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DISCLAIMER

BLOOD LIPIDS AND HUMAN ATHEROSCLEROSIS**

II. The Influence of Heparin upon Lipoprotein Metabolism.*

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An hypothesis that atherosclerosis is a disease associated with or caused by an error in the metabolism of fat and other lipids has been previously presented (1). The evidence supporting this hypothesis consists in the demonstration of the existence of certain special classes of lipoprotein molecules associated with atherosclerosis in several experimental animal species and in the human developing or manifesting atherosclerosis (1), (2), (3).

The further study of these lipoproteins as well as several other lipoprotein species has revealed (3, 4) that essentially all of the blood lipids (including fat, cholesterol, cholesterol esters, and phospholipids) exist in the blood only in the form of structural entities within several of these lipoprotein molecular classes. Current work is directed toward an understanding of the factors involved in the defect which results in the abnormal elevation of such molecules as the S_f 10-20 lipoproteins in certain individuals. Each lipoprotein, which can be identified in its native state by the ultracentrifugal analysis of serum, presumably has a different functional role in lipid metabolism, Its concentration in the blood is the resultant balance between formation and utilization at any particular steady state of physiological activity. Physiologic factors already reported to influence serum lipoprotein levels include (a) dietary intake of fats and cholesterol, (b) thyroid function, (c) experimental adrenal cortical hyperactivity (rabbit only), (d) age, (e) sex, (f) pregnancy (3, 5). Several errors in lipid metabolism have now been classified

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by the ultracentrifugal analysis of serum lipoproteins. Such are manifest in (a) atherosclerotic state, (b) nephrosis, (c) xanthoma tuberosum, (d), biliary obstruction, (e) acute hepatitis (6), (f) certain cases of diabetes, (g) hypothyroidism, (h) hypercholesterolemia, (i) xanthelasma (7). In addition, there are certain bizarre, but characteristic ultracentrifugal lipoprotein patterns which are seen in presumably normal individuals, but are as yet unclassified.

All individuals show some of the lipoprotein species in the serum. The individual variations encountered are derived from two variables, (a) the number of different lipoprotein species present, (b) the relative abundance of the different lipoproteins in a single serum. A variety of evidence (3) suggests that the typical "normal" of humans shows one or more lipoprotein species in the range up to 6 S_f units (including the S_f 2, S_f 4, S_f 6 lipoproteins). An analogous situation exists in the rabbit, chicken and dog, with minor differences in the S_f rate of the normally occurring lipoproteins. In atherosclerosis and certain other lipid metabolic derangements there exist in addition varying concentrations of lipoproteins in the classes S_f 8, S_f 10, S_f 13, S_f 17, S_f 17-20, S_f 20-40, S_f 40-40,000. In the range S_f 8 - S_f 40, the higher members of the group of lipoproteins make their appearance in serum only when appreciable levels of all lower members are present. However, the relative abundances of these various lipoproteins are variable from one disease category to another. In the range S_f 40-40,000 there are also characteristic patterns associated with disease states, but lipoproteins of the S_f 40 - 40,000 class also represent part of the alimentary "lipemia" of normals. Elevation of the S_f 10-30 range of lipoproteins has been shown to be essentially universally a part of the development of atherosclerosis in the human as well as other species. However, at least certain of the higher members may also be involved in this disease, but as the variability in concentration in a given individual approaches the variability of groups, they have not been used as a correlative guide.

As an extension of our studies of the factors involved in the maintenance of the levels of the various serum lipoproteins, it has now been found that heparin can produce a profound alteration in the lipoprotein pattern in a manner which may prove of value in the further understanding and management of atherosclerosis. Heparin, of course, has long been used clinically in the therapy of thromboembolic disease, but not for any possible influence it might have upon metabolic factors leading to atherosclerosis.

Hahn (8) reported that heparin abolished alimentary lipemia in vivo, but not in vitro. Weld (9) showed that the phenomenon is not restricted to any particular vascular bed by perfusing various body regions with heparinized lipemic plasma. Recently, Anderson (10) has shown that the turbidity of lipemic plasma may be cleared in vitro by mixing lipemic plasma drawn before heparinization with that drawn five minutes after heparinization. He hypothesized an "anti-chylomicronemic" factor which results from heparin injection and discussed the possibility of a heparin-phospholipid complex being a surface active agent responsible for the clearing of lipemic plasma. Waldron (11) reported that other anticoagulants (sulfonated polysaccharides and protemine fast pink B.L.) produced effects similar to heparin, and furthermore, that heparin enhances the absorption of fats from the intestine as evidenced by lipemia production.

