Title

EFFECTS OF TOTAL BODY IRRADIATION UPON LIPOPROTEIN METABOLISM

Permalink

https://escholarship.org/uc/item/61449576

Authors

Hewitt, John E.
Hayes, Thomas L.
Gofman, John W.
et al.

Publication Date

1951-11-01

UNIVERSITY OF CALIFORNIA – BERKELEY

RADIATION LABORATORY

DISCLAIMER

EFFECTS OF TOTAL BODY IRRADIATION

UPON LIPOPROTEIN METABOLISM*

John E. Hewitt, Thomas L. Hayes, John W. Gofman, M.D., Ph.D.,
Hardin B. Jones, Ph.D. and Frank T. Pierce, M. D.

from   the
Donner Laboratory of Medical Physics,
the Radiation Laboratory, and the Department of Physics,
University of California
Berkeley, California

A visible lipemia in the serum of rabbits 24 hours after total body
x-irradiation was noted by Rosenthal[1] in this laboratory. It was observed in tha
study that most of the animals in which the post-irradiation lipemia was marked die
within a 30 day period after exposure to radiation. The underlying mechanism re-
sponsible for this post-radiation lipemia was unexplained. Further, the opalescenc
observed in these radiation experiments was not characterized as to the actual
physico-chemical entities in the serum responsible for its appearance. The observa-
tion that the opalescence appears to be a highly unfavorable prognostic sign with
respect to survival following irradiation has prompted us to investigate the evi-
dently vital lipid metabolic derangement which has been produced as a result of
irradiation.

Recent ultracentrifugal investigations of Gofman and associates[2, 3, 4]
have provided a method for the intimate study in the native state of a "spectrum"
of lipoproteins. Serum opalescence may be due to the presence of one or more of
the lipoproteins characterizable in the ultracentrifuge. In fact, opalescence
itself may be a highly deceptive guide in the evaluation of lipoprotein alterations
that may occur. This is the result of the fact that trivial concentrations of very
high molecular weight lipoproteins or lipids can give rise to intense light scatter-
ing (and hence opalescence), whereas very high concentrations of much lower molec-
ular weight lipoproteins may give rise to much less light scattering. With the
ultracentrifugal technique this potential source of confusion is eliminated since
one is able to determine which classes of lipoproteins have been altered as a re-
sult of radiation, and the actual quantitative extent of alteration in lipoprotein
concentration may be measured directly. As will be detailed below, the ultra-
centrifugal method of lipoprotein study has led to the uncovering of important

prognostic alterations in a class of serum lipoproteins that would not have been detected by opalescence alone.

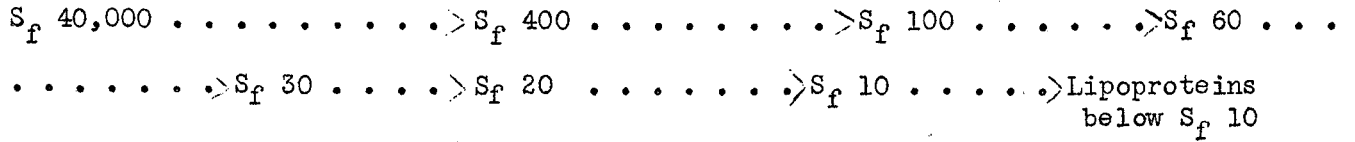## ULTRACENTRIFUGAL CHARACTERIZATION OF SERUM LIPOPROTEINS IN THE RABBIT.

By the procedures outlined in the section on "Methods", it is possible to characterize quantitatively serum lipoprotein transport in a single animal and to follow changes in this transport. From our own and other studies in this laboratory it appears that the normal rabbit shows lipoproteins characterized by flotation rates in the range S_f 5-15* at concentrations ranging up to 120 mg.%. In some normal rabbits one may find in addition low concentrations of several of the lipoprotein species in the range of S_f 15-30. In general higher S_f rates of the lipoproteins are associated with higher molecular weights and lower molecular densities.

Several experimental procedures are now known to alter both the concentrations of lipoproteins in rabbit serum and the distribution of lipoproteins among the various S_f classes. For example, cholesterol feeding in the rabbit results first in an alteration of concentration of lipoproteins in the S_f 8-12 region followed by progressive elevation in concentration of lipoproteins of successively higher S_f classes. In carbon tetrachloride intoxication of the rabbit[5], many of the members of the high S_f classes of lipoproteins appear in high concentration on normal diets. From these and other observations a concept has developed of the probable interrelationship of the various lipoprotein classes. This interrelationship may be described in terms of the diagram below.

---

* One S_f unit represents a flotation rate of 1×10^{-13} cm/sec/dyne/gm. in a sodium chloride solution of density 1.063 gm/cc at 26° C.

Figure I

Lipoprotein Interrelationships

S_f 40,000 . . . .$>S_f$ 400 . . . .$>S_f$ 100 . . . . . .$>S_f$ 60 . . .

