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SERUM LIPOPROTEINS AS AN INDICATION OF SURVIVAL TIME IN THE X-IRRADIATED RABBIT

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November 24, 1954

ABSTRACT

This communication presents a detailed analysis of the hyperlipoproteinemia of the x-irradiated rabbit, and correlates increases of certain classes of lipoproteins at 24 hours postirradiation with subsequent time of death.

INTRODUCTION

It has been shown previously that total body x-irradiation has a major effect on lipoprotein metabolism in the rabbit.[1] It was demonstrated that a strong relationship exists between an increase in total serum lipoprotein concentration at 24 hours postirradiation and subsequent death of the animal. Therefore, the analysis of the serum lipoproteins following irradiation presents a new and important avenue of investigation into the cause or causes of lethal radiation damage. In the original observations it was noted that the lipoprotein increase occurred in different S_f groups, and in extending the experiments to include more animals it was observed that a relationship existed between the specific class of lipoprotein showing the predominant increase and the subsequent time of death.

These studies have now been extended to a detailed analysis of hyperlipoproteinemia following x-irradiation, and a search for correlation between the increase of certain classes of lipoproteins and the time of death.

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METHODS

Serum lipoproteins were analyzed by the ultracentrifugal methods developed by Gofman and associates. These methods are described fully in a paper by Oliver deLalla and John W. Gofman.[2] Information from this paper is used in the following outline of ultracentrifugal methods.

The serum lipoproteins consist of a "spectrum" or series of substances composed of lipids in combination with protein. These substances vary considerably in molecular weight and hydrated density. The hydrated densities cover the range from <1.0 g/ml to 1.145 g/ml.

For determination of lipoprotein concentrations the lipoproteins are 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 g/cc by the addition of NaCl, is 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 at 52,640 rpm in an analytic ultracentrifuge, 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. Our analysis included lipoproteins characterized by flotation rates of 5 to 400 S_f units.

Forty New Zealand white rabbits received a single LD_{50} dose of total body x-irradiation. No anesthetic was used. The average weight of the animals was about 7 lb and the group consisted of 20 males and 20 females.

Each animal received total body irradiation from a 220 kv x-ray beam filtered through 0.5 mm of Cu and 1.2 mm of Al. At the target distance used, 60 cm, the machine delivered 35 r/min to the center of the animal. The dose, 835 r, was measured in a paraffin phantom using a portable Victoreen r-meter.

Blood samples for lipoprotein analysis were taken only twice during the experiment, once immediately prior to irradiation and once at 30 hours post-irradiation.

It was found convenient to divide the spectrum of lipoproteins thus analyzed into three S_f ranges: S_f 5 to S_f 15, S_f 15 to S_f 30, and S_f 30 to S_f 400. Thus, the S_f 5-15 value represents the sum of concentrations of all serum lipoproteins that float in the analytic ultracentrifuge with rates between S_f 5 and S_f 15 in the solution density of 1.063 g/ml.

RESULTS

Table I shows the concentration of lipoprotein in these three classes before and 30 hours after irradiation. Two of the 40 animals irradiated died at about 2 hours postirradiation, which, we have found, is before any significant lipoprotein changes occur. These two animals are omitted from the table. Also shown in Table I is the difference (Δ) between the preirradiation and postirradiation serum lipoproteins for the three classes as well as the time of death for each animal. An animal surviving past 30 days postirradiation is classified as "survived".

Prior to irradiation, most of the serum lipoproteins of the rabbit appear in the S_f 5-15 class. A typical rabbit prior to irradiation shows a lipoprotein analysis as follows: S_f 5-15 = 75.3 mg%, S_f 15-30 = 6.6 mg%, S_f 30-400 = 8.0 mg% (Rabbit RAD 332). At 30 hours postirradiation, the serum lipoprotein analysis shows marked changes. In general there is an increase in serum lipoprotein, but there is a striking difference between different animals in the amount of this increase and in its distribution among the three classes.

On the basis of the lipoprotein analysis at 30 hours postirradiation, the rabbits can be divided into three groups, as follows:

Group 1. Those animals showing only a small increase in any of the three classes, for example (RAD 332):

	5-15 (mg%)	15-30 (mg%)	30-400 (mg%)
Preirradiation	75.3	6.6	8.0
Postirradiation	94.5	27.1	22.5
Change (Δ)	+19.2	+20.5	+14.6

Group 2. Those animals showing a large increase primarily in the S_f 30-400 class, for example (RAD 312):

	5-15 (mg%)	15-30 (mg%)	30-400 (mg%)
Preirradiation	82.4	3.7	8.0
Postirradiation	78.6	104.8	1619.3
Change (Δ)	-3.7	+101.1	+1611.3

Note that these animals also show a significant increase in the S_f 15-30 class associated with the huge increase in the S_f 30-400.

Table 1

Lipoprotein Concentrations in the Rabbit Preirradiation and 30 Hours Postirradiation

