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ABSTRACT

Changes in lipoprotein concentrations following x-irradiation have been studied in the rabbit, dog, rat, and mouse. At the radiation doses employed, the alterations in serum lipoprotein concentrations occurred at a different time for each species.

Hyperlipoproteinemia is associated with higher average flotation rates for the lipoprotein molecules, indicating qualitative as well as quantitative changes in the metabolism of lipids in the irradiated animal.

A relatively large increase in the concentration of the low density lipoproteins precedes death from irradiation in the rabbit, rat, and dog.

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INTRODUCTION

The relationship between increased lipoprotein concentrations and radiation mortality has been demonstrated for the rabbit.[1,2] In that species measurements of the kind and amount of lipoproteins present 30 hours post-irradiation are significantly correlated with the time interval in which the animal dies after receiving an LD_{50} dose of x-irradiation. In this paper the nature of the lipoprotein changes in the rabbit are presented in more detail and the results of radiation studies in other species are given.

METHODS

Irradiation

All animals irradiated received total-body irradiation from a 220-kv x-ray beam. The filters employed were 0.5 mm of Cu and 1.2 mm of Al. The dose was measured in paraffin phantoms appropriate to the species being irradiated.

Seventy-three New Zealand white rabbits received 800 to 850 r at a dose rate of 40 r/min.

Eight dogs were irradiated; two received 425 r; six received 550 r. The dose rate for all was 6 r/min.

Thirty rats of the Long-Evans strain received 850 r; Seventy-five inbred Curtis Dunnings were given 750 r. The dose rate was 20 r/min.

One hundred and sixty A-strain mice received 800 r at a rate of 20 r/min.

Blood samples were drawn before and at various times after irradiation for all animals. Bleeding was done via the ear vein, neck vein, abdominal aorta, and heart for the rabbits, dogs, rats, and mice respectively.

Lipoprotein Analysis

The techniques for separating lipoproteins from other protein molecules of the serum on the basis of their hydrated densities are described fully by De Lalla and Gofman.[3] Their paper describes three types of preparative ultracentrifugations which result in the isolation of lipoprotein molecules of densities less than 1.063 g/ml, densities less than 1.125 g/ml, and densities less than 1.200 g/ml. Lipoproteins isolated in a flotation medium of density 1.063 g/ml are referred to as low-density lipoproteins, while the additional lipoproteins isolated from media of densities 1.125 g/ml and 1.200 g/ml are the high-density lipoproteins.

After preparative ultracentrifugation the isolated lipoprotein group is run in an analytic ultracentrifuge, which characterizes the molecules in terms of concentrations and flotation rates.

In this paper three flotation rates are used corresponding to the three types of preparative ultracentrifuge runs by which the samples to be analyzed were prepared.

(a) 1 S_f unit represents a flotation rate of 1×10^{-13} cm/sec/dyne/g in a sodium chloride solution of density 1.063 g/ml at 26°C.

(b) 1 $S_{f_{1.12}}$ unit. Defined as above except that the solution density is 1.125 g/ml and contains sodium nitrate and D_2O as well as the sodium chloride.

(c) 1 $S_{f_{1.20}}$ unit. In this case the solution density if 1.200 g/ml, with other factors corresponding to those of the $S_{f_{1.12}}$ unit.

Since the high-density lipoproteins are present in low concentration in the rabbit, only the low-density lipoproteins of flotation rates S_f 5–400 were analyzed in that species. In contrast, in the rats and mice the concentration of high-density lipoproteins is much greater than that of the low-density group. The concentrations of the high-density lipoproteins were measured between $S_{f_{1.20}}$ 2–12 and between $S_{f_{1.20}}$ 0–7 for the rats and mice respectively. For one group of rats the changes in concentration of the low-density molecules were also determined.

The lipoproteins of the dog were divided into three classes: Class 1 includes the low-density lipoproteins; Class 2 includes the additional lipo-

proteins of higher density isolated in a flotation medium of density 1.125 g/ml; Class 3 includes the lipoproteins of still high density, which are isolated in addition to Classes 1 and 2 in a flotation medium of density 1.200 g/ml.

These three classes can be measured approximately in terms of flotation rates as follows:

Class 1 ---$S_{f_{1.12}}$ 4-80

Class 2 ---$S_{f_{1.12}}$ 0-4

Class 3 --- When the $S_{f_{1.12}}$ 4 flotation rate is corrected to its equivalent $S_{f_{1.20}}$ value, all molecules of Class 2 and Class 3 have flotation rates lower than this $S_{f_{1.20}}$ value in the 1.200-g/ml medium. Subtraction of Class 2 from the concentration of these molecules gives the concentration of Class 3.

RESULTS

The Rabbit

Eighteen rabbits were used to study the changes in lipoprotein concentration with time after irradiation. They were divided into two groups of nine animals. For one group the 30-hour serum sample was markedly opalescent, for the other group the postirradiation serum was not visibly different from the preirradiation samples. The nature of the changes occurring in the S_f 5-400 lipoproteins of these animals is shown schematically in Fig. 1.

In general the lipoprotein levels at 12 hours postirradiation are unchanged from the preirradiation values. Exceptions are found, however, in some animals a slight reduction occurs before this time. In one animal, whose serum later became very opalescent, a slight increase in the lipoprotein concentrations was seen as early as 8 hours. Usually the first indication that large lipoprotein increases will occur is noted between 12 and 18 hours. In all animals a maximum in the concentration curves is reached at approximately 30 hours. This is followed by a decrease during the second and third days. From this point the curves of Fig. 1 show that individual

animals respond differently. Some animals of each group show a further increase in lipoprotein concentration, which reaches a maximum between 5 and 10 days postirradiation. And finally, a fall toward preirradiation values takes place during Days 12 to 20.

The nature of the lipoprotein concentrations at the 5-to-10-day maximum is qualitatively as well as quantitatively different for the two groups. For the nonopalescent group the increase at this time is found almost exclusively in the S_f 5–15 class. For the two animals of the opalescent group the increases are found in the higher S_f classes. These two animals died at seven days.

In order to study the nature of the 30-hour postirradiation lipoprotein increase more intensively, a series of 73 rabbits was used, including the 18 already discussed. All received 800 to 850 r of x-irradiation. For this study the changes in lipoprotein concentration between the preirradiation and the 30-hour postirradiation levels were determined, rather than the 30-hour levels themselves. For each of the four S_f classes, S_f 5–15, S_f 15–30, S_f 30–100 and S_f 100–400, the preirradiation concentration was subtracted from the postirradiation value.

For this larger series it was again found that an increase in total lipoprotein values occurred in all cases at this time. Previous reports have indicated that increases may occur in any one or all of the four S_f classes of which the total low-density lipoprotein level is composed. For this series of animals it was found that for the S_f 5–15, S_f 15–30, S_f 30–100 and S_f 100–400 classes the probabilities that the largest change will take place in the respective classes are proportional to 3:2:3:1.