EXPERIMENTAL

A single injection of sodium heparin intravenously in the cholesterol-fed rabbit produces dramatic changes in the lipoprotein spectrum, characterized in general by a decrease in concentration of molecules of the high S_f classes, with a concomitant increase in concentration in those of the lower S_f classes.

The association of these two changes suggests a progressive conversion of the higher S_f lipoproteins into those of lower S_f rates. An immediate response is observed within 5 minutes in the lipoproteins of the highest S_f classes, with

successive changes in those of the lower S_f classes, with maximum effect being observed at about three hours, Subsequently the pattern "reverts" to its initial state in approximately 24 hours.

To obtain some evaluation of chronic effects of heparin administration, a series of eight rabbits were fed one gram of cholesterol dissolved in Wesson oil in their Albers food and divided into two groups. One group of four animals received daily I.V. injections of fifteen mg of heparin, the other group received no heparin. The four animals receiving heparin showed levels of the S_f 10-50 class of lipoproteins three to six times lower than did three of the four control animals. One of the control animals was of the "refractory" type, developing only low levels of the S_f 10-50 class in spite of cholesterol feeding. The heparinized and control animals were paired and autopsied between 2-1/2 and 4 weeks. The four heparinized animals failed to develop gross atherosclerosis, whereas three of the four non-heparinized animals showed moderate atherosclerosis. The one non-heparinized animal which failed to develop atherosclerosis had also failed to develop appreciable level of the S_f 10-50 class of lipoproteins. Thus it appears that the effect of heparin in depressing the development of the S_f 10-50 lipoproteins is associated with a suppression of atherosclerosis.

Rabbits refractory to development of S_f 10-50 lipoproteins and atherosclerosis under comparable experimental conditions, are seen in our laboratory experience with a frequency no greater than 1 in 20. Thus it appears that the chance of seeing four such rabbits in this single group to which heparin had been administered is beyond reasonable expectation of chance ($p = < 0.01$). This effect is being studied further varying heparin dosage and fat and cholesterol feeding.

In the human, even more striking effects are seen following heparin administration. In Fig. 1 is shown the progressive changes in the spectrum of lipoproteins following intravenous injection of 100 mg of sodium heparin. This patient, a myocardial infarction survivor, showed initially high levels of

lipoproteins in the S_f 10-20 class, as well as in the S_f 20-100 class. Within 15 minutes there has been essentially a "wipe-out" of lipoproteins from S_f 20 - S_f 100, associated with an increase in the concentration of the S_f 10-20 class. Then, during the next 6 hours there has been an overall shift within the S_f 10-20 class, such that the S_f 12-20 lipoproteins are markedly reduced in concentration below their original level, while the S_f 10-12 and S_f 6-10 lipoproteins have increased in concentration over the initial level. Progressively over a period of 24 hours the entire pattern reverted toward the initial pattern, although there was still a lower S_f 12-20 level than initially. This same general effect has been observed in each of thirty subjects (normals and myocardial infarctions) studied in this manner, the only variations observed being in the degree of response and in the time relationships of the sequence of events which occur. Dosages ranging from 15 mg - 100 mg of sodium heparin I.V. have proven definitely effective, although graded dosage in a single patient requires further evaluation.

In vitro attempts to alter the lipoprotein pattern by heparin addition, have failed in all of numerous trials. However, samples of plasma from heparinized patients have been found effective in altering the lipoprotein spectrum of serum drawn from the same individuals before heparin administration or of serum of other individuals. Thus it appears that the injection of heparin results in the in vivo production of an "active principle" capable of producing these conversions of lipoproteins in vitro. This result is similar to the studies of the "anti-chylomicronemic" factor of Anderson. In an effort to elucidate the mechanism of the effect on lipoproteins, we have found that the "active principle" appears to reside in the ultracentrifugal globulin region. The serum albumin fraction and the low density lipoprotein group both appear inactive. Globulin fractions from post-heparin plasma when incubated with earlier post-heparin plasma appear to cause further changes in the lipoprotein distribution in such plasma in vitro.

We have as yet been unable to detect any of the "active principle" in serum from individuals who had not received heparin, although it is conceivable such a factor may circulate at low concentration.