No.	Time of Death	S_f 5-15 Pre-irrad.	S_f 5-15 Post-irrad.	S_f 15-30 Pre-irrad.	S_f 15-30 Post-irrad.	S_f 30-400 Pre-irrad.	S_f 30-400 Post-irrad.	△5-15	△15-30	△30-400
310	17 d	44.9	91.7	9.4	30.0	15.4	22.9	46.8	20.6	7.5
311	24 d	85.2	217.6	36.5	84.2	28.1	26.7	132.4	47.7	-1.4
312	3 d	82.4	78.6	3.7	104.8	8.0	1619.3	-3.7	101.1	1612.3
313	L	21.1	67.4	4.0	30.4	8.4	16.4	46.3	26.4	8.0
314	11 d	49.1	28.1	11.2	40.2	22.9	1591.2	-21.1	29.0	1567.8
315	L	29.7	96.9	5.9	72.5	10.3	127.3	67.2	66.7	117.0
316	20 d	30.7	78.2	4.4	65.1	8.7	47.7	47.5	60.6	39.1
317	L	29.5	59.9	4.2	25.7	7.0	39.3	30.4	21.5	32.3
318	11 d	70.2	210.6	10.8	55.2	12.6	29.0	140.4	44.5	16.4
319	L	16.4	137.6	1.4	18.3	0.9	20.1	121.2	16.8	19.2
320	L	61.2	179.7	32.4	254.0	22.2	167.2	118.9	221.8	145.1
321	7 d	33.7	110.0	7.0	70.7	7.0	48.2	76.3	63.6	41.2
322	L	134.2	159.7	13.1	305.8	16.2	252.1	25.3	293.0	235.9
323	4 d	129.2	133.8	10.8	138.5	17.3	1018.4	4.7	127.8	1001.1
324	9 d	183.0	212.9	31.4	57.1	28.1	48.2	30.0	25.7	20.1
325	L	31.4	77.2	22.9	46.3	95.9	198.0	45.9	23.4	102.0
326	20 d	140.4	185.3	11.2	75.8	13.6	38.8	44.9	64.6	25.3
327	12 d	116.1	116.1	17.3	83.3	11.2	100.6	0	66.0	89.4
328	L	68.3	187.2	4.7	30.0	0	17.3	118.9	25.3	17.3
329	L	120.3	194.7	13.1	97.8	7.5	41.7	74.4	84.7	34.2
330	L	23.9	26.7	2.3	19.2	0.7	52.4	2.8	16.8	51.7
331	10 d	40.2	86.1	4.7	65.5	1.2	51.5	45.9	60.8	50.3
332	L	75.3	94.5	6.6	27.1	8.0	22.5	19.2	20.6	14.5
333	L	58.5	272.8	4.7	20.6	10.3	17.3	214.3	15.9	7.0
334	L	63.2	51.5	10.8	55.2	15.0	1369.4	-11.7	44.5	1354.4
335	L	30.0	42.1	4.2	28.1	1.2	34.6	12.2	23.9	33.5
336	L	76.3	76.3	11.0	37.9	9.8	30.0	0	26.9	20.1
338	26 d	96.9	187.0	11.0	91.3	16.6	62.2	90.1	80.3	45.6
339	11 d	55.0	66.5	9.8	44.0	26.4	51.5	11.5	34.2	25.0
340	4 d	70.2	79.6	10.8	135.7	25.7	543.8	9.4	125.0	518.1
341	L	14.0	97.3	2.3	21.1	13.6	13.6	83.3	18.7	0
342	9 d	22.9	35.1	7.7	20.6	12.2	37.4	12.2	12.9	25.3
343	L	19.7	40.7	8.0	33.2	20.6	31.8	21.1	25.3	11.2
344	25 d	15.0	123.6	0	134.8	0.9	369.7	108.6	134.3	368.8
345	5 d	16.8	92.7	7.0	35.6	12.4	19.9	75.8	28.5	7.5
346	L	169.9	76.3	46.8	47.7	19.9	39.8	-93.6	1.0	20.0
347	6 d	36.5	74.4	6.1	47.3	15.2	32.3	37.9	41.2	17.1
348	4 d	44.5	40.2	10.8	74.9	41.2	721.6	-4.2	64.1	680.5

L = survived past 30 days
d = days

Group 3. Those animals showing a significant increase in the S_f 15-30 class without any large increase in the S_f 30-400, for example (RAD 338):

	5-15 (mg%)	15-30 (mg%)	30-400 (mg%)
Preirradiation	96.9	11.0	16.6
Postirradiation	187.0	91.3	62.2
Change (Δ)	+91.1	+80.3	+45.6

The ultracentrifugal patterns for these three groups are shown in Fig. 1 a, b, c respectively.

The times of death for all the 38 rabbits are shown in Fig. 2. For the purpose of this paper the rabbits have been grouped as follows:

1. Those animals which died in 1 to 4 days postirradiation (5 animals).
2. Those animals which died 5 to 30 days postirradiation (15 animals).
3. Those animals which survived more than 30 days postirradiation (18 animals).

Figure 3 shows the level of the S_f 5-15 class of lipoproteins plotted against survival times in terms of the three groups mentioned above. The preirradiation and postirradiation levels of this class show no correlation with time of death. The slightly lower values of the S_f 5-15 in those animals that died in 1 to 4 days can be accounted for by the high level of S_f 30-400 class lipoproteins in those animals. We have found previously that very large amounts of S_f 30-400 in the serum tend to lower the S_f 5-15 level.

The preirradiation levels of the S_f 15-30 or S_f 30-400 classes will not predict the fate of the animal. Figure 4 shows the distribution of the three survival groups as a function of the preirradiation levels of the S_f 15-30 and S_f 30-400 lipoproteins.

At 30 hours postirradiation, however, the levels of S_f 15-30 and S_f 30-400 very definitely correlate with subsequent time of death. This correlation is shown in Fig. 5. Animals that died in 1 to 4 days postirradiation tend to show high levels of the S_f 30-400 class lipoprotein, 30 hours postirradiation. Associated with this is usually a large increase in the S_f 15-30 class. Animals that subsequently died in from 5 to 30 days postirradiation showed high levels of S_f 15-30 class at 30 hours postirradiation, without the high level of the S_f 30-400. Animals that subsequently survived showed only a low level of either class at 30 hours. Thus the three types of lipoprotein changes shown at 30 hours postirradiation in the rabbit (Fig. 1 a, b, c) can be correlated with the general time of death, i.e., survival past 30 days, death in 1 to 4 days, death in 5 to 30 days, respectively.