Further information on the nature of the changes induced by irradiation has been obtained by examining the changes occurring within the S_f 5–15 class itself. The change in concentration for a whole class does not completely describe the effects observed within that class. Within any class is a group of lipoprotein molecules that are distributed according to their flotation rates. The curve given by the analytic ultracentrifuge, at a given time after the rotar has reached its operating speed, can be considered as an inverted frequency-distribution curve. An ordinate of the curve is proportional to the concentration of lipoprotein molecules of a species characterized by the S_f rate at the point at which the ordinate is measured. The position of the maximum ordinate of the curve at a given time during an ultracentrifugal run defines the S_f rate of

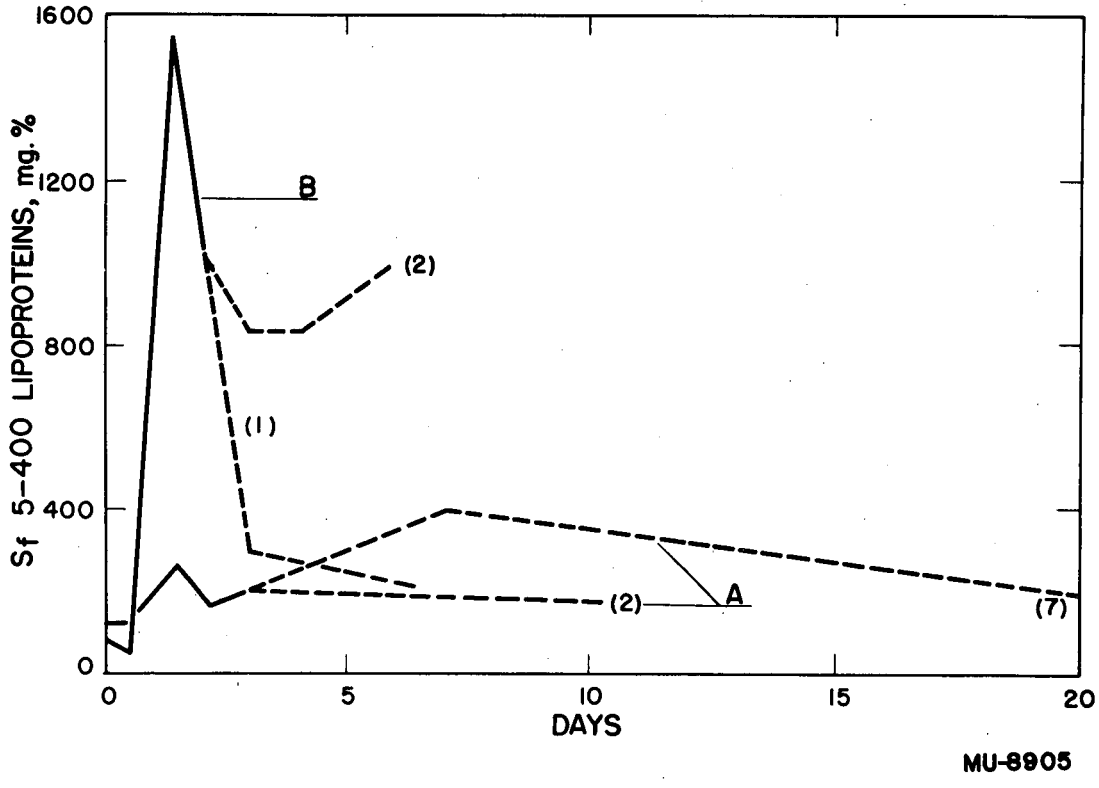

Fig. 1 Schematic graph of the serial changes in concentration in irradiated rabbits. The maxima are time as well as concentration averages of the maxima from individual rabbit curves. Curve B represents the average values for 9 animals showing markedly opalescent serum at 30 hours postirradiation. Curve A represents the lipoprotein concentrations of 9 animals whose sera did not become opalescent. The dotted lines indicate alternative types of changes possible after the maximum increase at 30 hours. The number of animals following each path is indicated in parentheses. Six of the 9 animals of Curve B died in less than 3 days.

