Spontaneous and Induced Variations in Serum Lipoproteins

By H. L. Chandler, M.D., E. Y. Lawry, Ph.D., K. G. Potee, M.D., and G. V. Mann, M.D.

The diurnal variability of several classes of serum lipoproteins and cholesterol has been measured in human subjects. The $S_1$, 12-20 class of lipoprotein and the cholesterol were found stable in reference to the technical limitation of the method, but the $S_1$, 20-100 class showed significant variation although without evidence of a trend. The effect of intravenous heparin treatment was described. The buccal administration of heparin had no effect. The heparinoid substance Treburon produced a decrease in the $S_1$, 20-100 material at three and five hours and decrease in serum total cholesterol and phospholipids at five and seven hours when administered buccally.

The concept that atherosclerosis is a progressive metabolic disease is now widely accepted. That lipid metabolism is directly involved in this condition is suggested both by the prominence of fatty constituents in the lesions and the frequency of associated abnormalities of serum lipid concentrations. The significance of the circulating lipids in the pathogenesis of this disease is not established. During the past several years much interest has been evoked by the reports of Gofman and his group suggesting a close relationship between high concentrations of certain ultracentrifugally identified and quantitated low density serum lipoprotein molecules and atherosclerosis.1,2 Keys, after independent statistical treatment of the same data, has concluded that these specific lipid particles show no greater correlation with atherosclerosis than does the serum cholesterol level.3 A large scale investigation by several cooperating ultracentrifuge laboratories is in progress to clarify these claims. Whatever the relative merits of these measurements it is apparent that if only one or at most a few measurements of the serum lipids are available, interpretation of such data is dependent upon some knowledge of the variability of the material measured.

The conditions which induce alterations in the serum cholesterol and lipoprotein levels are incompletely known. Most observers have found that restriction of the total lipids of a usual American diet to about 50 Gm. per day has led to a reduction of the serum cholesterol.4 Experiments in this laboratory have shown that when individuals are placed on such a low fat diet and are at the same time placed in negative caloric balance both the serum cholesterol and all classes of lipoproteins studied were sometimes lowered.5 The serum lipid changes were restricted to those subjects with initially elevated levels; for example, with cholesterol greater than 300 mg. per 100 cc. and $S_1$ 12-20 greater than 59 mg. per 100 cc. Another experiment revealed significant increases in both the serum cholesterol and lipoprotein content of serum when normal young males were placed in strong positive caloric balance with a fat free diet.6 It appeared that changes could be induced in these serum lipids either by reducing the dietary fat or by altering caloric balance. Experimental work in animals and human subjects done in this laboratory indicated that the serum cholesterol is sensitive to physiologic stress.6 It was found that such agents as cold, fever, and inanition lowered the serum cholesterol. It was then demonstrated that corticotropin (ACTH) would produce the same effect although cortisone was shown not to be the effective adrenal steroid mediating this action. These experimental observations are supported by the finding that the serum cholesterol and the $S_1$ 12-100 classes of lipoproteins are transiently lowered following a myocardial infarction.7

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The use of thyroid extract in patients with hypothyroidism is well known to lower serum cholesterol levels, and in a small group of such patients it has been reported to lower the levels of serum $S_f$ 12-20 lipoproteins. Heparin, administered intravenously or intramuscularly, has been shown to result in a sharp decrease in the serum lipoproteins but to have little or no effect on the serum cholesterol.

It has been stated by Gofman and his co-workers that in individuals on a steady diet and without specific therapy there is a "reasonably stable level of concentration" of molecules of the $S_f$ 12-20 group, regardless of whether the concentration is low or high, or whether the analysis is performed on blood drawn in the fasting state or during alimentary lipemia. This conclusion was based on a second measurement two days to three months after the first measurement on 65 individuals. The measurements were made on blood drawn at any time of day and without particular reference to meals. In a subsequent communication from the same laboratory the standard error of any single obtained value was defined as 15 mg. per 100 cc. for $S_f$ 12-20 lipoproteins and 29 mg. per 100 cc. for cholesterol. This statement was derived from unpublished data consisting of determinations repeated on the same individual within a one-year period. We have found no reported data dealing with spontaneous changes in concentration of these lipoproteins within a 24-hour period.