. . . .$>S_f$ 30 . . . .$>$ S_f 20 . . . .$>S_f$ 10 . . . . .$>$Lipoproteins
                                                                                    below S_f 10

(The dotted lines indicate that there may be numerous lipoproteins of
intermediate S_f classes.)

It appears that lipids of dietary and/or endogenous sources enter into
this serum lipoprotein transport pathway somewhere in the higher S_f classes (S_f
100 - S_f 40,000). The direction of metabolism then seems to be that of actual
successive transformation into lipoproteins of lower S_f classes. Evidence support-
ing this transformation sequence has been obtained in this irradiation study.
(See below.)

In general the actual lipoprotein level observed in any S_f class is the
resultant steady state balance between influx into this class and removal from
this class. Under normal conditions very low levels of lipoproteins above S_f 12
are observed. This is still consistent with lipid metabolism going through the
transformation sequence described above, except that conversion rates of the higher
S_f classes are high enough to prevent the build-up of appreciable concentrations
of the high S_f lipoproteins. One might anticipate the appearance of elevated
concentrations of the high S_f lipoproteins by either of two mechanisms:

(a) A decrease in the utilization rate of a particular S_f class of
lipoproteins, or -

(b) an excessive influx of lipid into a particular S_f class of
lipoproteins.

In a particular case of disturbance of serum lipoprotein pattern, an evaluation of which mechanism is operative would be valuable in understanding the underlying physiologic defect.

METHODS

For the irradiation studies a total of 49 adult New Zealand White rabbits were used. The average weight was 3 kg. The number of animals of each sex was approximately equal.

Each animal received total body irradiation from a 220 KV x-ray beam filtered through 0.5 mm Cu. At the target distance used, 60 cm, the machine delivered 40 r/min. to the center of the animal. The dose was measured in a paraffin phantom using a portable Victoreen r-meter. The doses given ranged from 800 to 900 r.

Lipoprotein concentrations were determined in the following manner: The lipoproteins were separated as a group from the other large molecules of the serum by preparative ultracentrifugation as follows: The serum, with its density raised to 1.063 gm/cc by the addition of NaCl, was spun at 30,000 RPM for 12 hours. Under these conditions all the lipoproteins of density less than 1.063 float to the top of the tube and can be pipetted off in the top fraction. This separation results in a concentration of the lipoproteins. The top fraction so obtained is then run in an analytic ultracentrifuge at 52,640 RPM; where a series of pictures is taken of the moving lipoprotein boundaries. From these pictures the flotation rate (S_f rate) and concentration of the component lipoproteins can be determined (Fig.2) Our analysis included lipoproteins characterized by flotation rates of 5 to 400 S_f units.

RESULTS AND DISCUSSION

Lipoproteins of the S_f 5-400 group show a general increase after total body irradiation (Table 1). Illustrative ultracentrifugal patterns of the serum are given in Figure 2. In general, it may be said that the changes fall into three classes. In some animals the increase in any class is very small (Fig. 2A); in others there is a large increase in the S_f 30-400, (Fig. 2B) and in the third group there is an increase in the S_f 5-30 group of lipoproteins (Fig. 2C).

Figure 3 shows an analysis of the serial changes in the various lipoprotein components with time for one rabbit. A period of 8 to 12 hours elapsed before any change occurred. This was found to be true for all animals. At about 12 hours there is a sharp increase in the S_f 100-400 class which reaches a maximum at 30 hours and falls back toward normal at 3 days. The S_f 30-100 class builds up as the S_f 100-400 falls, reaching a maximum at 45 hours.

As the S_f 30-100 class of lipoproteins falls the S_f 5-15 class increases reaching a maximum at three days. The observed sequence of post-irradiation events is consistent with the transformation scheme outlined in Figure 1. The character of these changes is similar for all animals showing initially a large increase in concentration of lipoproteins of the high S_f classes. Not all animals first showed the lipoprotein increase in the S_f 30-400 group. In some cases the initial change was an increase in the S_f 5-30 lipoproteins. It may well be that in this latter group the lipids were actually introduced in the S_f 30-400 class or higher, but that rapid transformations down to the S_f 5-30 class prevented an appreciable build-up in S_f 30-400 concentration. In either case, the total lipoprotein level reached a maximum at 30 hours. Forty of 49 irradiated rabbits survived at least 12 hours. It is this group of 40 in which lipoprotein changes were assessed.

A series of histograms is given in Figure 4, plotting mg.% of lipoproteins vs. the number of rabbits having a given lipoprotein value both at the outset and at 30 hours. A shift of distribution is seen after irradiation for each S_f class and for the total S_f 5-400 range. In each case the mean of the distribution is increased after irradiation.