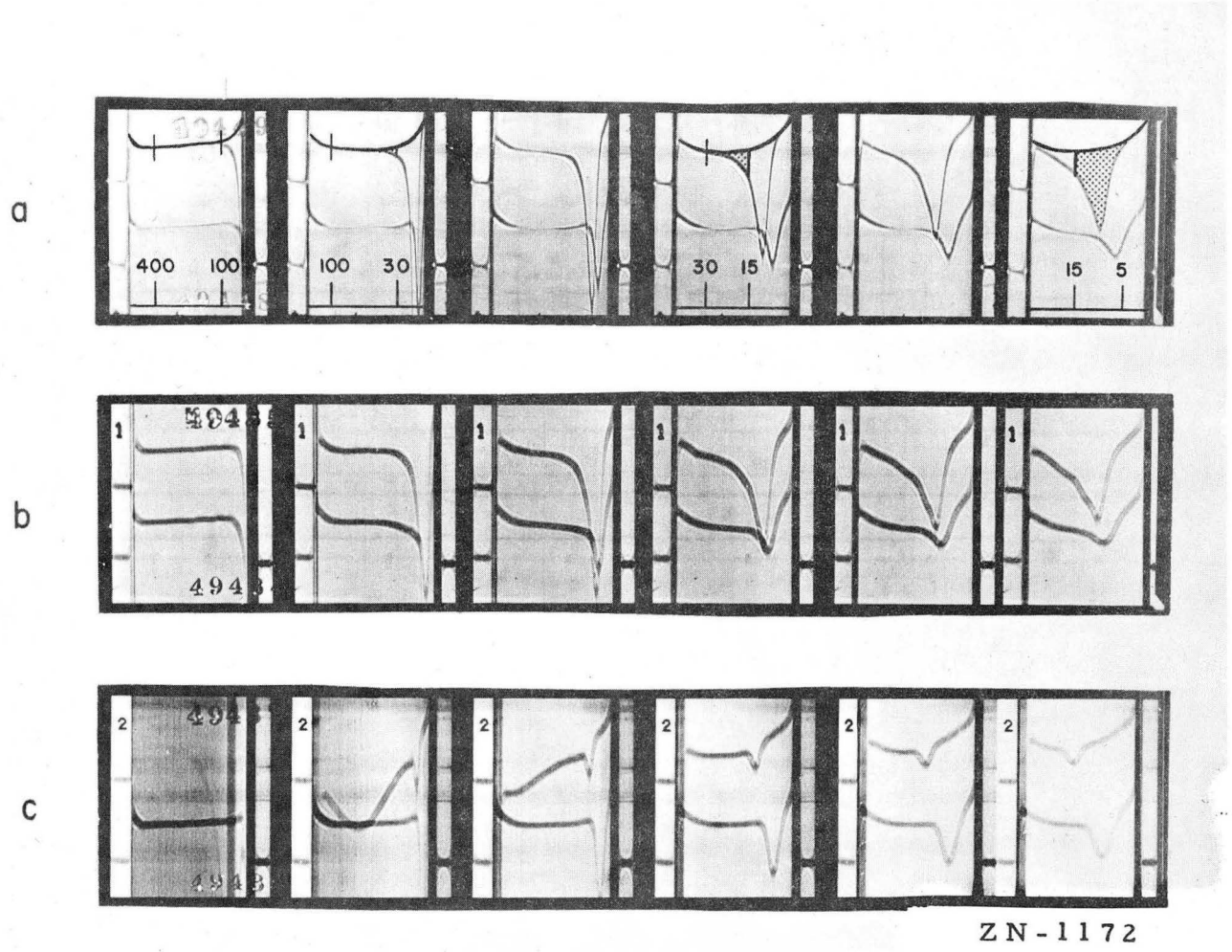

Fig. 2 Ultracentrifugal patterns of lipoproteins present in rabbit serum before and after x-irradiation. From left to right sucessive frames are at 0, 6, 22, 30, and 38 minutes after the rotor has reached 52,640 rpm. A flotation-rate scale in S_f units is drawn at the top of the figure. On each film the preirradiation pattern is on the bottom with the 30-hour postirradiation pattern from the same rabbit immediately above. In (a) the limits of the 4 S_f classes are indicated by vertical lines through the top pattern in frames 1, 2, 4, and 6. The smooth curves drawn in above the patterns in (a) represent the position of the top of the pattern when no lipoprotein is present. The area bounded by this reference curve, the pattern, and 2 S_f rate lines is proportional to the concentration of the lipoproteins characterized by this S_f range.
(a) and (b) In the rabbits represented by these two sets of patterns the major postirradiation increase occurred in the S_f 5–15 region.
(c) The postirradiation pattern shows a large increase in the S_f 30–400 group.

of the molecule present in highest concentration. In this sense it is possible to speak of the S_f rate of the maximum ordinate of the distribution.

Figure 2 illustrates the manner in which the S_f rate of the maximum ordinate in the S_f 5-15 class increases after irradiation. In Fig. 2 (a) the maximum ordinate changes from S_f 6.1 before to S_f 10.5 after irradiation. For Fig. 2 (b) the increase is from S_f 8.6 to S_f 10.1. In Fig. 2 (c) the rate increases from S_f 6.5 to S_f 13.2.

To examine the effects of bleeding alone on the change in S_f rate, nine control animals were bled before and after a 30-hour interval and compared with 10 animals that were bled before and 30-hours after receiving 800 r. The changes in lipoprotein concentrations and flotation rates are compared in Table I. For the control animals changes in S_f rates of the maximum ordinate within the S_f 5-15 class ranged from -1.6 to +0.7 S_f units. For the irradiated animals the range was from +1.1 to +5.8. Although changes do occur in the S_f rate of the maximum ordinate in the control rabbits, they are, with one exception, only about 1/2 the value seen in the group that has been exposed to x-rays. Also they are distributed between plus and minus values, whereas the change is consistently positive for the latter group. In 90% of the 73 animals irradiated the molecule of the S_f 5-15 class present in highest concentration after irradiation had a higher S_f rate than did the species predominating in the same class before irradiation. In those cases where increases occur in the concentration of the S_f 5-15 class itself this change in S_f rate may be explained by assuming that new molecules of lower density have been introduced into the class. Many cases are found, however, where almost no change in the concentration of the S_f 5-15 class takes place.

It has been repeatedly observed that when a very large increase occurs in either the S_f 5-15 class or the S_f 30-400 class the change in lipoprotein level is restricted mainly to that class. This inverse relationship is plotted in Fig. 2.2. Figure 2 (c) gives an example of an animal in which a large increase in the S_f 30-400 class is found while the concentration of the S_f 5-15 molecules is almost unchanged. (The postirradiation sample was run at one-half the concentration of the preirradiation sample.) Indirect evidence supports a postulate that in this and similar cases the animal may be producing the same molecules of the S_f 5-15 class after irradiation as before, but that they are modified in the blood in such a manner as to increase their average S_f rate. We have shown[4] that oleic acid, lipid emulsions, and egg lipoprotein

Table I

Difference in S_f rates between the molecule in the S_f 5–15 class present in highest concentration at 30 hours postirradiation and the molecule present in highest concentration in the same class prior to irradiation. These values are given for 10 irradiated rabbits and also for 9 nonirradiated rabbits, bled at the same times. The changes in concentration for each S_f class are also presented.

Rabbit	S_f rate difference	Differences in concentrations, mg%				
		S_f 5–15	S_f 15–30	S_f 30–100	S_f 100–400	S_f 5–400
		Nonirradiated				
1	0.3	12	7	7	11	37
2	0.7	12	18	14	6	50
3	-0.2	-10	-6	-4	7	-13
4	0.3	-8	16	0	0	8
5	-0.2	14	6	24	4	48
6	-1.6	2	14	2	2	20
7	-0.4	33	10	22	0	65
8	-0.4	0	6	4	2	12
9	-0.8	39	-6	-70	6	-31
		Irradiated				
1	2.5	51	22	8	0	81
2	1.2	144	52	-1	2	197
3	5.5	- 4	111	1000	751	1860
4	5.8	52	66	38	5	161
5	1.6	33	23	26	9	91
6	2.9	153	48	15	3	219
7	3.2	83	69	35	10	197
8	1.6	49	70	25	2	146
9	1.4	81	90	38	- 1	208
10	1.1	129	27	15	4	175

