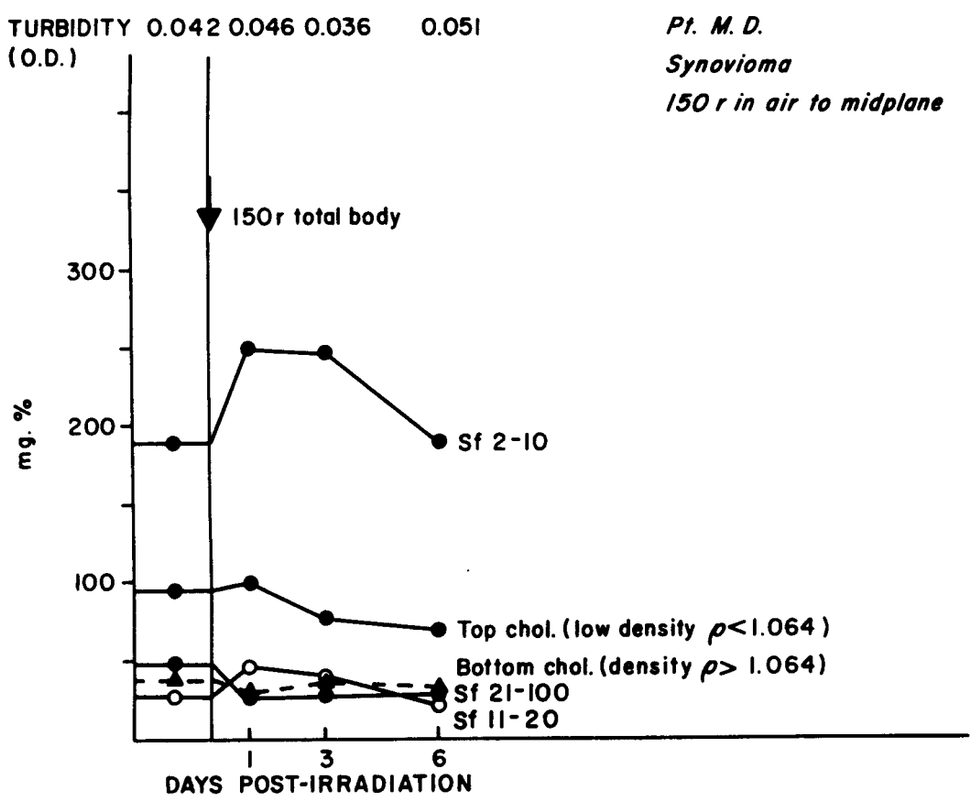

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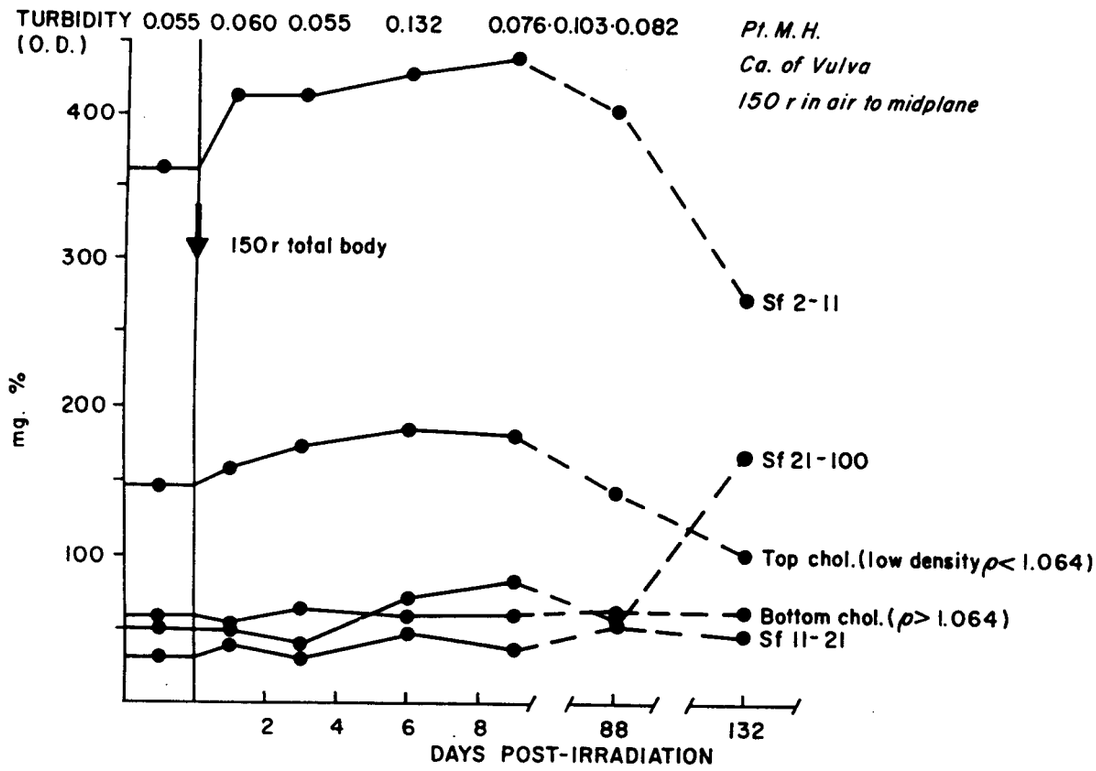