An even better correlation is seen if the differences between preirradiation and postirradiation levels are considered (Δ). For example, the ΔS_f 15-30 for an animal would be obtained by subtracting the preirradiation value of the S_f 15-30 class from the value for this class obtained at 30 hours postirradiation.

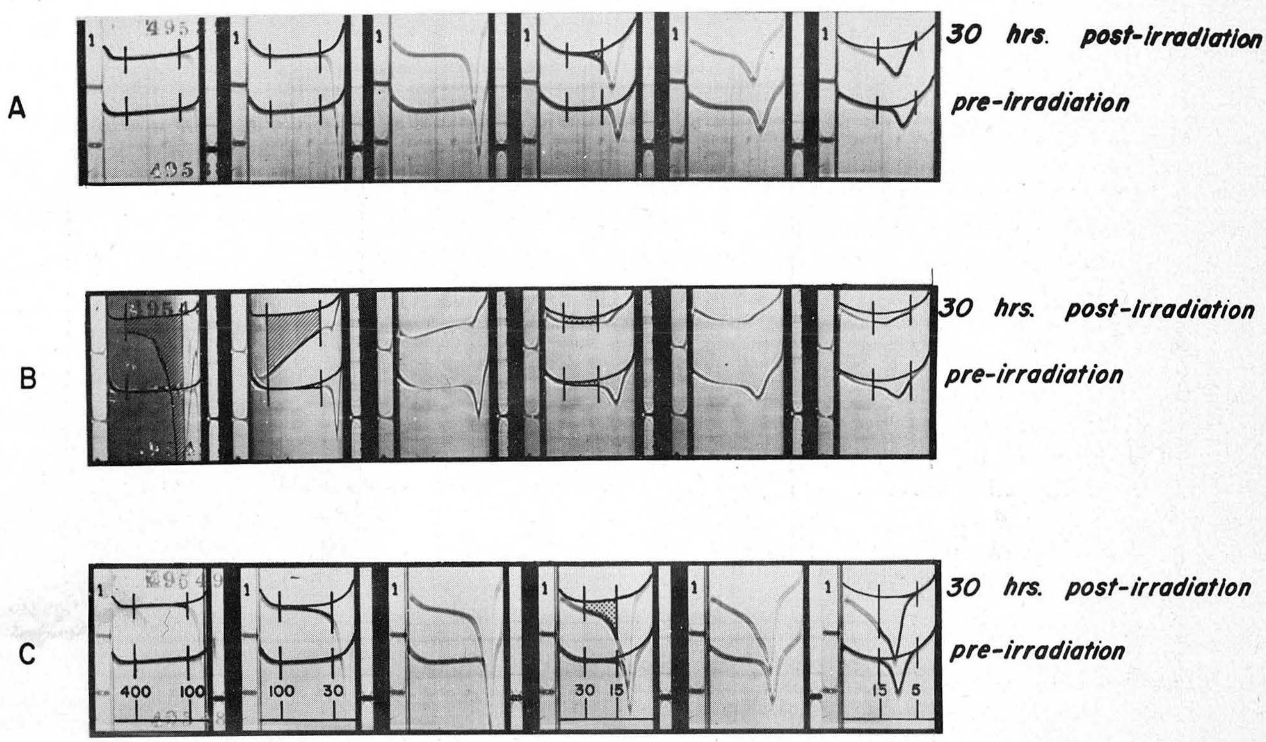

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Fig. 1. Lipoprotein patterns before and after radiation. From left to right successive frames are at 0, 6, 12, 22, 30, and 38 minutes after the rotor has reached 52, 640 rpm. A flotation rate scale for each frame is drawn at the bottom of the picture. The smooth curves drawn above the patterns represent the positions of the patterns when no lipoprotein is present. The area bounded by the reference curve, the pattern, and any two vertical lines is proportional to the concentration of the lipoproteins characterized by this S_f range.