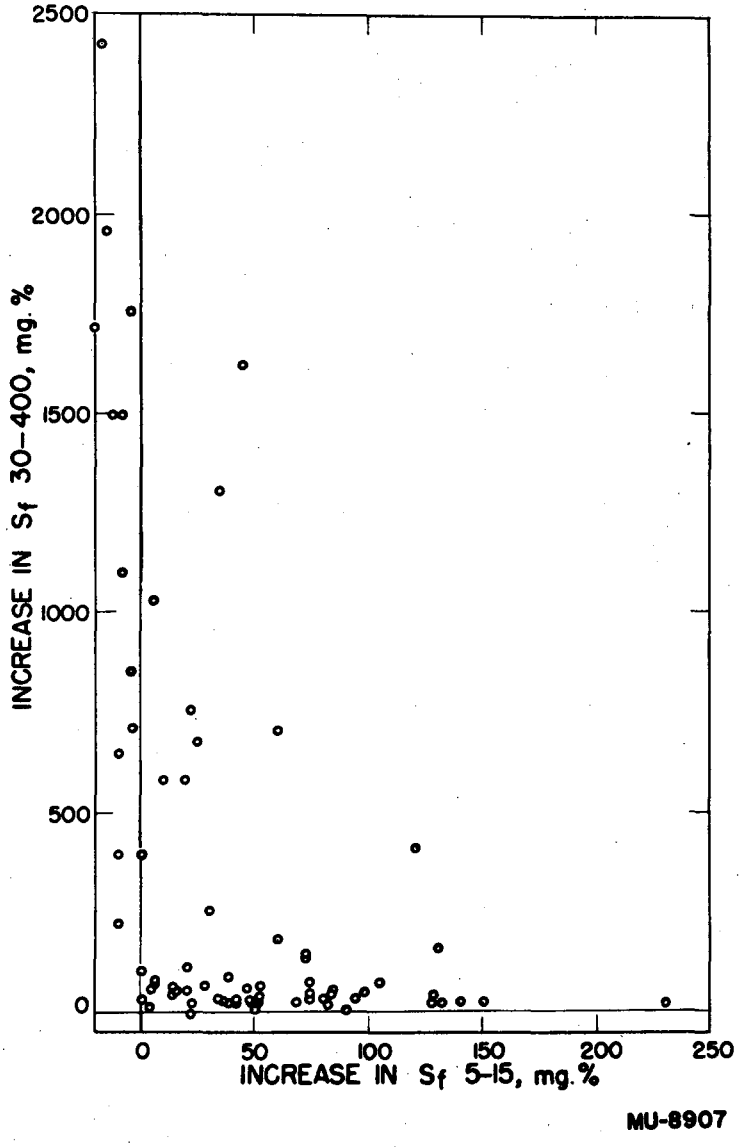

Fig. 2.2 Plot of the concentration increase in the S_f 30–400 lipoproteins as a function of the increase in the S_f 5–15 class in 73 rabbits at 30 hours postirradiation.

can increase the S_f rates of rabbit and human lipoproteins in vitro without appreciable concentration changes. Evidently the density of the serum lipoproteins is lowered either by the incorporation of more lipid into the molecules or by a process of exchanging the lipid already present for lipid material of a lower density. The increase in the average flotation rate of the S_f 5–15 class which is seen in the postirradiation pattern of Fig. 2 (c) may be thus explained as being due to the presence of large concentrations of the low-density S_f 30–400 class in the serum.

The Dog

The lipoprotein spectrum in the dog differs markedly from that in the rabbit. Although the rabbit has some high-density lipoproteins, the concentration of such molecules is very low. In contrast, the dog has large concentrations of high-density lipoproteins. Evidently the method of handling lipids differs in the two species. Figures 3(B), 3 (C) and 3(D) present the analytic patterns of the lipoproteins present in the serum of three dogs at the terminal period, 12 to 14 days postirradiation. These may be compared to Fig. 3 (A), which is a representative control. In general there is an increase of concentration of Classes 1 and 2 and a decrease in Class 3. The distribution of molecules within Classes 1 and 2 has broadened, with increasing concentrations of molecules of higher flotation rates appearing. In Figs. 3 (B-2) and 3(C-2) double "peaks" are seen in Class 1, indicating that new types of lipoprotein molecules are being produced.

The serial changes in the three classes are given in Fig. 4 for an animal that received 550 r. A sharp rise is shown in Class 1 and Class 2 from 8 to 10 days postirradiation, and a sharp drop in the Class 3 lipoprotein concentration, beginning at 10 days. Qualitatively similar results are reported for the dog by Entenman.[5] Large lipoprotein changes in the dog evidently coincide with its general condition, becoming apparent in only those animals which die, and at a much later time than in the rabbit.

Representations of the lipoprotein patterns are given in the form of histograms in Fig. 5. Each histogram is divided into three parts horizontally to represent the three lipoprotein classes. Histograms are given for each of the 10 animals studied, both before and at 10 to 13 days after irradiation. The large concentration in Class 3 relative to the concentrations for Classes 1 and