These figures illustrate the correlation of death with lipoprotein level. In the pre-irradiation graphs there is a random distribution of the animals which subsequently died. Therefore, apparently the lipoprotein levels prior to irradiation have no appreciable correlation with the radiosensitivity of the animal. In the 30 hour graphs, however, it is seen that there is an excellent correlation between high lipoprotein levels and subsequent death. Since the animals show high levels in general in either the high (S_f 30-400) or low (S_f 5-30) lipoprotein groups, the correlation for total lipoprotein values is better than with any single lipoprotein group.

A x^2 analysis is given in Figure 5. Before irradiation the x^2 is approximately 0, whereas measuring the lipoprotein values at 30 hours and correlating these values with the subsequent death of the animal gives a x^2 of 22.0. Thus the probability that these two variables, lipoprotein levels and death, are related is extremely high. There are four exceptions to the test. Three animals lived with very high total lipoprotein values (Nos. 19, 32 and 46). One animal died with a low value (No. 60)*. All of these exceptions were males. There is some suggestion that males are more susceptible to this lipoprotein increase after irradiation. The x^2 of 4.8 for sex vs. lipoprotein increase indicates a possible relationship between these two variables. There is no indication that the time

---

* This animal, No. 60, lived 3 weeks, 8 days longer than any other animal which died.

of death is a function of lipoprotein level.

A high concentration of S_f 30-400 lipoproteins is associated with serum opalescence (Table I). However, the S_f 5-30 group of lipoproteins may be high enough to produce a significantly high level of total lipoproteins and the serum will remain clear. Compare, for example, Rabbit No. 91, showing no change in opalescence after irradiation, with Rabbit No. 30 which showed a moderate increase. The lipoprotein values for these rabbits are approximately equal, but in Rabbit No. 91 the increase was manifest in the S_f 5-30 group whereas Rabbit No. 30 showed an increase in the S_f 30-400 group. Thus, increases in the S_f 5-30 group which are correlated with death are not detectable by means of serum opalescence.

Since the metabolic defect in lipoprotein metabolism after irradiation is so closely associated with death, it is of interest to study any agent capable of influencing lipoprotein metabolism. The importance of heparin in producing lipoprotein transformations has been reported by Graham, et al.[6]. Figure 6 shows the effect of a single heparin injection on the abnormal lipoprotein pattern produced by radiation. In terms of the transformation concept discussed above, it appears that heparin accelerates the progressive conversion of the lower density molecules into those of higher density. This graph (Figure 6) may be compared with Figure 3. The sequence of events following the appearance of the high S_f rate component is similar in that there is a fall of the very low-density molecules accompanied by an increase in the higher density components. However, after the injection of heparin this process is greatly accelerated. Without heparin, it required 3 days for the fast component to drop to an approximately normal level; with the injection of 10 milligrams of heparin this change occurred in 15 minutes.

In view of the observed reduction by heparin of lipoproteins of S_f 12 and above, the possibility is suggested that the post-irradiation levels of

lipoproteins could be accounted for by an aheparinemia. Several heparin precipitants (Toluidine blue, Protamine sulfate, and Quinine) were injected into normal rabbits to test this hypothesis. A pronounced elevation in the levels of lipoproteins above S_f 12 was found at 30 hours. A typical pattern is shown in Figure 2D.

It is very unlikely that these three chemical substances are causing the effect on lipoprotein metabolism by any other mechanism than that they have a very strong affinity for heparin, and in the doses administered probably bind all available heparin for many hours. Under these circumstances, changes occur in the serum lipoproteins identical with the severe post-irradiation changes. This strongly suggests that the irradiation effects on serum lipoproteins come about through the unavailability of heparin.

The mechanism of the heparin control of blood lipoproteins is being studied currently by others in this laboratory (Graham, Nichols, Lindgren, Pierce and Ruben). It is certain that heparin initiates the release of another substance in the blood stream. This substance may contain heparin andas it has notidentified chemically it is convenient to refer to it as the "active factor". The active factor causes some lipoprotein transformations from higher S_f to lower S_f in vitro. It is always present in detectable amounts in the blood following intravenous administration of heparin and at the same time as the acceleration of transformation of lipoproteins from higher classes to lower S_f classes in vivo. The active factor is formed in appreciable quantities by very small injections of heparin. Current methods of detection can identify the active factor generated by less than 100 micrograms of heparin per kilo of body weight. No active factor circulates in so-far-detectable quantities in the normal rabbit or apparently in the post-irradiation rabbit up to the third day. At this time it appears that the active factor is being generated and this most probably represents the release of unusual quantities of free heparin.

This phase is being investigated for possible relationship to the severity of irradiation damage on the theory that following lethal levels of irradiation heparin becomes unavailable for the control of normal lipoprotein transformations. The lack of available heparin causes a piling up of lipoproteins in the blood stream in molecules that are blocked from metabolic conversion to lower S_f molecules. These transformations can still occur in these animals if small quantities of heparin are injected into the animal. It is not yet explained why the site of greatest inhibition of transformation should be at somewhat different S_f levels in different animals.