The studies described here were designed to define the spontaneous diurnal variations in these classes of lipoprotein and cholesterol. Such data then provided a frame of reference for measurement of the acute effects of various agents in altering the concentrations of lipoprotein classes and cholesterol in the serum. Another complementary study will be published which deals with the spontaneous variability of these quantities over a 10-week period.

METHODS

Venous blood was used in all determinations. For cholesterol and lipoprotein analyses serum was obtained by permitting drawn blood to clot at room temperature for about two hours. This was then centrifuged and the serum separated and stored at 0 to 5 C. for not more than four days until the laboratory measurements were performed. Clotting times were done at room temperature by a modified Lee-White technic with two 8 mm tubes and using 1.0 cc. of blood from the total 25 to 30 cc. drawn. There was undoubtedly some error introduced because of turbulence, but the data have relative value. Serum lipoproteins of the $S_f$ 12-20, 20-100 classes were measured by the method of Gofman and his associates; when analyses were made in duplicate the mean value was used. Serum cholesterol was determined by the technic of Abell and co-workers. Fresh serum was used in the measurement of phospholipids by the procedure of Schmidt and associates.

DIURNAL VARIATIONS OF LIPOPROTEINS AND CHOLESTEROL

Three female and five male hospitalized patients, ranging in age from 41 to 72 years and one healthy, young male were bled before breakfast, before lunch, before or after dinner, and again before breakfast on the second day for lipoprotein and cholesterol analyses. Five male and four female healthy young adults ranging in age from 21 to 28 were bled before each of the three meals of one day for lipoprotein, cholesterol, and phospholipid determinations.

Table 1 shows the daily means and standard deviations for each of these 17 subjects, the diurnal variations for the healthy and hospitalized groups and the pooled diurnal variation for the entire series. The variation of the lipoproteins of the hospitalized group appears greater than that of the healthy group; the difference is not statistically significant for the $S_f$ 20-100 class of lipoproteins and is barely significant at the 5 per cent level for the $S_f$ 12-20 class. The variabilities of the serum lipids of the two groups of subjects may differ because of difference in state of health, but age and initial levels are more likely factors contributing to the difference. It was anticipated that the older group with higher initial levels would show greater variability since the error of measurement increases with increases in the concentration being measured, and variability studies extending over a longer period of time show that biologic variation (variability corrected for measurement error) also increases with level. In the present series the effects of these two sources of variability could not be separated. The data of table 1 suggested that
### Table 1.—Diurnal Variations of Serum Lipoproteins, Cholesterol and Phospholipids

(All values in mg. per 100 cc.)

<table>
<thead>
<tr>
<th>n</th>
<th>Sf 12-20</th>
<th>Sf 20-100</th>
<th>Cholesterol</th>
<th>Age</th>
<th>Sex</th>
<th>Diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>S.D.</td>
<td>Mean</td>
<td>S.D.</td>
<td>Mean</td>
<td>S.D.</td>
</tr>
<tr>
<td>1</td>
<td>4</td>
<td>92</td>
<td>8.5</td>
<td>138</td>
<td>28.2</td>
<td>239</td>
</tr>
<tr>
<td>2</td>
<td>4</td>
<td>75*</td>
<td>4.2</td>
<td>110</td>
<td>32.4</td>
<td>234</td>
</tr>
<tr>
<td>3</td>
<td>4</td>
<td>30</td>
<td>3.4</td>
<td>27</td>
<td>6.3</td>
<td>211</td>
</tr>
<tr>
<td>4</td>
<td>4</td>
<td>30</td>
<td>5.6</td>
<td>30</td>
<td>5.2</td>
<td>180</td>
</tr>
<tr>
<td>5</td>
<td>4</td>
<td>29</td>
<td>5.4</td>
<td>66</td>
<td>15.5</td>
<td>201</td>
</tr>
<tr>
<td>6</td>
<td>4</td>
<td>19*</td>
<td>4.4</td>
<td>21</td>
<td>11.9</td>
<td>196</td>
</tr>
<tr>
<td>7</td>
<td>4</td>
<td>16*</td>
<td>8.5</td>
<td>13</td>
<td>6.4</td>
<td>139</td>
</tr>
<tr>
<td>8</td>
<td>4</td>
<td>15*</td>
<td>4.0</td>
<td>13</td>
<td>5.8</td>
<td>321</td>
</tr>
</tbody>
</table>

### Hospital Group

| Pooled Variation, Hospital Group | 5.8 | 17.2 | 15.8 |

### Healthy Group

<table>
<thead>
<tr>
<th>Phospholipids</th>
<th>Mean</th>
<th>S.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### (Both Groups)

| Pooled Diurnal Variation: | 5.1 | 15.3 | 13.8 |
| Technical Error of Measurement: | 4.2† | 8.4† | 14.5‡ |

n = Number of bleedings within 24-hour period.
* Bled after, rather than before, evening meal.
† Based on 125 pairs of duplicates. ‡ 98 pairs.