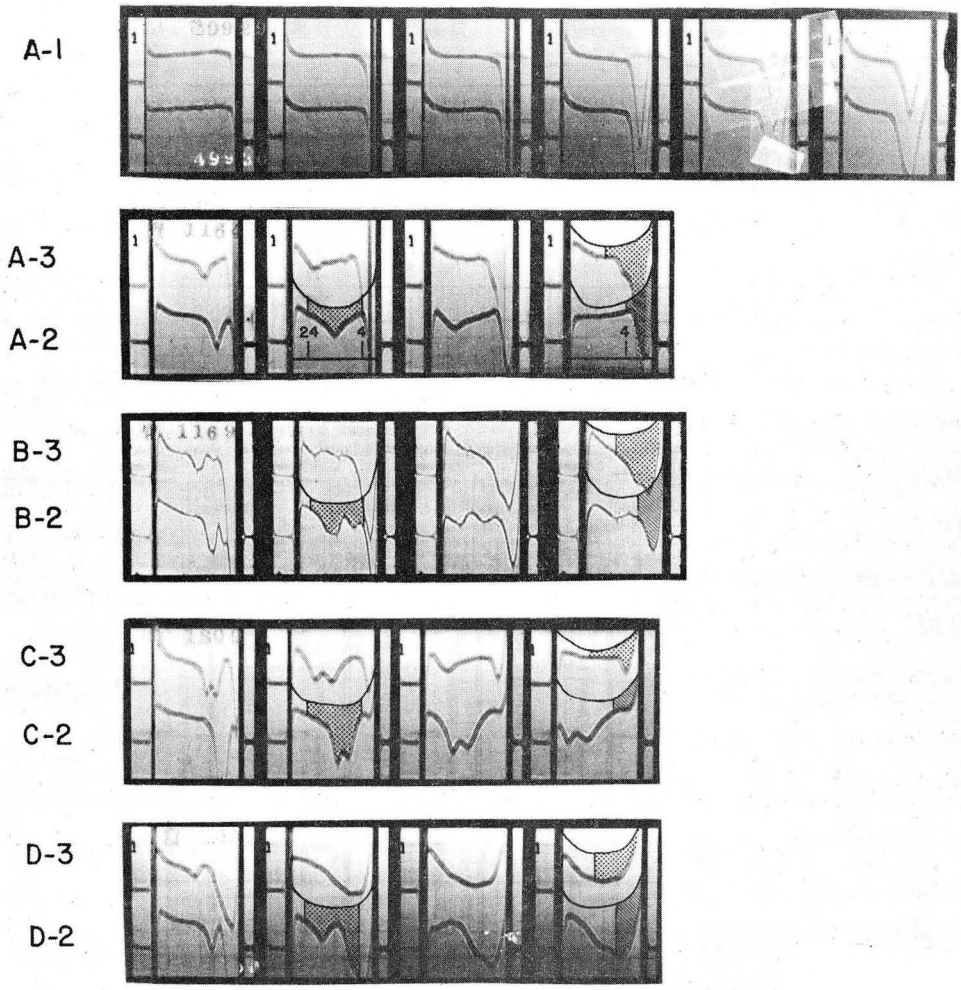

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Fig. 3

Fig. 3 Ultracentrifugal patterns of the serum lipoproteins in the dog before and after 550 r of x-irradiation. A, B, C, and D represent different dogs. The number qualifying each letter refers to the type of preparative run made on that sample. Thus A-1, A-2, A-3 were isolated in densities of 1.063, 1.125 and 1.20 g/ml from portions of the same serum sample. All analytical runs were made at 52,640 rpm. For A-1 the successive frames have the same time sequences as the patterns in Fig. 2. For the other four films the frames from left to right were taken at 16, 32, 48, and 64 minutes after the rotor reached 52,640 rpm. A flotation-rate scale in units of $S_{f\ 1.12}$ has been placed at the bottom of the A-2 pattern. In the 32-minute frame of the A-2 pattern the area of the $S_{f\ 1.12}$ 4–24 range is outlined. This represents most of the area associated with the Class 1 lipoproteins. The remaining area ($S_{f\ 1.12}$ 24–80) is measured in earlier frames and is not shown here. Class 2 ($S_{f\ 1.12}$ 0 — 4) is outlined in the 64 minute frame of A-2. The sum of the areas of Class 2 and Class 3 is found in A-3 at 64 minutes. These areas may be seen more distinctly in B, C, and D.

(A) Lipoprotein patterns found in a normal dog.
(B), (C), and (D) Lipoproteins of three dogs 10 to 13 days after receiving 550 r of x-rays.

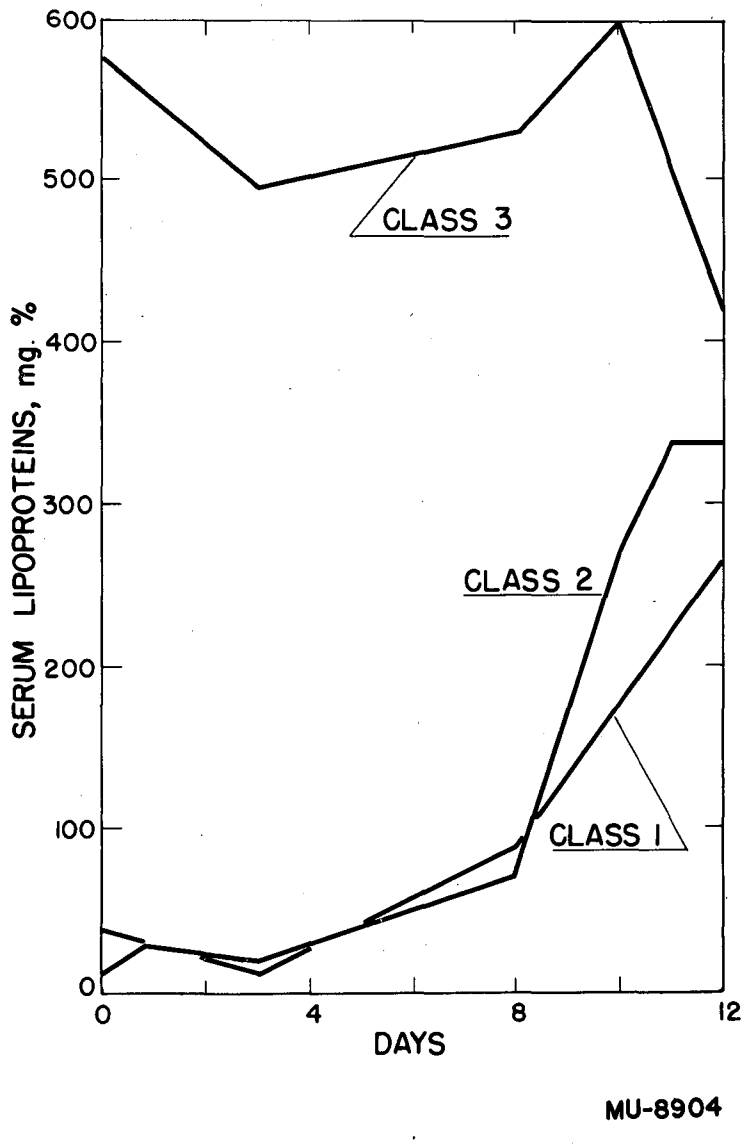

Fig. 4 Serial changes of three classes of serum lipoproteins in a single dog with time after irradiation.