A. Rabbit 332. Showing only a small increase in any class.

B. Rabbit 312. Showing a large increase primarily in the S_f 30-400 class.

C. Rabbit 338. Showing a significant increase in the S_f 15-30 class without any large increase in the S_f 30-400 class.

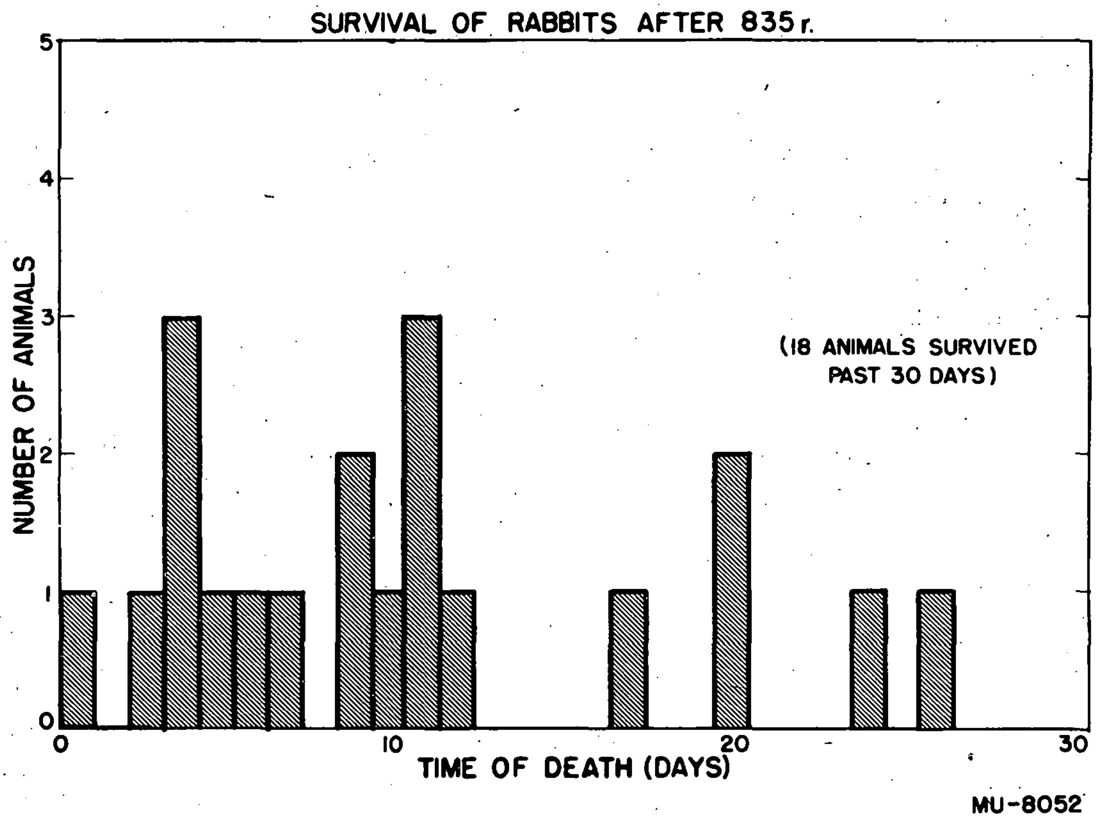

Fig. 2. Survival of rabbit after 835 r total body x-irradiation.

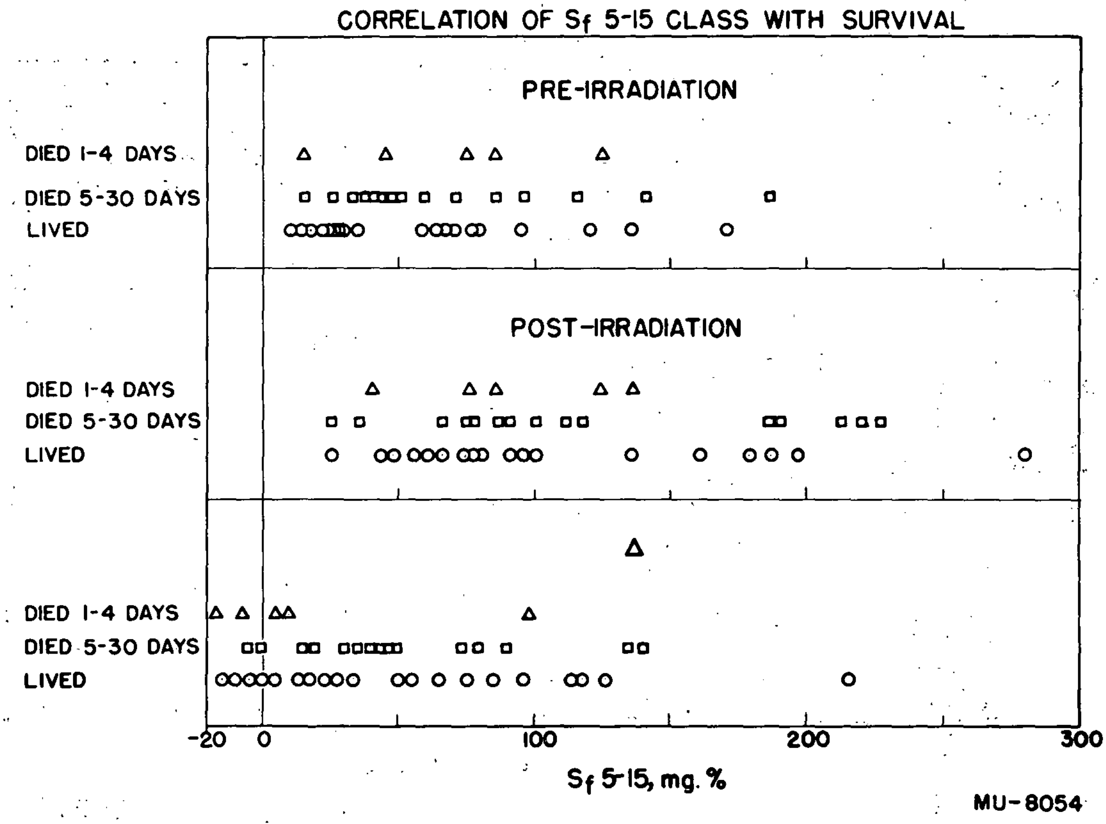

Fig. 3. Correlation between the level of S_f 5-15 class and subsequent death of the animal.