S.E. = \( \sqrt{\frac{\sum \Delta^2}{2R}} \) in which \( \Delta \) = difference between duplicates; \( R \) = number of pairs.

The standard deviation increased with the mean value, and this may well have been great enough to explain any difference between the two groups. In the pooled data for the entire group the distribution of lipoprotein and cholesterol levels among these persons was comparable to that among the subjects used in the other studies to be described.

The above discussion also applied to the variability of serum cholesterol, with the dif-
ference that the variability for the hospital group was significantly different from that for the healthy group if the data for two hospital subjects with levels above 300 mg. per 100 cc. were included, but not significantly greater if these two were excluded. The effect of level on variability was here more conspicuous.

The standard deviations for the Sf 12-20 and total cholesterol measurements computed from the combined data of all 17 persons did not differ significantly from the corresponding standard errors of measurement. Greater variation was observed in the Sf 20–100 lipoproteins than could be attributed to technical error alone. This confirmed reports that this class of lipoprotein was more variable than was the Sf 12–20 class. These measurements revealed no trends in either class of lipoproteins or in the cholesterol throughout the day.

Two subjects, 1 and 7, showed variations in the Sf 12–20 class significantly greater than the technical error. Subject 7 may be rejected as perhaps due to error since none of the measurements were in duplicate. All the measurements on subject 1 were made in duplicate, however, and an analysis of variance of these data showed a significant difference between measurements on sera drawn at different times. This emphasized the fact that although the diurnal variation for most individuals or for a group may not exceed the standard error of measurement, it may for some persons, especially those with elevated levels. This possibility, along with the significant variations in the Sf 20–100 class of lipoproteins, indicated that the variations observed in a group of subjects after any treatment should be compared with the variations observed in the same or a control group observed over a comparable period of time, and not solely with the standard error of measurement derived from consideration of duplicate analyses of samples; moreover, the distribution of initial levels in a control group must be similar to that of the treated group in order to make such comparisons valid.

HEPARIN EFFECT

Following the allegation of a highly positive correlation between increased levels of serum Sf 12–20 and 20–100 classes of lipoproteins with atherosclerosis, attempts have been made to reduce these serum lipid levels by dietary and pharmacologic means with the hope that the atherosclerotic process would be halted. On the basis of Hahn’s observation that heparin clears the lactescence of lipemic serum, this agent was studied for its effect on the serum lipoproteins.9 A marked alteration of the lipoprotein constitution of serum was described in general terms, but adequate specific data regarding this effect was lacking in the published reports. Such data have been sought in the present study.

Immediately after blood for analysis had been drawn from each of 10 hospitalized patients, 50 mg. of heparin* was administered intravenously to each subject. Three successive blood samples were then secured from each patient according to one of several overlapping time schedules. In this way observations of the effect of heparin in the several subjects were obtained at six different times after the heparin treatment without excessive bleeding of any single subject.

The data were analyzed by comparing the changes induced in the serum lipoproteins and cholesterol levels by the heparin treatment with the changes observed over a comparable period of time in those subjects studied for diurnal variation, described above. For each individual the difference between his own control measurement and that observed at a given time after treatment was obtained. The mean of these differences for the treated subjects was then compared with the mean difference obtained in a similar manner from those untreated subjects of the diurnal variation study who were observed after the time interval nearest to that under consideration in the heparin study. The range of the Sf 12–20 control levels for the heparin-treated subjects was 18 to 64 mg. per 100 cc.; for Sf 20–100, 13 to 103 mg. per 100 cc.; for cholesterol, 145 to 249 mg. per cent. These ranges were similar to those of the untreated subjects, as table 1 suggests.