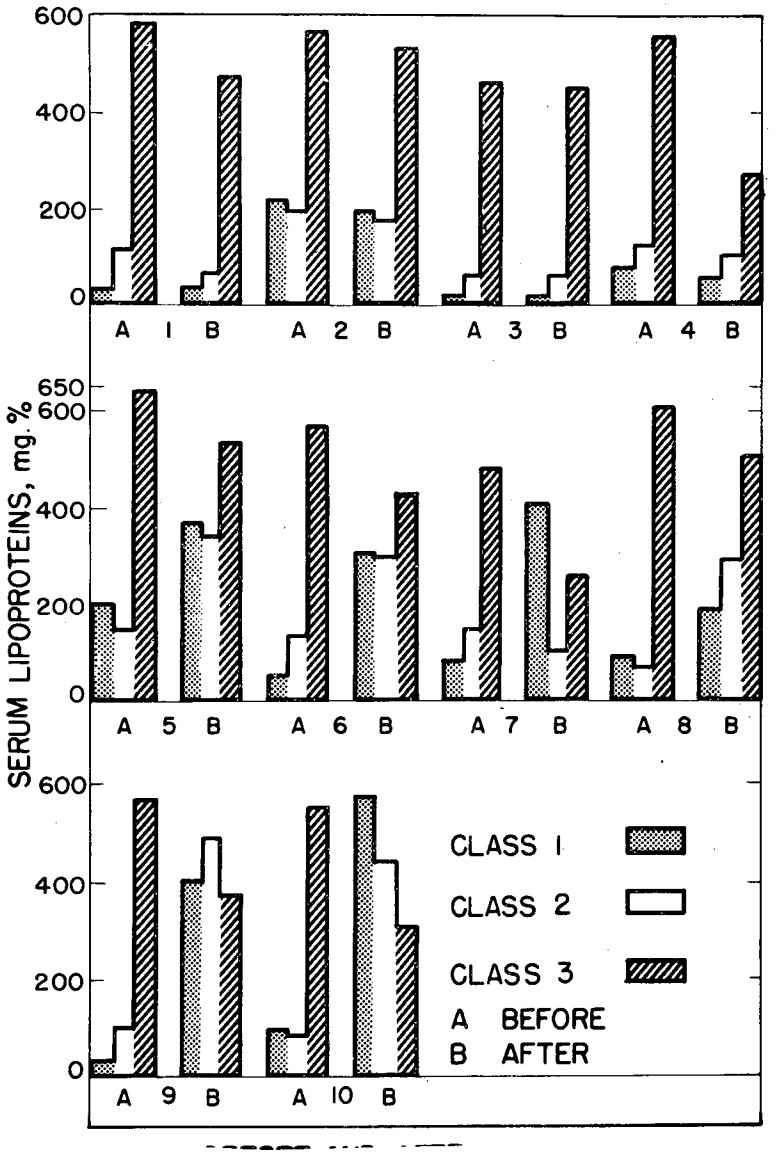

Fig. 5 Histograms showing the relationship between the concentrations of lipoproteins in Class 1, Class 2, and Class 3 in the dog before and at 10 to 13 days postirradiation. The A figures are preirradiation figures. The B figures are the postirradiation figures. Dogs 1 and 2 received 425 r. Dogs 3 and 4 were nonirradiated controls. Dogs 5 to 10 received 550 r. The decrease in concentration in Class 3 for Dog 4 is unexplained.

2 can be observed in all the preirradiation figures. For one of the control animals and the two animals receiving 425 r the lipoprotein classes remain almost unchanged after irradiation, as shown by almost identical pre- and postirradiation figures. The observed loss of concentration in the high-density class of the other control is believed to be due to a technical error. The histograms for Dogs 5 to 10 in Fig. 5 show the postirradiation changes occurring in the six animals that received 550 r. In addition to the general increase in Classes 1 and 2 and the fall in concentration in Class 3, the relative changes in each class can be seen. Thus, Dogs 7 and 10 showed a large increase in Class 1 relative to Class 2, whereas in the other animals receiving 550 r the increases are more equally distributed between the two classes.

Rat and Mouse

It has been shown[6] that a large increase in serum cholesterol concentration occurs in rats on the fourth day after the animals have received an x-ray dose of 600 to 900 r. Since cholesterol is a major component of lipoproteins, it might be expected that lipoprotein increases would occur at this time.

In the normal rat, lipoproteins of high and low densities are found. However, the concentration of molecules present with hydrated densities greater than 1.063 g/ml is much higher than the concentration of molecules of lower density. In this respect the situation in the rat is similar to that in the dog. The two species of animals differ in total lipoprotein concentration. In the normal dog, a value for the total lipoproteins may be 700 mg%, compared to an average value of 100 mg% in the rat. In addition, the high-density lipoproteins of the normal rat are characterized by a higher average flotation rate than those found in the dog.

The nature of the high-density lipoprotein patterns of the rat is shown in Fig. 6. Figure 6 (a) is representative of the control samples. It indicates lipoproteins distributed around a maximum ordinate whose $S_{f\ 1.20}$ rate is 5. It is found that, for any sample, almost all the high-density lipoproteins are included in a range of $S_{f\ 1.20}$ values from 2 to 12. This range was used to measure all samples. Figure 6 (b) shows the high-density lipoprotein pattern