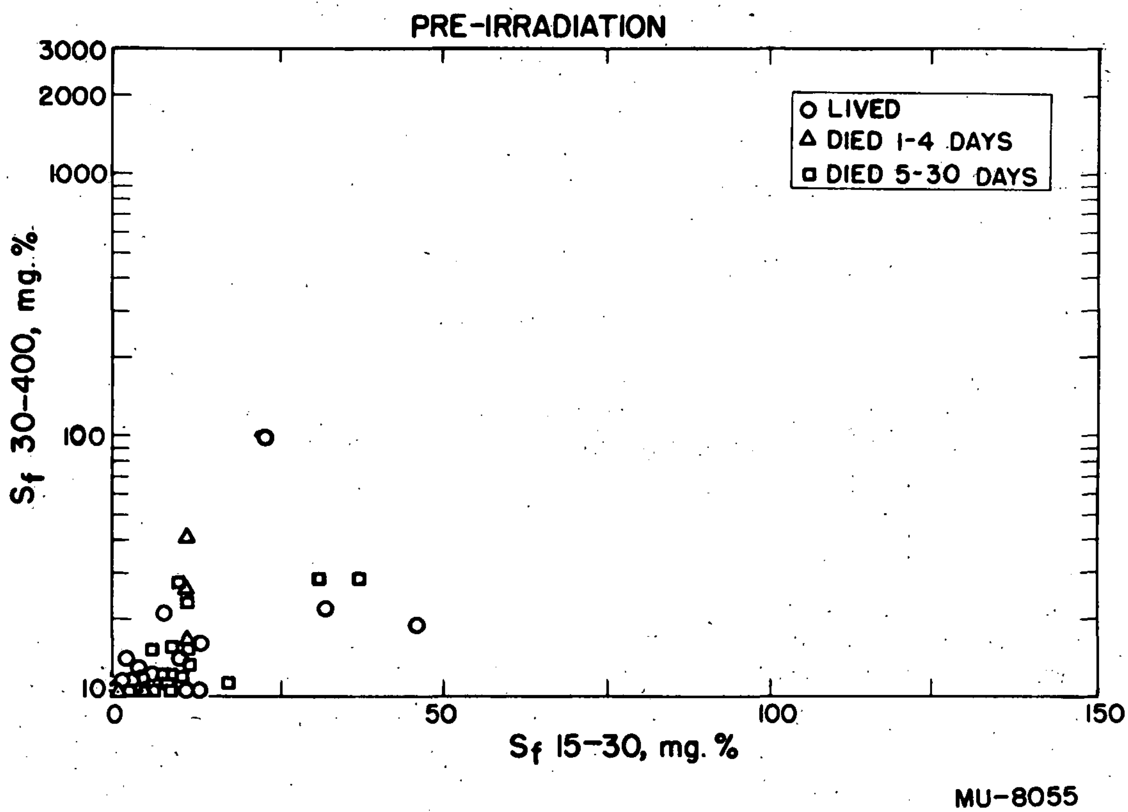

Fig. 4. Survival as a function of preirradiation levels of the S_f 15-30 and S_f 30-400.

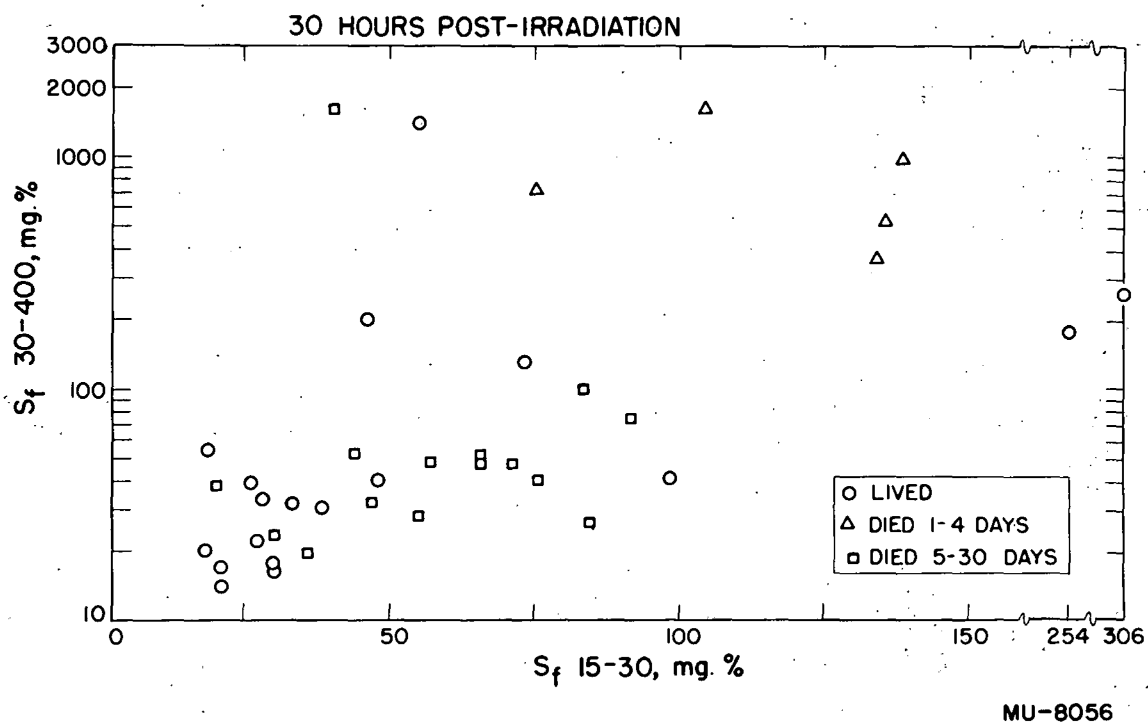

Fig. 5. Survival as a function of the 30-hour postirradiation levels of the S_f 15-30 and S_f 30-400.

A plot of the distribution of the survival groups as a function of the ΔS_f 15-30 and ΔS_f 30-400 is shown in Fig. 6. In order to statistically analyze the correlation the following definitions will be used:

	S_f 30-400	>	300 mg%	=	+ S_f 30-400
	S_f 15-30	>	27 mg%)		
but			)	=	+ S_f 15-30
	S_f 30-400	<	300 mg%)		
	S_f 15-30	<	27 mg%)		
			)	=	- both classes
	S_f 30-400	<	300 mg%)		

Figure 6 may now be tabulated as follows:

	+ S_f 30-400	+ S_f 15-30	- both classes	
died 1—4 days	5	0	0	5
died 5—30 days	1	11	3	15
survived past 30 days	1	4	13	18
	7	15	16	38

$$x^2 = 27.1$$

$$p < 0.001$$

This table may be broken down into three separate correlations. First, the correlation between a large increase in the S_f 30-400 class and death in 1 — 4 days.