In an effort to determine the effect of a maintained heparin level on the serum lipoprotein pattern we have made some studies of individuals receiving repository heparin (R). Fig. 2 shows the effect of heparin repository in the same patient as studied in Fig. 1 with I.V. heparin. It is noted that in 4 hours with repository heparin, this patient has lost essentially all of her lipoproteins above S_f 10 units, representing in essence a reversion to a "normal" lipoprotein pattern. After 24 hours there has been a partial return of S_f 10-20 molecules (50%), but a much lower fractional reappearance in the S_f 20-100 class of molecules. (Even after 2 and 4 days there is a significant depression of the S_f 10-20 level.)

A series of twenty patients have received intravenous heparin at intervals varying from 2-14 days. There appeared to be no reduction of ability of patients to show the response described in Fig. 1, even after numerous heparin injections. A small proportion of the patients showed depression in S_f 10-20 levels which persisted for the full 3 to 14 day intervals between I.V. injections of heparin, but in general the levels observed 3-14 days after injection showed no average trend toward reduction. In this particular series samples were drawn throughout just prior to each new heparin injection. From what has been observed in the 24 hour period following such injections, we may anticipate that averaged over the entire period between injections, the S_f 10-20 level may have been appreciably lower than its pre-heparin value. In one patient studied over a one month period, who had shown a maintained S_f 10-20 depression for the 3 day interval between heparin injections, showed a slow progressive rise approaching his original S_f 10-20 levels over a 1 month period after cessation of heparin injections.

R - Lederle Repository Heparin.

An incidental and unexpected clinical observation made by Lyon and Yankley in the patients receiving repeated heparin injections was that 22 patients with moderate or severe angina pectoris reported marked relief from this symptom with a drastic decrease in nitroglycerin requirement. Reports on the effect of heparin in relieving pain of myocardial infarction and as a coronary vasodilator have appeared in the literature (12). However, the presently reported responses in angina have been observed with small single doses of heparin (20-100 mg) given at intervals of several days. All of seven patients whose severe angina had been relieved by the heparin injections complained of return of angina when saline placeboes were injected instead of heparin. Most of the patients in this series were on a partially fat-restricted diet before and during the heparin administration period. However, the immediate and dramatic effect of heparin was in contrast to the slower and less marked reduction in angina accompanying the dieting itself. However, six of the patients who responded well to heparin had not been on a restricted diet.

We are cognizant of the many difficulties in evaluating objectively efficacy of drug relief of angina. However, the striking character of the response in patients with relatively fixed anginal patterns and nitroglycerin requirements, plus the loss of response when saline placeboes were used would appear to militate against the response being in any way psychogenically determined. Extended studies of this response are in progress now. We are not able at this time to provide any suggestion of a possible relation of the heparin effect on blood lipoproteins to that in relieving angina pectoris, although such may be the case.

DISCUSSION

Heparin appears to act profoundly and rapidly in altering the blood lipoprotein spectrum. Shifts among the lipoproteins are observed both in the human and the rabbit from molecules of the higher S_f classes to those of successively

lower classes in times of the order of minutes to hours following a single heparin injection. The decrease in concentration of a particular S_f range of molecules, accompanied by an increase in the concentration of molecules in the next lower S_f classes suggests that the former molecules may be actually transformed into the latter by the influence of heparin. This appears to occur in several successive stages over a period of hours. This represents one of the first clues on the possible interrelationships of the various classes of lipoproteins of serum. Inasmuch as the lipoproteins of the S_f 17-100 classes represent in the human the major glyceryl ester (fat) bearers of serum, the observed interconversion accentuated by heparin, may represent steps in the normal pathway of transport and metabolism of fat. It is appropriate to consider the possibility that heparin itself, or some substance of similar properties, may normally be involved in the physiological interconversion of lipoproteins. Thus, in individuals who usually show high levels of S_f 10-20 and S_f 20-100 lipoproteins there may be a blockage in the utilization pathways of such molecules (possibly due to a deficiency of heparin-like substance), such that a piling up in concentration of such molecules occurs in the blood. In supposedly normal individuals (especially young adults and children of both sexes) all these molecules, if present, are in very low concentration, which would be expected if utilization pathways were greatly facilitated in these individuals.