SUMMARY

1. There is an excellent correlation between high level of total lipoprotein 30 hours after irradiation and subsequent death of the animal.

2. A serum opalescence is associated with low density lipoprotein only, not with total lipoprotein level.

3. Changes in the lipoprotein levels after irradiation are consistent with the theory of conversion of low density lipoprotein to higher density components.

4. The injection of heparin after irradiation hastens the return of lipoprotein levels to normal values.

5. The injection of toluidine blue, protamine sulfate or quinine produces changes in the lipoprotein pattern similar to those shown after irradiation.

REFERENCES

1. Rosenthal, R.L. Opalescence of Serum after Total Body X-Irradiation as a Prognostic Sign of Death. Science 110: 43, 1949.

2. Gofman, John W., Lindgren, Frank, Elliott, Harold, Mantz, William, Hewitt, John, Strisower, Beverly, and Herring, Virgil. The Role of Lipids and Lipoproteins in Atherosclerosis. Science 111, 166, 1950.

3. Gofman, John W., Jones, Hardin B., Lindgren, Frank T., Lyon, Thomas P., Elliott, Harold A., and Strisower, Beverly. Blood Lipids and Human Atherosclerosis. Circulation 2, 161, 1950.

4. Lindgren, Frank T., Elliott, Harold A., and Gofman, John W., The Ultracentrifugal Characterization and Isolation of Human Blood Lipids and Lipoproteins, with Applications to the Study of Atherosclerosis. Journal of Physical and Colloid Chemistry, 55, 80, 1951.

5. Pierce, F.T. and Gofman, John W. The Effect of Carbon Tetrachloride Poisoning in Serum Lipoproteins Associated with Atherosclerosis. Circulation 4, 29, 1951.

6. Graham, Dean M., Lyon, Thomas P., Gofman, John W., Jones, Hardin B., Yankley, Alexander, and Simonton, John. Blood Lipids and Human Atherosclerosis II. The Influence of Heparin upon Lipoprotein Metabolism. Circulation. (In Press).

TABLE I

Table I shows a summary of the lipoprotein changes for each rabbit used.
Areas from the ultracentrifuge patterns have been converted into milligrams
percent by means of an appropriate proportionality factor. Animals classified
as "lived" have lived at least 30 days after irradiation. Deaths occurring
beyond that period are not considered attributable to radiation damage. A
group of 9 animals shown at the end of the table died within 1 to 3 hours
post-irradiation. They were therefore not included in this study. The last
column gives a purely subjective estimation of the degree of opalescence in
the serum before ultracentrifugation. It is based on a 1 to 10 scale.

TABLE I   UCRL-1549

Males

#	Dose	Lived	Died	Time of Death	Visual Estimation of Opalescence Irradation Pre	Post	Total Lipoprotein (S$_f$ 5-400) mg% Irradation Pre	30 hr. Post
4	800r		x	40 hr			19	692
12	"		x	40 hr			107	1500
16	"		x	43 hr	2	9	71	2940
19	"	x					285	504
22	"	x					38	119
24	"		x	35 hr			142	880
26	"		x	7 da	1	10	73	1720
32	"	x			2	9	55	2300
33	"		x	20 hr	2	8	119	1010
38	"	x			2	2	78	225
46	"	x			2	7	90	1840
50	"		x	5 da	1	9	140	2060
55	"	x			2	2	12	341
56	"		x	11 da	2	2	17	355
60	"		x	21 da	1	1	17	90
66	900r		x	12 da	2	4	168	632
76	825r		x	10 da	2	5	55	1070
80	825r	x			2	2	83	277
86	900r		x	13 da	2	3	64	545
87	900r		x	10 da	2	2	237	376
95	900r	x					19	159

TABLE I
(cont.)

UCRL-1549

Females

#	Dose	Lived	Died	Time of Death	Visual Estimation of Opalescence		Total Lipoprotein (S_f 5-400) mg%	
					Irradation		Irradation	
					Pre	Post	Pre	30 hr. Post
6	800r	x					140	289
8	"	x					43	152
18	"	x			1	1	142	180
21	"	x					47	214
28	"		x	63 hr	1	5	66	937
29	"	x			2	2	325	256
30	"		x	7 da	1	4	33	784
42	"	x			2	2	28	192
43	"	x			2	2	66	204
44	"	x			2	2	92	263
48	"	x			2	3	194	192
49	"		x	3 da	2	4	109	672
58	"	x			2	2	33	284
63	"	x			2	2	126	301
74	825r	x			2	2	47	171
78	"	x			2	2	52	312
91	900r		x	8 da	2	2	168	708
92	900r		x	5 da	2	2	35	476
93	900r		x	4 da	2	4	97	793

TABLE I
(conc.)