		+S_f 30-400 Yes	No
died 1 - 4 days	Yes	5	0
	No	2	31

$$x^2 = 20.2$$

$$p < 0.001$$

Second, the correlation between an increase in the S_f 15-30 class without an increase in the S_f 30-400 class and death in 5 — 30 days:

		+S_f 15-30 Yes	No
died 5 - 30 days	Yes	11	4
	No	4	19

$$x^2 = 9.7$$

$$p < 0.01$$

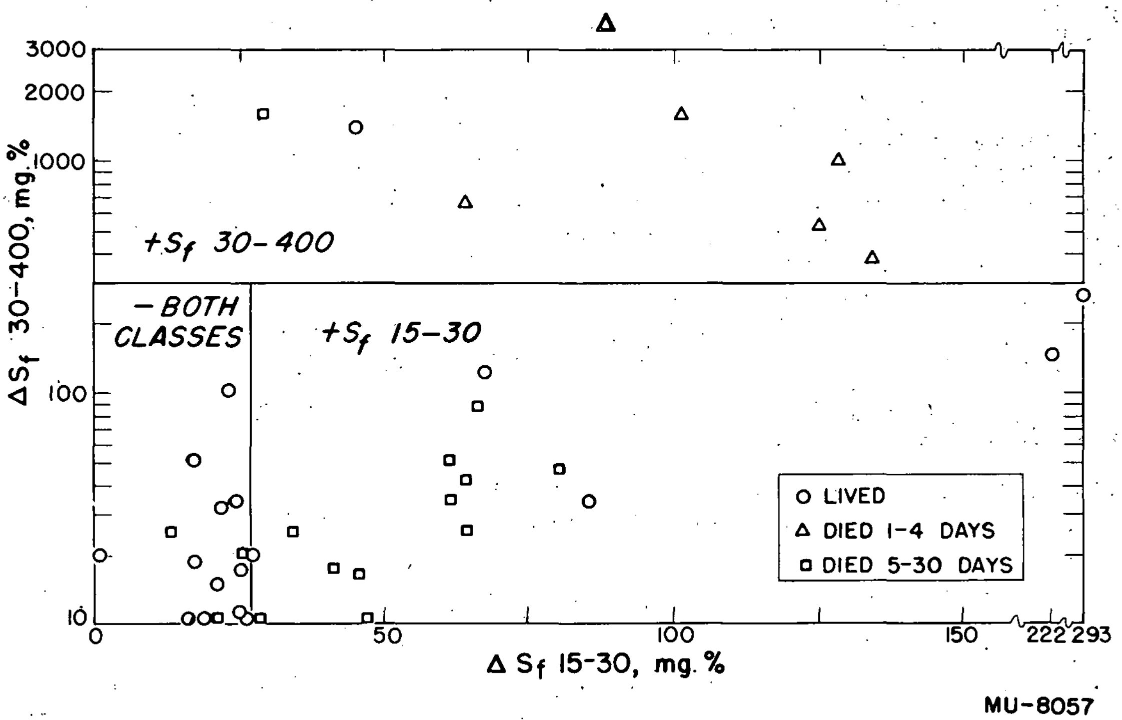

Fig. 6. Survival as a function of the ΔS_f 15-30 and ΔS_f 30-400 classes. The horizontal and vertical lines divide the area of the graph into 3 subareas which form the mutually exclusive groups against which the time of death was tested. It can be seen that animals in one of the survival periods shown fall, in general, into one of the 3 areas on the graph.

Third, the correlation between only small increase in either class and survival past 30 days:

		- both classes	
		Yes	No
survived past 30 days	Yes	13	5
	~~No~~ No	3	17

$x^2 = 10.5$

$p < 0.01$

There was essentially no difference in the response of the two sexes for this group of rabbits. Figure 7 shows the distribution of the two sexes as a function of the ΔS_f 15-30 and ΔS_f 30-400.

It is interesting to note that, as shown in Fig. 8 a and b, animals showing a large increase in either the S_f 15-30 or S_f 30-400 class do not die sooner than those showing only a moderate increase in the class. In fact, an extremely large increase in either of these classes seems to enhance the animal's chances for survival. This observation is in agreement with the results reported by Steadman[3] who stated that in a series of rabbits given 1,000 r the ones which survived over 30 days had the highest lipid values. These facts suggest the hypothesis that the hyperlipoproteinemia following irradiation represents a need of the animal for increased fat mobilization and that in those animals showing extremely large amounts of lipoprotein this need has been satisfactorily met.

Such an hypothesis is attractive, but considerably more data are necessary to test its validity. Experiments are now in progress in an attempt to gain such data.

DISCUSSION

The usefulness of serum lipoprotein concentration measurements as an index of radiation damage has been enhanced by the discovery that the time of death within the 30-day period is related to increased concentration of individual S_f classes of lipoproteins. If the deaths are subdivided into early (1—4 days) and late (5—30 days) deaths, it is found that an increase of concentration in one class of lipoprotein is associated with the early deaths, while the late deaths are associated with elevated concentrations in another class.