As reported by Anderson for the in vitro clearing of alimentary lipemia by in vivo heparinized plasma, the present work indicates that heparin in free form does not directly induce any alteration in lipoprotein spectrum. However, plasma from heparinized patients contains a factor that is effective. This factor resides in the ultracentrifugally determined globulin fraction and induces changes in vitro which simulate, at least in part, the changes in lipoproteins which follow in vivo administration of heparin. Further search for such an "active factor" in the sera of "normal" individuals who show low levels of S_f 10-100

lipoproteins appears warranted. If present, such a factor might be anticipated at very low concentration, from the negative results of our searches for it to date. Again, such a factor might not normally reside in appreciable concentration in plasma, but might be called forth in response to lipid loading.

Heparin administered to rabbits protects the animal from development of atherosclerosis under circumstances which otherwise induce atherosclerosis. Since the protective effect of heparin is accompanied by a suppression of development of high levels of the S_f 10-50 lipoproteins, the observation strengthens the previously reported evidence linking these molecules with atherosclerosis, and further suggests the value of maintaining low levels of these molecules in prevention of experimental atherosclerosis. The effect of lowering the similar classes of molecules in the human on progression of the clinical manifestations of atherosclerosis is being evaluated (13).

The dramatic relief of angina pectoris by intermittent heparin administration parallels the profound effect of this substance upon lipoprotein metabolism. However, at this time we have been unable to <u>demonstrate</u> that these two simultaneous effects are actually related. It may be that the relief heparin provides in angina pectoris due to its vasodilator or to its anti-thrombotic activity. The effect on angina pectoris is seen with small doses of heparin and persists much longer than the anti-coagulant effect of the doses used. Currently under study are the effects of heparin in several diseases which are marked by extreme elevation of lipoproteins of the S_f 10-100 classes (e.g., nephrotic syndrome, hypothyroidism and xanthoma tuberosum).

SUMMARY

1. Heparin administered to rabbits and humans causes profound reorientation in the distribution of low-density lipoproteins, characterized by a shift of lipoproteins of high S_f rates to those of successively lower S_f rates.

2. Heparin administered to cholesterol-fed rabbits prevents the development of high levels of S_f 10-50 lipoproteins and retards the development of atherosclerosis in such animals.

3. Following heparin administration the plasma contains an "active principle" associated with the ultracentrifugal globulins, which produces similar re-orientation of the lipoprotein spectrum in vitro.

4. Heparin added directly to serum is ineffective in vitro.

5. Intermittent heparin administration to patients with severe angina pectoris results in dramatic and uniform relief from his symptom for periods of several days beyond a single injection.

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SYNOPSIS

"The Influence of Heparin upon Lipoprotein Metabolism"

Heparin administered to humans and rabbits causes profound re-orientation in the distribution of low-density lipoproteins, characterized by a shift of lipoproteins of high S_f rates to those of successively lower S_f rates. The observations appear to indicate that this agent has actually caused a transformation of the former group into the latter. Heparin administered to the rabbit prevents the usual build-up of high concentration of the S_f 10-50 lipoproteins during cholesterol feeding and retards the development of atherosclerosis. In the human, accompanying the redistribution of lipoproteins, there was observed a marked reduction in angina pectoris in all patients studied who presented this symptom. The relation between the heparin effect upon lipoproteins and its effect upon angina cannot be assessed at present.