UCRL-1549

Animals dying in less than 12 hours post irradation

#	Dose	Lived	Died	Time of Death	Visual Estimation of Opalescence		Total Lipoprotein (S_f 5-400) mg%	
					Irradiation Pre	Post	Irradiation Pre	30 hr. Post
20	800r			2 hr			111	
25	"			11 hr			109	
36	"			2 hr			118	
40	"			1 hr				
68	900r			2 hr				
70	900r			2 hr				
73	825r			2 hr			118	
77	825r			3 hr			76	
88	900r			2 hr				

FIGURE 2

Lipoprotein patterns before and after radiation. From left to right successive frames are at 0, 6, 12, 22, 30 and 38 minutes after the rotor has reached 52,640 RPM. A flotation rate scale for each frame is drawn at the top of the figure. The smooth curves drawn in above the patterns represent the position of the top of the pattern when no lipoprotein is present. The area bounded by the reference curve, the top of the pattern and any two vertical lines is proportional to the concentration of the lipoproteins characterized by this S_f range. To measure the S_f 5-400 lipoproteins considered in this paper, areas were measured in four frames and added together.

A. Rabbit #74, shows very little increase of total lipoprotein 30 hours post-irradiation.

B. Rabbit #50 shows an increase of total lipoprotein due to a large increase of the S_f 30-400 class.

C. Rabbit #92. Here the lipoprotein increase is found mainly in the S_f 5-30 component.

D. Lipoprotein changes due to single injection of 10 mg. of toluidine blue. Note similarity to post-irradiation changes. (Figure 2B above)

A   Pre - irradiation

      30 hours post - irradiation

B   Pre - irradiation

      30 hours post - irradiation

C   Pre - irradiation

      30 hours post - irradiation

D   30 hours post - injection
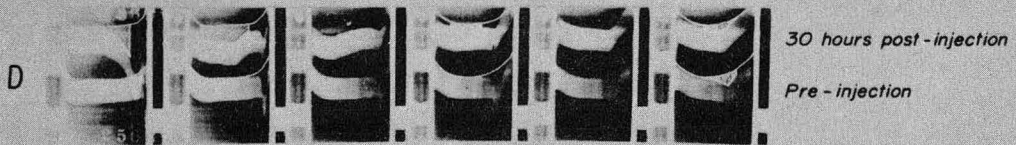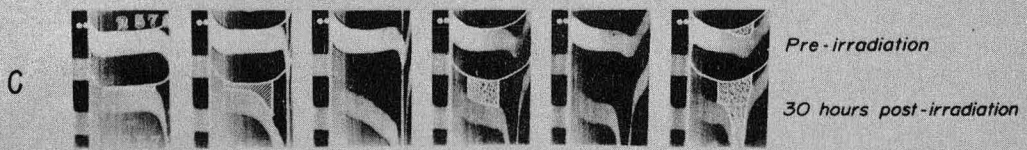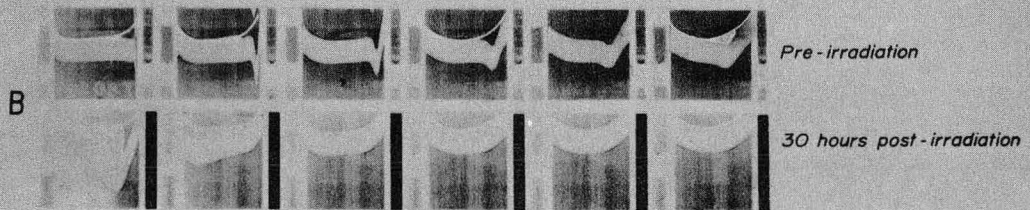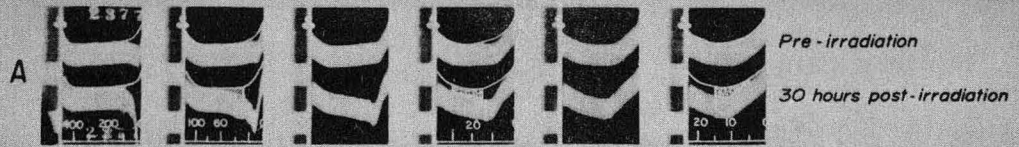
      Pre - injection