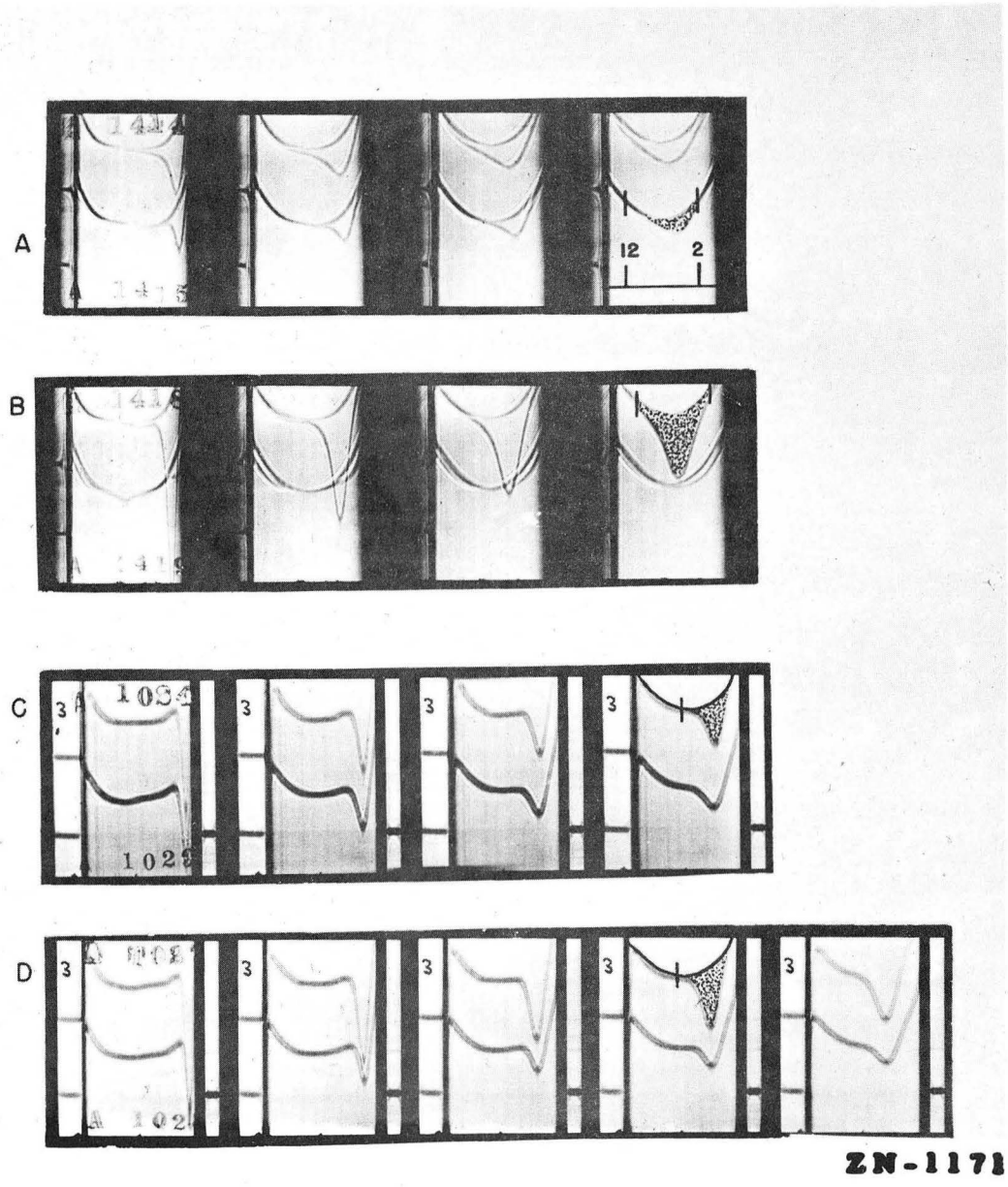

Fig. 6 Ultracentrifugal patterns of the high-density serum lipoproteins in the rat and mouse before and after x-irradiation. Four films are shown with two patterns on each film. The successive frames from left to right were taken at 16, 32, 48, and 64 minutes after the rotor reached 52,640 rpm. The area associated with the $S_{f\ 1.20}$ 2–12 lipoproteins of the rat is outlined in the fourth frame of A (lower pattern) and B (upper pattern). The $S_{f\ 1.20}$ 0–7 class in the mouse is similarly indicated in C (upper pattern) and D (upper pattern).

(A) The normal rat.
(B) A pool of seven rats at 3 days postirradiation.
(C) A pool of normal mice.
(D) A pool of 20 mice at 2 days postirradiation.

at three days postirradiation. This may be compared directly with 6 (a), since the preparative concentration factor is the same. The concentration in 6 (b) is obviously several times as high as in 6 (a).

The changes of lipoprotein concentration with time after irradiation were studied for two groups. Group 1 included 30 Long-Evans strain rats. Animals in this group received 850 r. At various intervals thereafter five rats at a time were exsanguinated and their sera pooled. This experiment was terminated at the end of three days by the death of the remaining animals.

Group 2 consisted of 75 inbred Curtis-Dunning males, which were given 750 r, and 45 controls. Ten animals were used to determine the time of death, which was found to be 9 to 13 days at this dose. The remaining irradiated animals were exsanguinated seven at a time at periods of 1, 2, 3, 4, 6, and 8 days postirradiation. Each set of seven sera was pooled. Similarly, the controls were divided into six pools containing six sera each.

Figure 7 illustrates the changes of lipoprotein level for the two groups of animals after irradiation. The lipoprotein concentration reaches a maximum at three days, which is well above three standard deviations from the mean of the six control samples of Group 2. Group 1 closely parallels Group 2, so that reducing the dosage by 100 r does not change the time sequence of the response. The concentration of lipoproteins of density less than 1.063 g/ml is also plotted for Group 2. The values for these low-density lipoproteins are very small for the first 6 days, so that variations are not significant. The value at 8 days, however, though still small, is apparently well above control levels. The upward trend of these two classes at 8 days may well be the forerunner of another major peak occurring just prior to death at 9 to 13 days.

One consistent finding was that the rats showed some diarrhea on the third day, coincident with the time of the largest lipoprotein increase. Of the 75 irradiated animals of Group 2, 24 were irradiated separately after the nature of the curve had been determined. These were sacrificed at 3 days and three pools of seven animals each were formed. Thus the peak at 3 days actually is an average of four pools of seven animals each. All of the last group irradiated had diarrhea at 3 days.

As was characteristic of the dogs, in addition to the increase in area de-

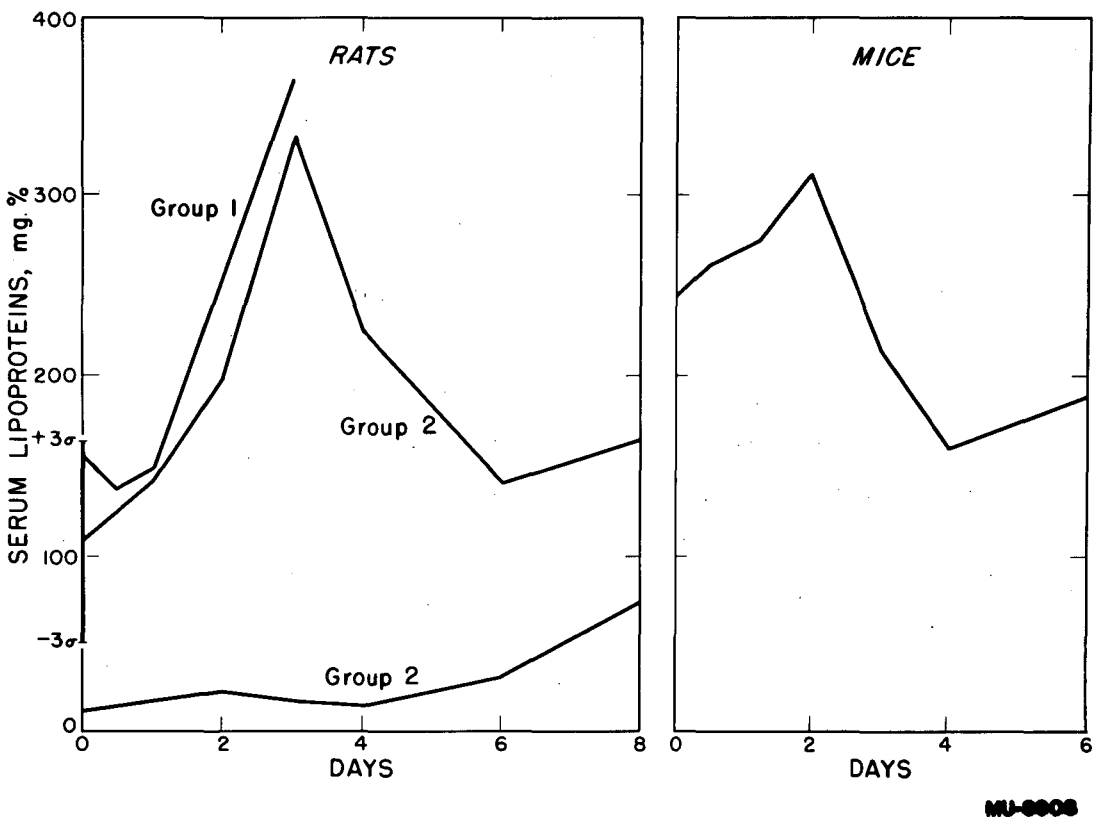

Fig. 7 Serial changes in the high-density serum lipoprotein concentrations with time after irradiation for the rat and the mouse. Two groups of rats were used. Group 1 received 850 r. Group 2 received 750 r. The variability in six control pools of Group 2 is indicated by the 3σ positions on the axis. The lower curve gives the changes in the low-density lipoproteins for Group 2.

fined by the ultracentrifugal patterns, the maximum ordinate of the curve is seen at a higher S_f rate at 3 days postirradiation. The S_f rate of the molecule present in highest concentration is $S_{f_{1.20}}$ 6.6 at 2 days and $S_{f_{1.20}}$ 6.4 at 3 days, compared to a range of $S_{f_{1.20}}$ 5.1 to 5.5 for six control samples.