Figure 25



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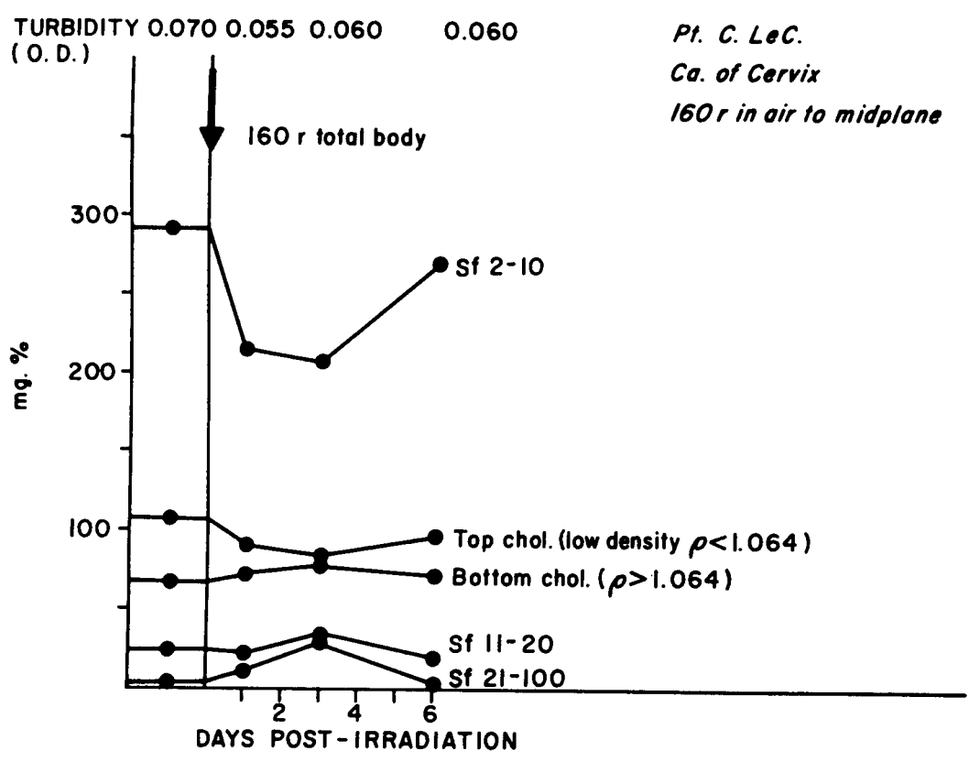