This suggests that lipoprotein measurements may be used to distinguish at least two types of radiation injury which are responsible for the total number of deaths during the 30-day period. As a corollary, if the grouping of the times of death is accepted as an indication of qualitative differences in radiation damage, increased concentrations in various lipoprotein classes may be due to different primary causes. This is further supported by the fact that animals

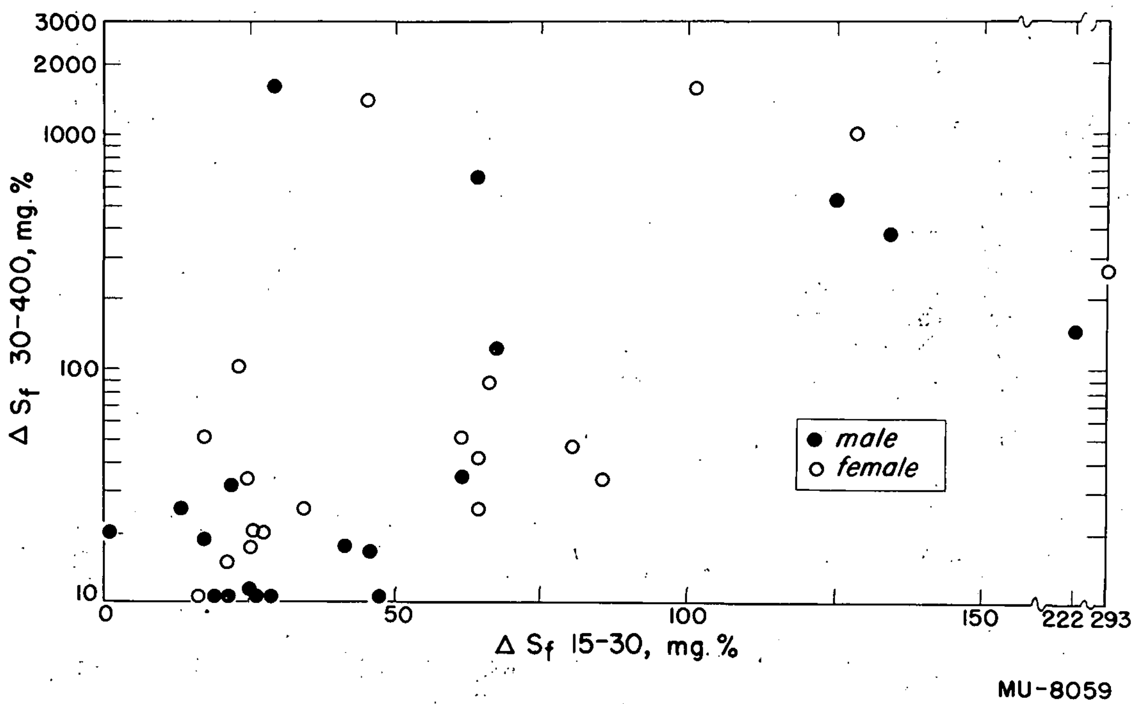

Fig. 7. Postirradiation lipoprotein increase in the two sexes.

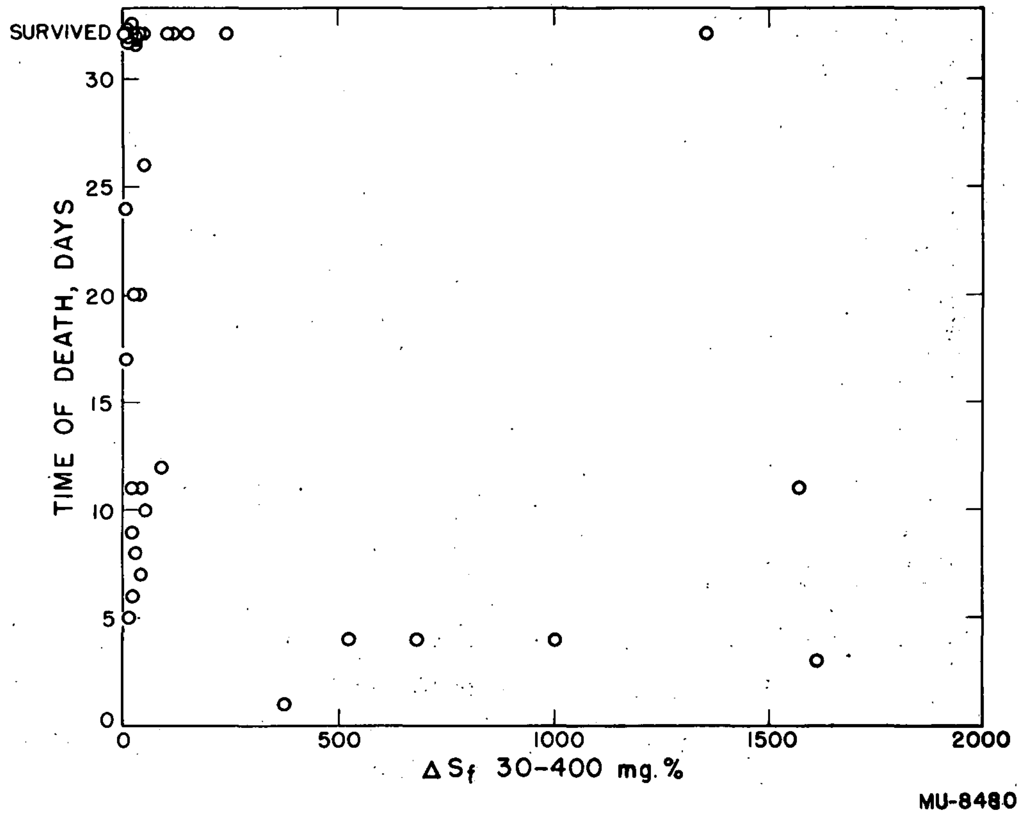

Fig. 8a. Absolute lipoprotein levels versus time of death. Time of death of all rabbits plotted against the ΔS_f 30-400.