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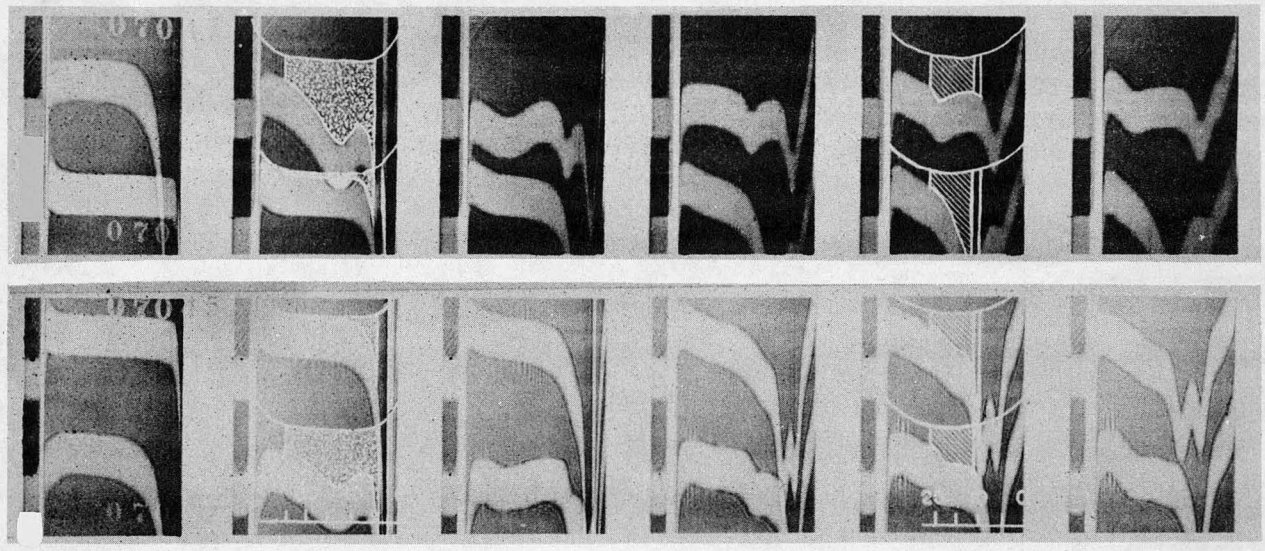


M-4b (M)

LEGEND for Figure 1 a and b

The Effect of a Single Intravenous Injection of Heparin on Human Low-Density Lipoproteins

The accompanying ultracentrifugal flotation patterns show the progressive changes in the lipoproteins of a 63 year old female patient with coronary artery disease treated with a single 100 mg dose of heparin given intravenously.

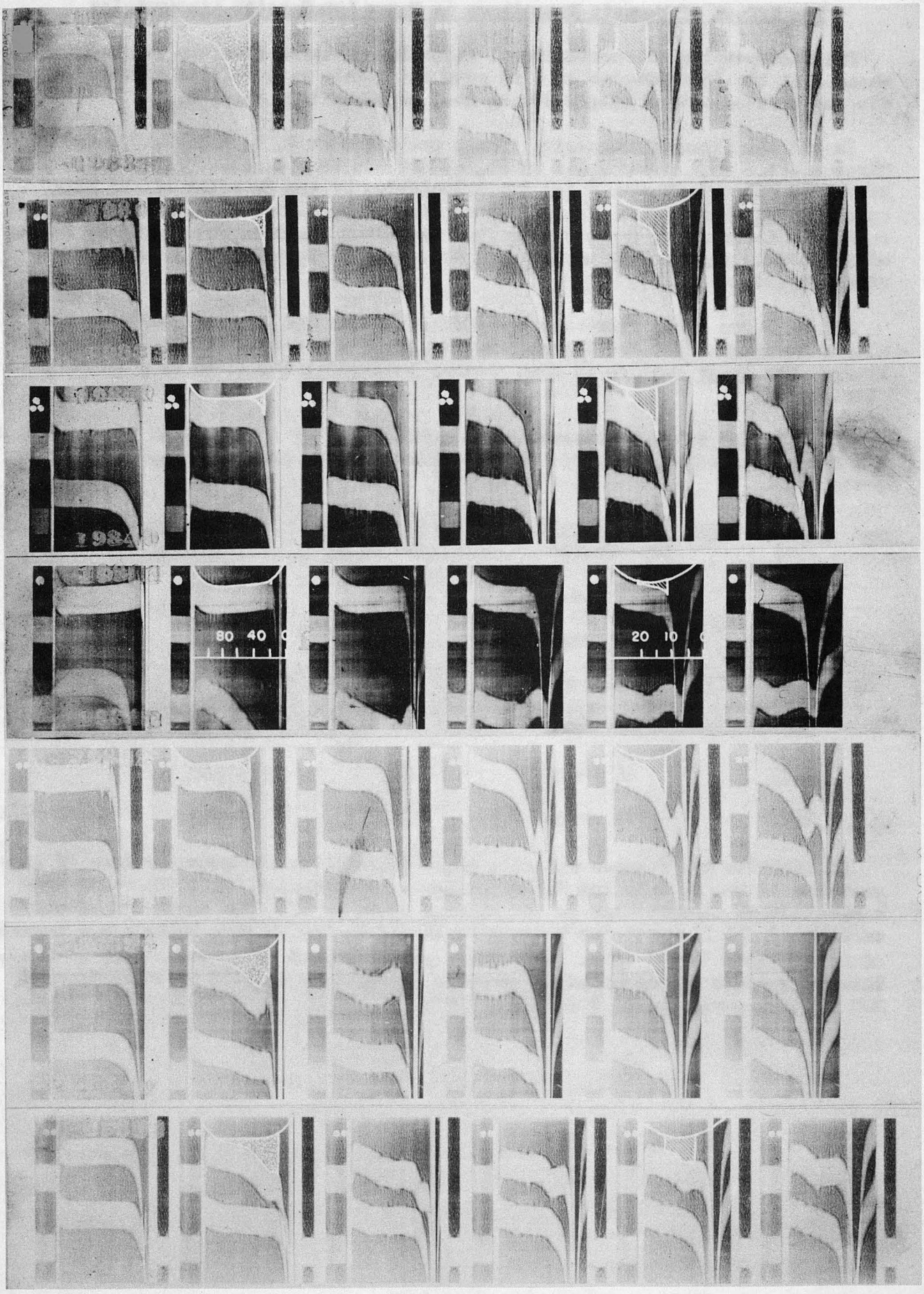
In all the figures successive frames are taken at 0, 6, 12, 22, 30, and 38 minutes after full rotor speed of 52640 RPM has been reached. Frames 2 and 5 in Figure 1(b), lower pattern are ruled, for the calculation of the S_f rates of anypeak appearing in these frames, respectively. These rulings can be used to calculate S_f rates in the corresponding frames of the other figures.