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Figure 27

ELECTROLYTE CONCENTRATION VS TIME. DOG NO. 77 (JEJUNUM), SOLUTION: 5 X ISOTONIC $MgSO_4$

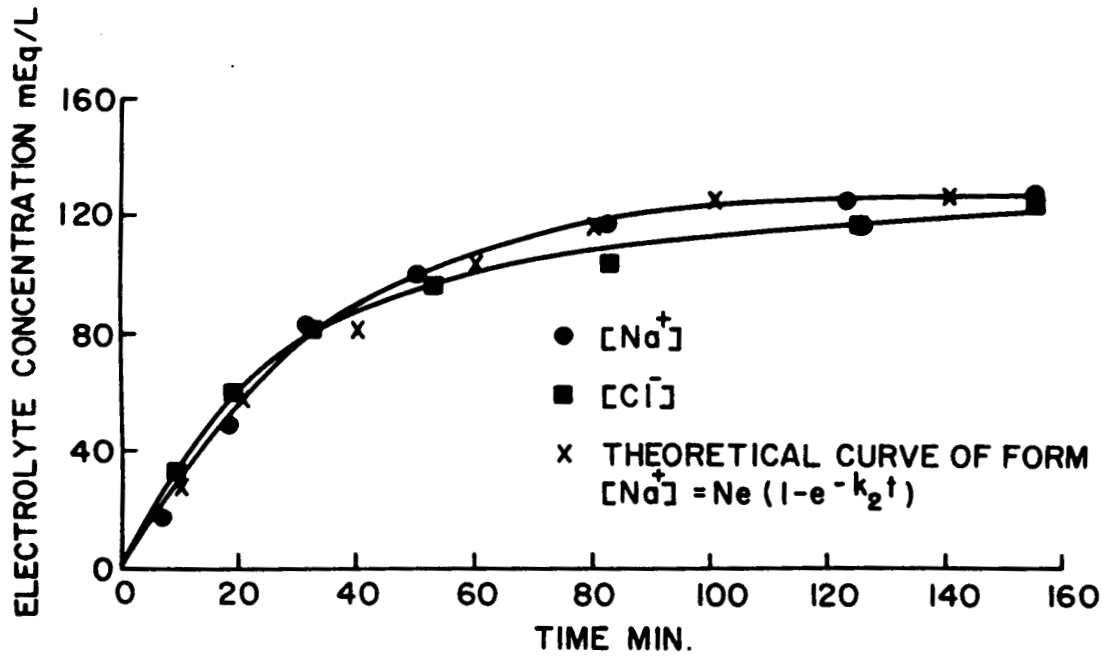

Figure 28

CHANGE IN SODIUM CONCENTRATION WITH TIME IN HYPOTONIC $MgSO_4$ AND IN ELECTROLYTE MIXTURE CHARACTERISTIC OF THE BOWEL SEGMENT. DOG NO. 74, (JEJUNUM)