ZN 126

FIGURE 3

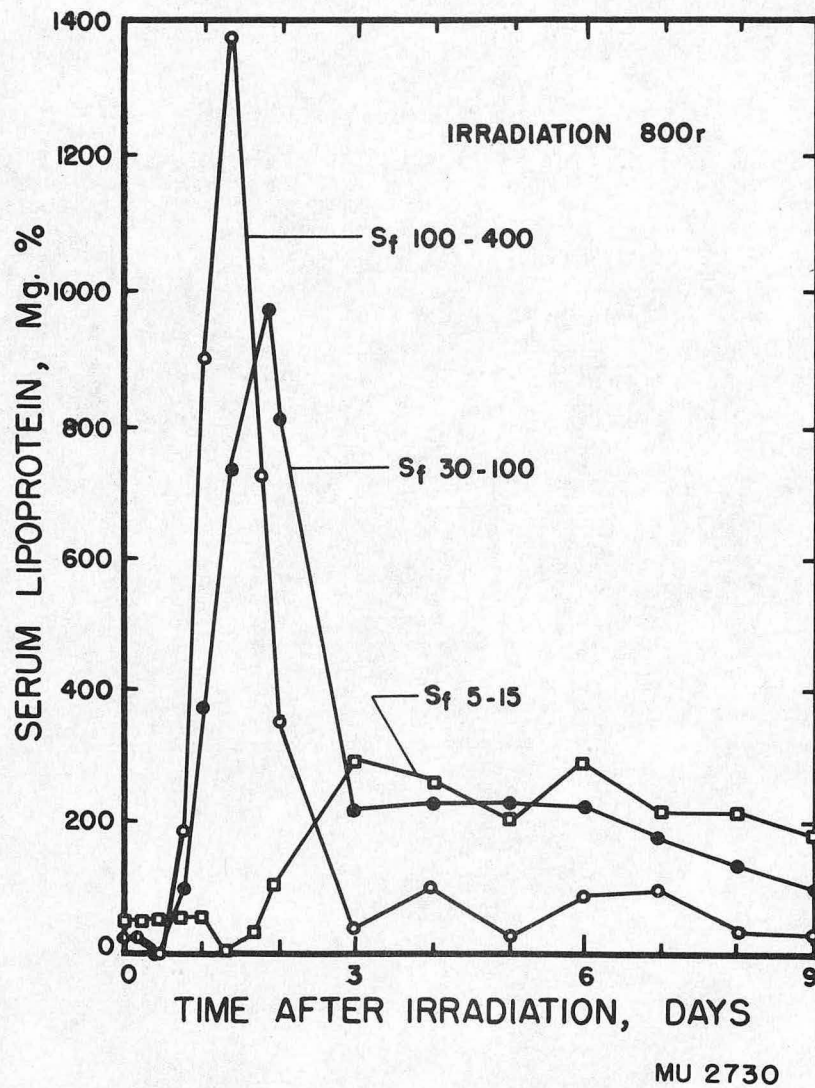Serial lipoprotein changes with time for a single animal. (Rabbit #32)