A group of 160 mice, all males of the A strain, was irradiated with 800 r at a dose rate of 20 r/min. It had previously been determined that a similar group all died by the eighth day at this dose.

At various postirradiation times groups of 10 to 21 animals were exsanguinated. The animals were bled from the heart with the chest cavity open, under ether anesthesia. The sera from each group were pooled and subjected to ultracentrifugal analysis. In Fig. 7, the control and 4-day points are the average lipoprotein concentrations from three pools each, one pool of 16 mice and two pools of 10 mice. The 3-day point is an average of the concentrations found in two pools of 10 mice each. The 1-, 2-, and 6-day points were determined from single pools of 16 to 21 mice each. The lipoproteins of the normal mouse yield analytic patterns similar to those of the rat (Fig. 6). Again high-density lipoproteins predominate, with small traces of low-density molecules also present. The high-density lipoproteins of the mouse are restricted to a narrower $S_{f_{1.20}}$ range than those of the rat. Also the maximum ordinate of the analytic patterns is 2 or 3 $S_{f_{1.20}}$ units slower. In Fig. 7 the concentration of the high-density lipoproteins is shown to rise to a maximum at 2 days. The increase here is only about 25% of the initial value, compared to an increase of 200% in the rat. The most striking change is found at 4 days, where a concentration definitely below the control values is found. The low-density lipoproteins were not measured quantitatively because of their low concentration, but an inspection of the ultracentrifugal patterns indicated that a slight increase in concentration may occur at 6 days.

DISCUSSION

Any critical study of the alterations in serum lipid concentrations must take into account the fact that these lipids exist in the blood in the form of lipid-protein complexes. In human serum we find a whole series of lipoproteins whose hydrated densities range from less than 1.0 g/ml to 1.15 g/ml. While these various molecules all contain lipids and thus permit the general classification of lipoprotein to be applied, they are nonetheless separate and distinct molecules, possibly with widely different metabolic roles.

Each of the four species studied here has a characteristic lipoprotein pattern before irradiation. In the rat, mouse, and dog the molecules present in highest concentration are high-density lipoproteins, characterized by different $S_{f_{1.20}}$ rates for each species. A difference in flotation rate implies that these molecules are different with respect to size, shape, or hydrated density. Recent evidence indicates that the normal rabbit may also have appreciable concentrations of high density lipoproteins. However, in the rabbit the low density lipoproteins constitute a larger fraction of the total lipoprotein concentration than in any of the other species.

As more evidence is accumulated about the metabolism of the various lipoprotein molecules it may be possible to relate the nature and extent of the lipoprotein derangement produced by a given stimulus to the type of lipoproteins normally occurring in the animal. It is of interest that the rabbit and the dog, which are respectively the most radioresistant and the least radioresistant of the four species, show the greatest difference in their normal lipoprotein patterns. The relative importance of the low density lipoproteins in the rabbit is in contrast to the high concentration of lipoproteins of very high density found in the dog.

All the species showed alterations in their lipoprotein concentrations during the postirradiation period. Perhaps the most striking changes for each species would be the large increase of low-density lipoproteins in the rabbit at 30 hours postirradiation, the increased concentrations of high-density lipoproteins in the rat at 3 days, the decreased concentrations in the mouse at 4 days, and the increases in the low-density classes of the dog at 10 to 13 days. In addition, the concentrations of the low-density lipo-

proteins of the rat are elevated at 6 days postirradiation.

The results of these experiments indicate that, at the radiation doses used here, a relationship exists between the time of death and the time at which specific changes in the lipoprotein patterns appear. The three species--the rabbit, rat, and dog--have in common a relatively large increase in the concentration of the low-density (less than 1.063 g/ml) lipoproteins from 1 to 3 days before the expected time of death.

For the rats it cannot be proved from these experiments that the increase is an indication that an individual animal will die, since the observations are determined from pooled samples and the dose was such that no animal had an expectancy of living. However, we have shown[2] that in the rabbit no animal dies between 30 hours and 4 days postirradiation without showing large increases in the concentrations of the S_f 30–400 class of low-density lipoproteins at 30 hours. And of the eight dogs irradiated here, the two that survived 425 r showed no increases in the low-density lipoproteins at 10 to 13 days postirradiation. These results indicate that relatively large increases in the concentrations of this class of lipoproteins may have value in predicting the postirradiation fate of individual animals of the three species.

Differences are found in the general condition of the species at the time at which the largest lipoprotein concentration increases occur. The rabbit seems to be in excellent condition at 30 hours postirradiation, as judged by external appearances. Severe diarrhea accompanies the large concentration increase of the high-density lipoproteins of the rat at 3 days, and the dogs exhibit gross hemorrhage in the gums and intestinal tract at 10 to 13 days.

The extent to which lipoprotein concentration changes are related to other changes occurring in the blood remains to be investigated. It might be expected that the dehydration accompanying diarrhea would produce increased concentrations of all the serum proteins. But the large lipoprotein increases seen in the rat are restricted to the high-density group. The low-density lipoproteins remain relatively unchanged at this time.

Electrophoretic studies by Muntz[7] have shown a serious reduction of the A/G ratio occurring in the x-irradiated dog at 10 to 14 days postirradation. He has shown by lipid extraction that the increased globulin concentration found at that time are not primarily due to an elevation in lipid content. We have confirmed this observation by separating the lipoproteins from the other serum molecules ultracentrifugally. Electrophoretic analysis of lipoprotein-

free portions of the serum show clearly that the A/G ratio is reduced in the irradiated dog at the same time that the maximum lipoprotein changes are found. These events would indicate that further investigation of the relationship between the lipoproteins and other serum proteins is warranted.

SUMMARY

1. The changes in lipoprotein concentration following x-irradiation have been presented for the rabbit, dog, rat, and mouse.

2. The most striking lipoprotein changes in the four species were:

A. A large increase in the concentration of the low-density lipoproteins of the rabbit at 30 hours postirradiation.

B. Increased concentrations of the two lower-density classes and decreased concentrations in the highest-density class in the dog at 10 to 13 days postirradiation.

C. Elevated concentrations of the high-density lipoproteins at 3 days and the low-density lipoproteins at 8 days in the rat.

D. A pronounced decrease in the concentrations of the high-density lipoproteins of the mouse at 4 days.

3. Hyperlipoproteinemia is associated with higher average flotation rates for the lipoprotein molecules, indicating qualitative as well as quantitative changes in the metabolism of lipids in the irradiated animal.

4. A relatively large increase in the concentration of the low-density lipoproteins precedes death from irradiation in the rabbit, rat, and dog.

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