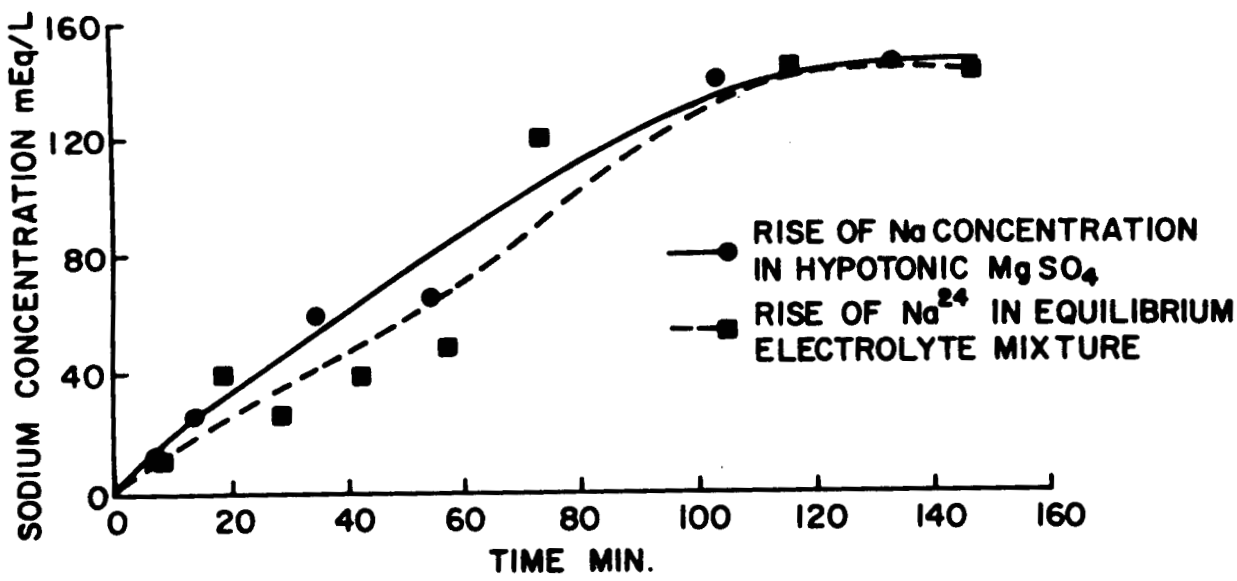

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Figure 29

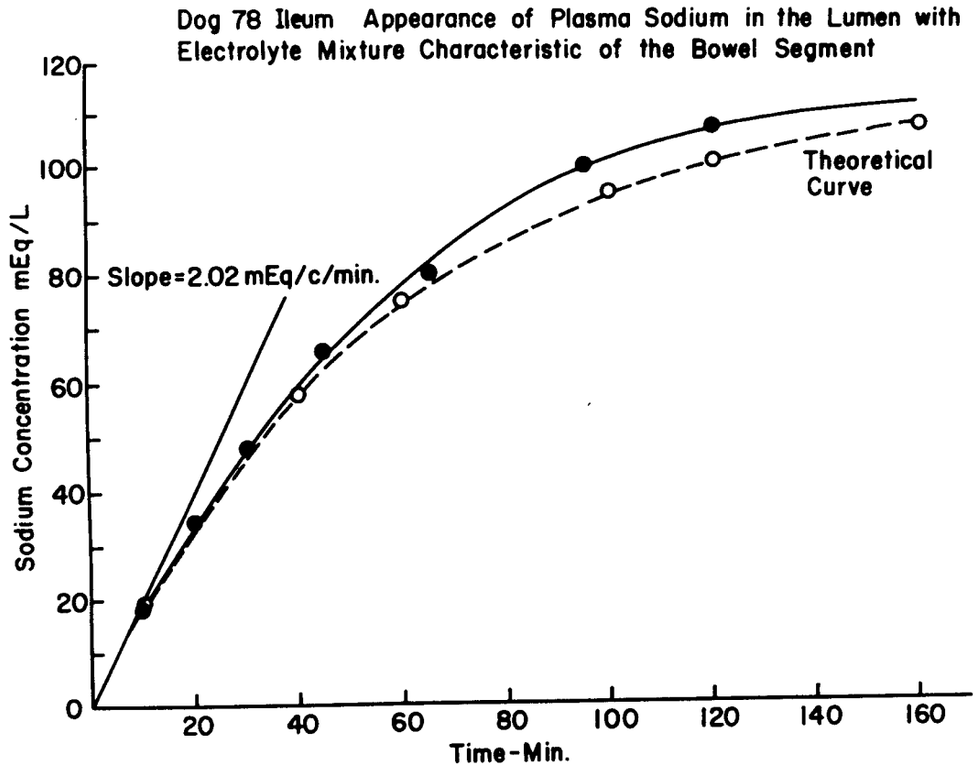

Figure 30