Fig. 3

FIGURE 4

Distribution of lipoprotein concentrations before and after irradiation in
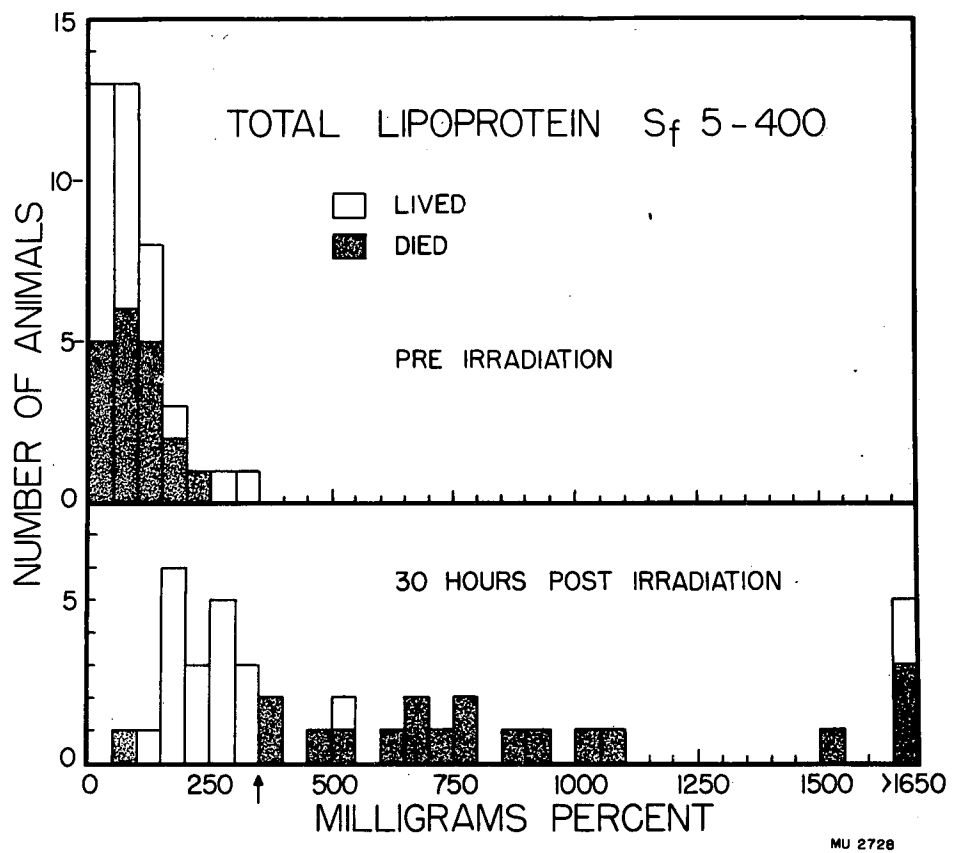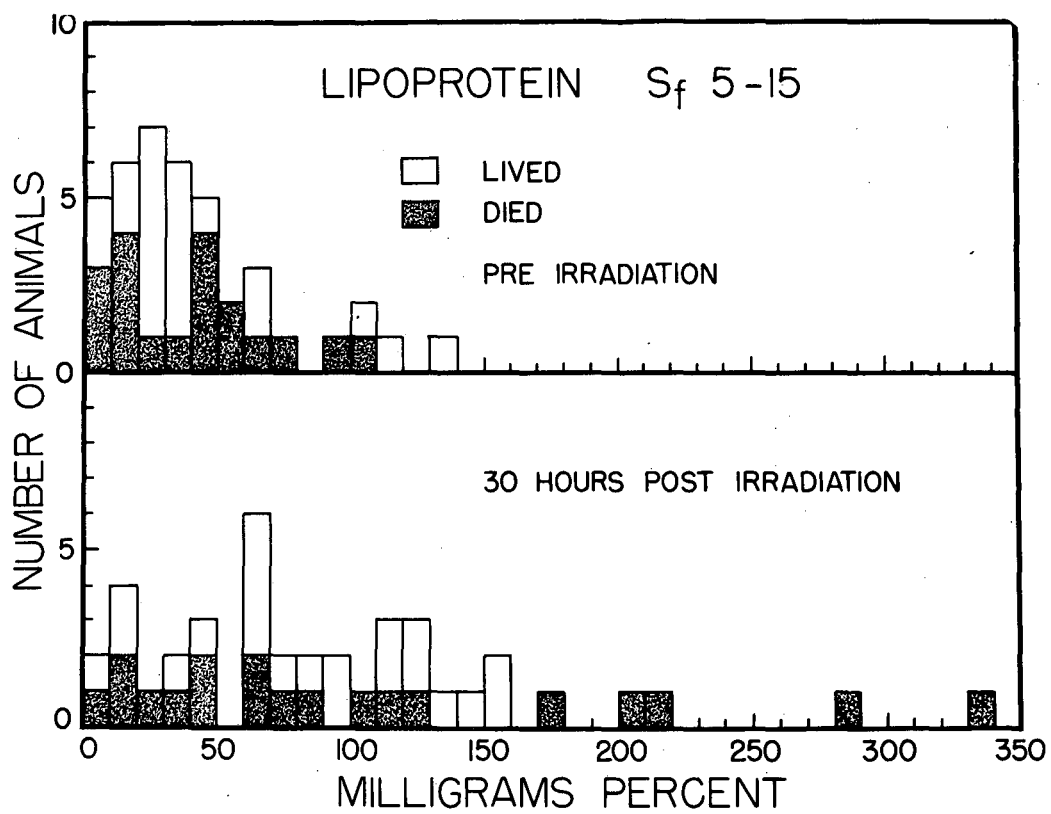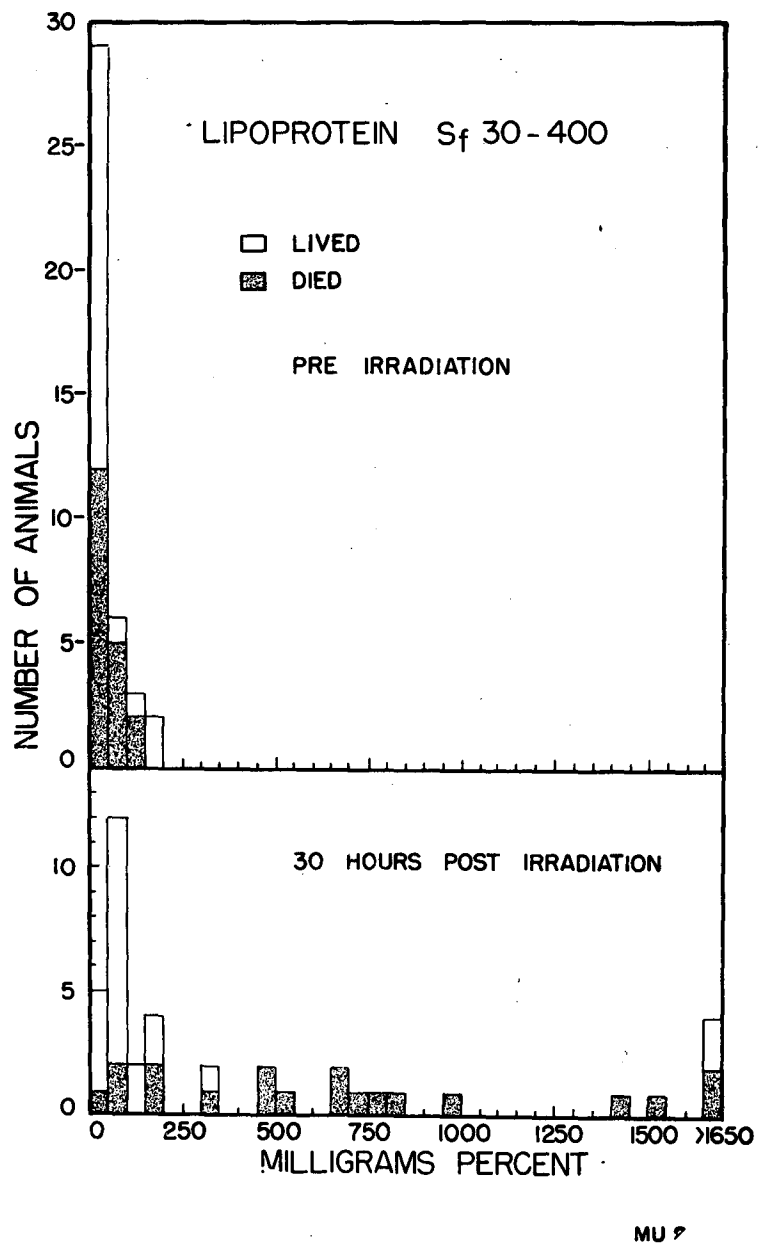40 rabbits. Each square represents one animal.