The comparisons of the mean changes after heparin treatment with those of the untreated

* The heparin used in this study was supplied by the Upjohn Company through the courtesy of Dr. Joseph Webb.
controls are shown in Table 2. The significant
changes observed were a decrease in the Sf
20–100 class of lipoproteins 20 minutes after
heparin treatment and a decrease in the Sf
12–20 group six hours after treatment. By six
hours after heparin the Sf 20–100 class was back
to the control level, and by 12 hours after
treatment the mean of Sf 12–20 group had re-
turned to the original level. Observations 18 and
24 hours after treatment showed no changes in
either quantity. No appreciable variations were
observed in the serum cholesterol at any time.

This observation that changes in the Sf
20–100 class preceded those in the Sf 12–20
parallels the experience of Graham and co-

workers, and is consistent with their hypothesis
that the lipoproteins are converted into pro-
gressively smaller molecules of higher density
and lower flotation rate. This process, assumed
to be a normal and continual aspect of fat
transport and metabolism, is accelerated by
heparin. From this point of view we can exam-
ine these data in greater detail. At 20 minutes
after heparin injection the Sf 20–100 levels of
all five subjects examined had dropped to
values of less than or equal to 12 mg. per 100
cc., which is the limit of resolution of these
molecules by our method; that is, all five sub-
jects had Sf 20–100 levels too low to be measured
accurately. Three of these persons, all with
control Sf 20–100 levels less than 45 mg. per
100 cc., showed decreases of 10 mg. per 100
cc. or more in their Sf 12–20 levels at 20 minutes,
while the other two, who showed no change in
their Sf 12–20 levels, had Sf 20–100 control
levels above 45 mg. per 100 cc. If the rate at
which the larger Sf 20–100 molecules are trans-
formed to the smaller Sf 12–20 lipoproteins is
roughly the same for most individuals, more
time would be required to clear the Sf 12–100
groups of lipoproteins in those persons with
higher initial levels; this may be a partial ex-
planation for the differing individual responses
at 20 minutes after heparin. Furthermore, if
the heparin effect is progressive from the
higher Sf classes toward the lower, then the
concentration of the Sf 12–20 at a given time

| Table 2.—Average Changes in Serum Lipoproteins and Cholesterol in Subjects Treated with 50 mg. Heparin Intravenously Compared with Untreated Control Subjects (Mean changes in mg. per 100 cc.) |
|---|---|---|---|
| Time | n | Sf 12-20 | Sf 20-100 | Cholesterol |
| 20 min. after heparin | 5 | -13.2 | -42.8 | 0.0 |
| 3 hr. controls* | 8 | -0.5 | -1.1 | -0.3 |
| 6 hr. after heparin | 8 | -12.1 | -7.5 | +2.0 |
| 6 hr. controls | 8 | +1.6 | +2.4 | -2.9 |
| 12 hr. after heparin | 9 | -3.1 | +14.4 | -5.1 |
| 12 hr. controls | 9 | -1.5 | +2.2 | -5.0 |

n = number of observations.

p = probability that the observed difference is due to chance as calculated by Student's "t" test.

* Controls, untreated, taken from diurnal variation study.
the case, then the concentration of the larger Sf 20–100 lipoproteins would be expected to increase while the level of the smaller Sf 12–20 class was still low. This sequence was observed at six hours after heparin when all but one of the eight persons observed had Sf 20–100 levels which were definitely measurable; i.e., above 12 mg. per 100 cc. This group as a whole showed levels not significantly different from the controls, whereas the Sf 12–20 lipoproteins showed a significant lowering from the control levels. It appears that the lipoproteins of the Sf 20–100 class may be precursors of those in the Sf 12–20 class. All these changes were transitory and had disappeared by the twelfth hour after treatment.

The most prolonged clotting times were observed at 20 minutes after heparin treatment, when the Sf 20–100 lipoproteins showed a significant fall, but the clotting defect had disappeared after six hours, when the Sf 12–20 class was decreased. This experiment gives no information concerning the relation of the induced clotting defect to the period of reduction of lipoproteins. Obviously the clotting disturbance had disappeared before all the lipoprotein alterations induced by heparin were corrected.

Transmucosal Heparin*

An effort was made to ascertain whether the lipoprotein active factor of heparin could be absorbed transmucosally. After bloods were drawn for control measurements, three patients were each given 250 mg. of heparin in tablet form, placed in the usual position for buccal absorption. Blood specimens were obtained 20 minutes, one hour, and three hours after the midpoint (30 minutes) of tablet disappearance. No alteration in the concentration of any of the groups of lipoproteins, serum cholesterol, or serum phospholipids was demonstrated. Nor was there a significant change in the clotting times of blood drawn at these intervals.