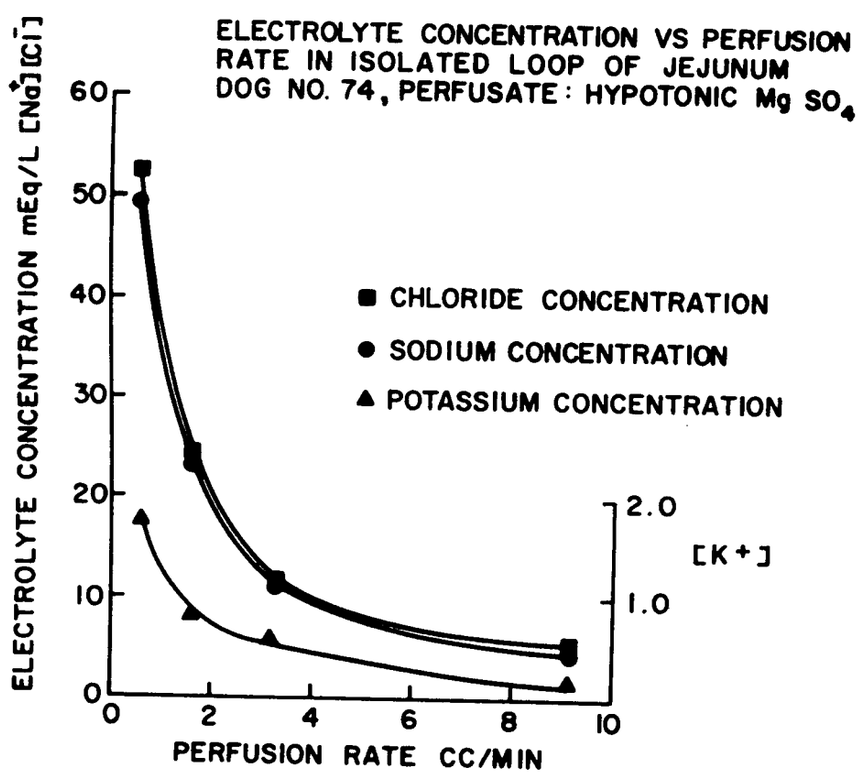

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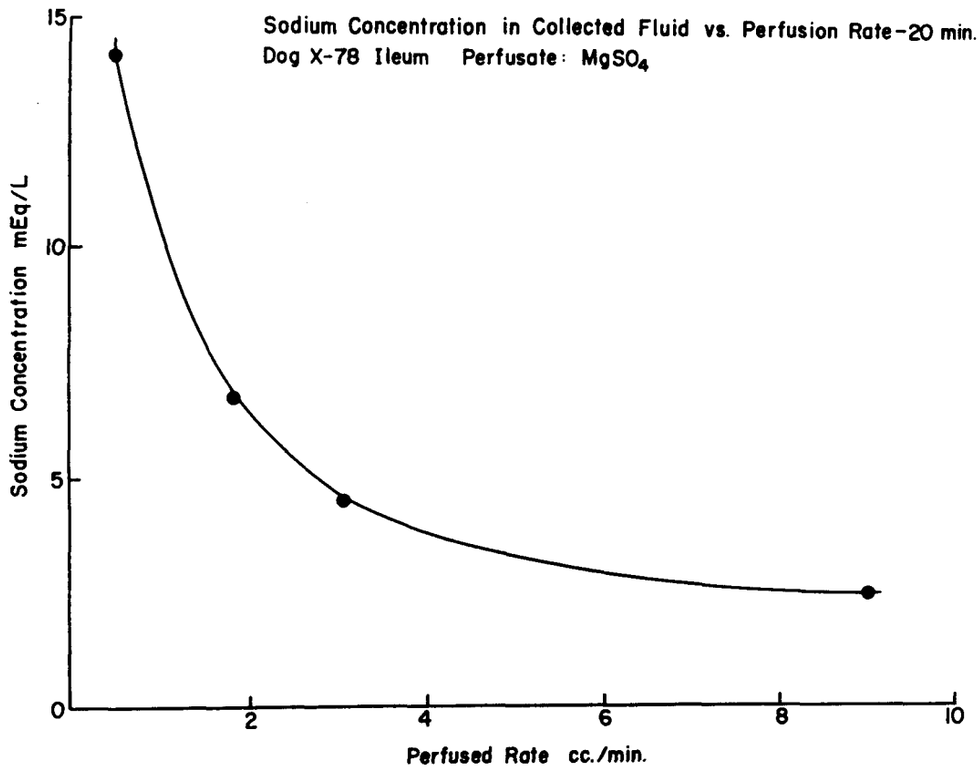
Figure 31