Fig. 4

Fig. 4

Fig. 4

FIGURE 5

ASSOCIATION TABLES

A. Association of lipoprotein levels with death.

Pre-irradiation
Lipoprotein mg.%.

	< 73 ⇐		
lived	11	10	21
died	9	10	19
	20	20	40

$$x^2 = 0$$
$$p = 1$$

30 hrs. post-irradiation
Lipoprotein mg.%.

	< 350 ⇐		
lived	18	3	21
died	1	18	19
			40

$$x^2 = 22.8$$
$$p < .001$$

B. Correlation of sex with lipoprotein level 30 hours after irradiation.

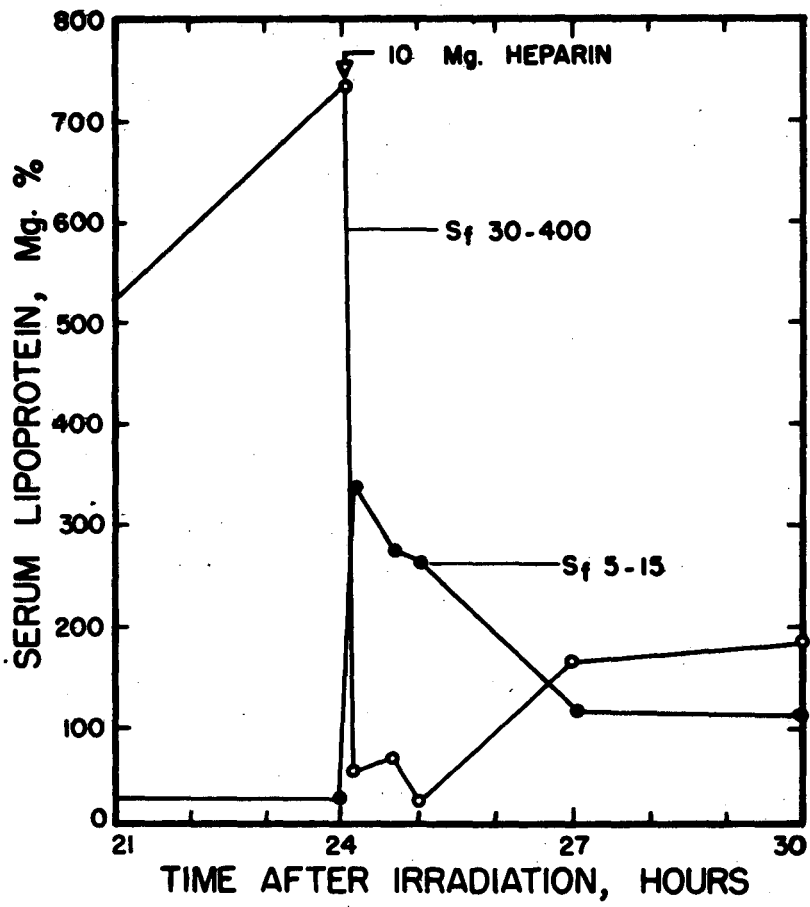Lipoprotein mg.%.

	< 350 ⇐		
Female	13	6	19
Male	6	15	21
	19	21	40

$$x^2 = 4.8$$
$$p = .03$$

In each case the dividing line was arbitrarily chosen to give the best
correlation. The 350 mg. dividing line is shown graphically by an arrow
in Figure 4.

FIGURE 6

Effect of a single injection of heparin on post-irradiation lipoprotein
changes for one animal.

Fig.6