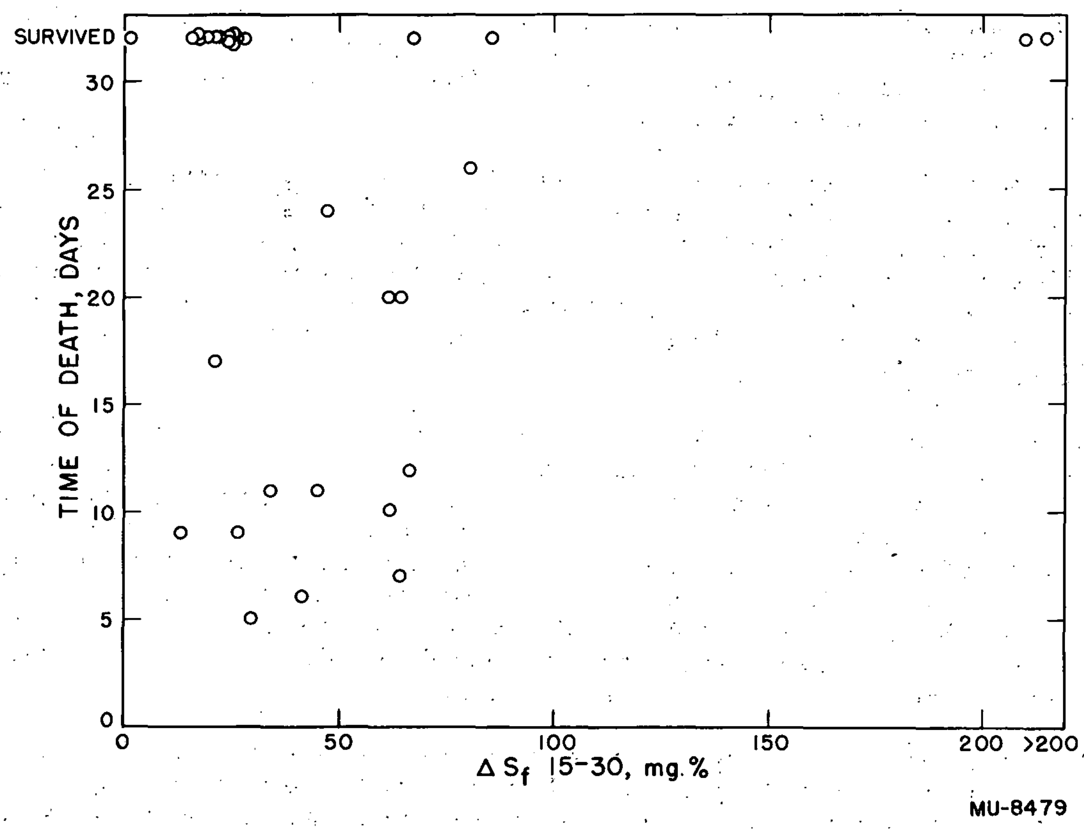

Fig. 8b. Absolute lipoprotein levels versus time of death. Time of death of animals not showing a large increase in the ΔS_f 30-400 class plotted against the ΔS_f 15-30.

showing a large increase in a particular class of lipoprotein die no sooner than those showing only a moderate increase in the same class. This tends to indicate that the time of death is determined more by the type of injury sustained than by the degree of such injury.

If it is true that deaths after radiation are due to two or more types of damage, it would be advantageous to study each type of death separately instead of putting them all together in an expression such as the LD_{50} 30 days. The serum lipoproteins appear to offer a means of distinguishing at least two of these types of damage at an early time (24 hours) in the radiation syndrome.

It is interesting to note that Pierce[4] has shown that lipoprotein increases somewhat similar to those related to death in 1 to 4 days postirradiation can be produced by the injection of cortisone and ACTH. It is possible that hyperlipoproteinemia in the higher S_f classes reflects an increased production of these hormones following irradiation.

In view of this response to cortisone the possibility presents itself that the animals showing the increase in the higher S_f classes of lipoproteins may be in a shock state. Milch[5] has shown increases in serum lipoproteins in the rabbit following crush, burn, and localized radiation. Similar results have been obtained by Thomas in the dog[6]. Also it has been shown by Pierce[7] that extensive bleeding causes elevations in the lipoproteins, particularly in the higher S_f classes.

As was reported previously,[1] intravenously injected heparin tends to hasten the return of the postirradiation lipoprotein levels to normal values. Preliminary experiments on the effect of heparin on survival in the rabbit seem to indicate no effect on the over-all survival but possibly a decrease in the number of animals dying in the 1- to 4-day period. Since heparin has been found to be particularly effective in lowering the levels of that class of lipoprotein which is associated with death in 1 to 4 days, the possibility should be evaluated that if a normal lipoprotein spectrum could be maintained following irradiation, survival could be enhanced.

Experiments are in progress to determine the mechanism of the post-irradiation hyperlipoproteinemia, and in view of the apparently vital significance of lipoprotein levels it is hoped that these experiments will yield important information on the nature of radiation injury.

SUMMARY

1. In rabbits given an LD_{50} dose of x-irradiation, it has been shown that there is a strong relationship between increases in certain S_f classes of serum lipoproteins and the time of death.

a. Elevation of the S_f 30-400 class is correlated with death in from 1 to 4 days postirradiation.

b. Elevation of the S_f 15-30 class is correlated with death in from 5 to 30 days postirradiation.

c. Animals showing only minor changes in these lipoprotein classes survive past 30 days.

2. The fact that an extremely large increase of serum lipoproteins apparently enhances survival may indicate a need for increased fat mobilization in the irradiated animal.

3. The lipoprotein spectrum following irradiation can be used to distinguish at least two of the several types of injury sustained by the irradiated rabbit.

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