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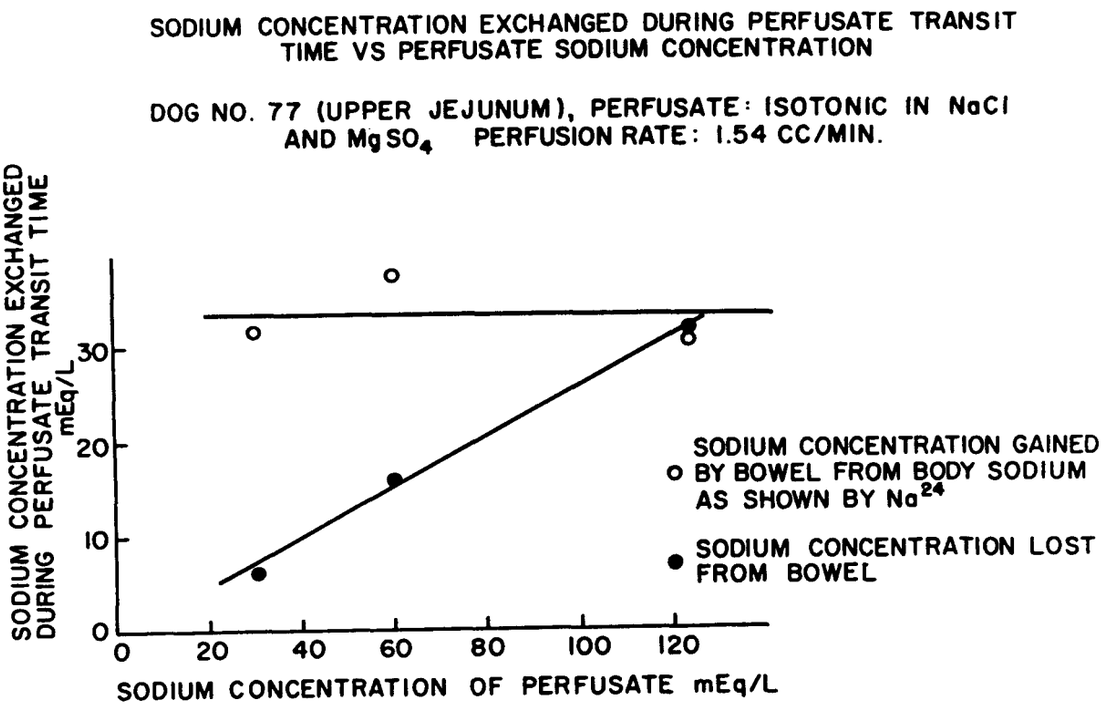