All stippled areas represent the measure of the S_f 20-100 class of lipoproteins; all cross-hatched areas represent the measure of the S_f 12-20 class of lipoproteins.

Figure	Time after heparin	S_f 12-20 (mg%)	S_f 20-100 (mg%)
1(a) upper pattern	Pre-heparin	167 mg%	530 mg%
1(a) lower pattern	20 minutes	239	50 (See note below
1(b) upper pattern	6 hours	128	52
1(b) lower pattern	26 hours	143	344

Note: The sample recorded in 1(a) lower was ultracentrifugally concentrated only to 3/4 the level in the other samples. Hence the measured area from the diagram was corrected for this in preparing the tabulation of results.

It is seen that while the 6 hour post heparin sample shows an S_f 12-20 level not over 25% lower than the preheparin sample, there is a marked shift in distribution of the lipoproteins of the S_f 12-20 class toward the lower ranges of this class.



M-4a (M)

LEGEND for Figure 2, a through g.

The Effect of Heparin Repository on Human Low-Density Lipoproteins

The accompanying ultracentrifugal flotation patterns show the progressive changes in the lipoproteins of a 63 year old female patient with coronary artery disease treated with a single 200 mg dose of repository heparin.

In all the figures successive frames are taken at 0, 6, 12, 22, 30, and 38 minutes after full rotor speed of 52,640 RPM has been reached. Frames 2 and 5 have been ruled in Figure 2 (d) for calculation of the S_f rates of any peak appearing in that frame. The ruling of frame 2 in figure 2(d) may be used to calculate S_f rates in frame 2 of any of the other figures. Similarly the ruling of frame 5 in figure 2(d) may be used to calculate S_f rates in frame 5 of any other figures.

All patterns shown represent two different sera run simultaneously in the ultracentrifuge. In each figure only the upper pattern is involved in this study. The lower pattern is from another individual and is to be completely disregarded.

All stippled areas represent the measure of the S_f 20-100 class of lipoproteins; all cross-hatched areas represent the measure of the S_f 12-20 class of lipoproteins.

Figure	Time after heparin	S_f 12-20 (mg%)	S_f 20-100 (mg%)
2(a)	Pre-heparin	197 mg %	357 mg%
2(b)	70 min.	147	18
2(c)	3 hours	102	14
2(d)	4 hours	21	0
2(e)	6 hours	83	18
2(f)	24 hours	81	160
2(g)	72 hours	107	164

In addition the figures reveal a great increase in the concentration of lipoproteins of the S_f 6-10 and S_f10-12 classes accompanying the decrease in concentration of lipoproteins in the classes above S_f 12. The change in concentration of all lipoproteins between S_f 12 and S_f 100 in four hours after heparin administration is 533 mg %. Assuming a plasma volume of approximately 2500 cc, this represents a minimum of 13 grams of lipoprotein cleared by the action of 200 mg of heparin in the repository form.