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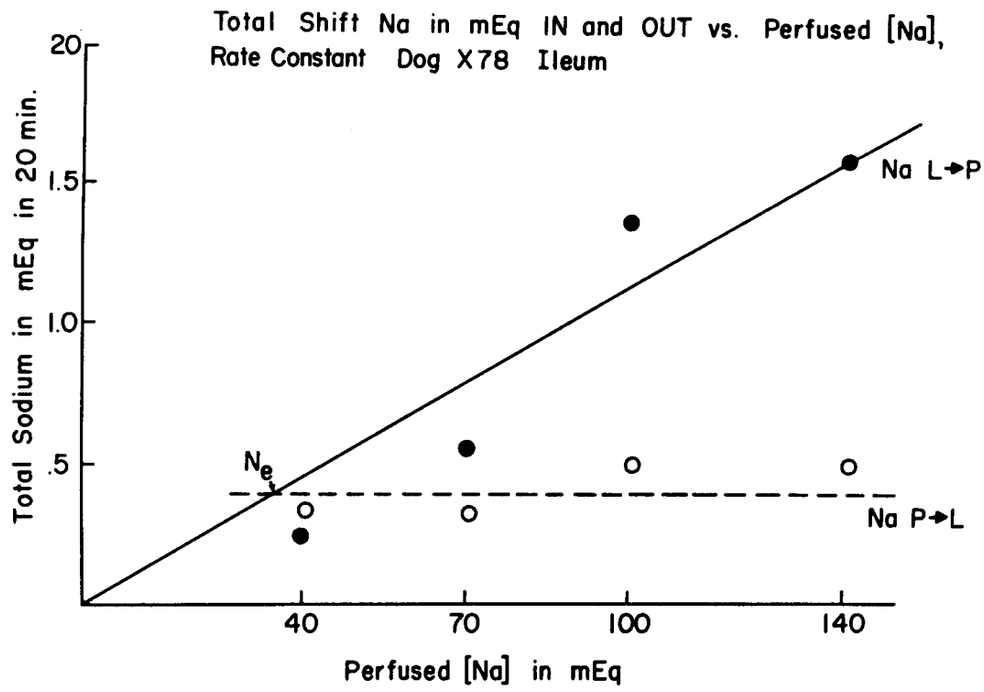
Figure 33



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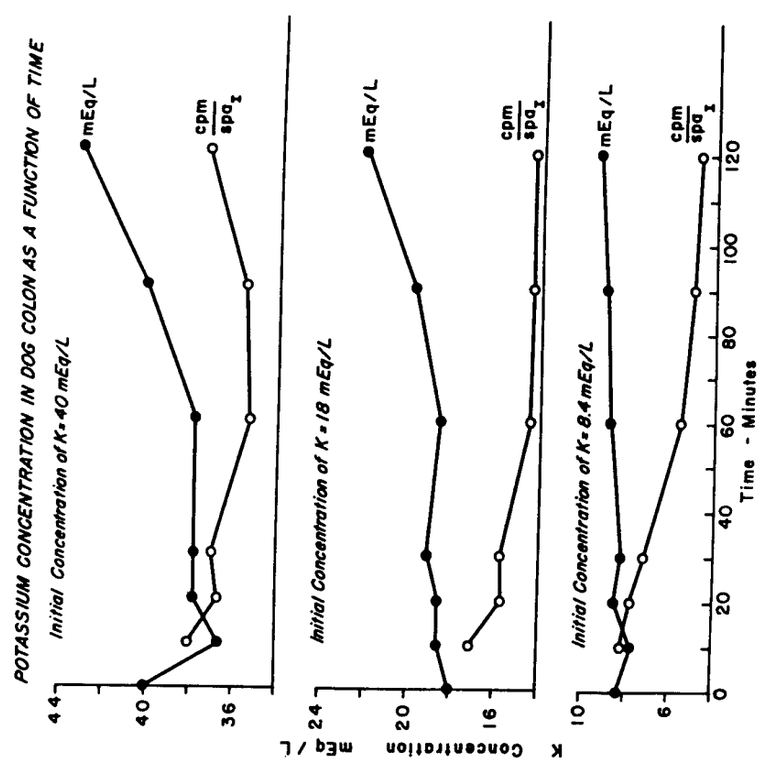

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