* The tablet form of heparin was provided by the Abbott Laboratories through the courtesy of Dr. L. F. Josselyn.

Treburon†

Treburon is a pectic acid derivative with heparin-like properties chemically identified as the sodium salt of sulfated polygalacturonic acid methyl ester methyl glycoside.15 In animal tests it has been found to have about one-fourth the anticoagulant and antithrombin effects of heparin on a weight basis with both agents administered intravenously. In human subjects 150 mg. of Treburon administered intravenously was about as effective as 50 mg. of heparin.16 Oral ingestion of a dose of 2.0 Gm. in man and 1.0 Gm. per kilogram of body weight in rabbits did not alter the coagulation time. No toxicity was demonstrated in an initial study of 138 patients given Treburon parenterally in dosages sufficient to render the blood therapeutically hypocoagulable. However, during the course of the present study, Treburon was withdrawn from parenteral use in clinical investigation because of certain undesirable side effects associated with its use.

In this experiment nine hospitalized patients with a variety of illnesses were utilized. Three of these subjects were selected because they were known to have moderately high lipoprotein levels; the other six were unselected. After obtaining blood samples for control purposes, each patient was given two 500 mg. tablets of Treburon, one on each side of the mouth, for transmucosal absorption. Using the midpoint of tablet disappearance as the zero of time, three subsequent blood samples were drawn from each subject according to either of two schedules so that at least three observations were obtained at 20 minutes and one, three, five, and seven hours after treatment.

As in the treatment of the heparin data, the changes from the individual control levels observed at each interval after Treburon treatment were compared with the spontaneous changes observed in the untreated subjects studied for diurnal variations and described above. The results are shown in table 3. No significant changes were observed in the Sf 12–20 class; the Sf 20–100 lipoproteins were

† Furnished by Hoffmann-La Roche through the courtesy of Dr. Leo A. Pirk.
significantly decreased three and five hours after Treburon. Both the total cholesterol and phospholipids were significantly reduced five and seven hours after Treburon; this reduction could possibly extend beyond seven hours, at which time our last observations were made. No appreciable changes in clotting times were observed.

**Table 3.** The Effects of 1.0 Gm. of Transmucosal Treburon on the Serum Lipoproteins, Cholesterol, and Phospholipids of Human Subjects

(Average changes in mg. per 100 cc.)

<table>
<thead>
<tr>
<th>Time after Treburon</th>
<th>n</th>
<th>Δ 12-20</th>
<th>Δ 20-100</th>
<th>Δ Cholesterol</th>
<th>Δ Phospholipid</th>
</tr>
</thead>
<tbody>
<tr>
<td>20 min. after Treburon</td>
<td>3</td>
<td>+2.7</td>
<td>−12.3</td>
<td>−19.3</td>
<td>−6.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>.02 &lt; p</td>
<td>.2</td>
<td>.05 &lt; p</td>
<td></td>
</tr>
<tr>
<td>3 hr. controls*</td>
<td>8</td>
<td>−0.5</td>
<td>−1.1</td>
<td>−0.3</td>
<td>−4.0</td>
</tr>
<tr>
<td>1 hr. after Treburon</td>
<td>3</td>
<td>+1.7</td>
<td>−18.3</td>
<td>−9.3</td>
<td>−3.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>.02 &lt; p</td>
<td>.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 hr. controls</td>
<td>8</td>
<td>−0.5</td>
<td>−1.1</td>
<td>−0.3</td>
<td>−4.0</td>
</tr>
<tr>
<td>3 hr. after Treburon</td>
<td>9</td>
<td>+0.6</td>
<td>−18.2</td>
<td>−10.0</td>
<td>−3.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>.02 &lt; p</td>
<td>.05</td>
<td>.05 &lt; p</td>
<td></td>
</tr>
<tr>
<td>5 hr. after Treburon</td>
<td>6</td>
<td>+0.7</td>
<td>−21.8</td>
<td>−23.3</td>
<td>−39.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>.02 &lt; p</td>
<td>.05</td>
<td>.01 &lt; p</td>
<td></td>
</tr>
<tr>
<td>6 hr. controls</td>
<td>8</td>
<td>+1.6</td>
<td>+2.4</td>
<td>−2.9</td>
<td>−4.0</td>
</tr>
<tr>
<td>7 hr. after Treburon</td>
<td>6</td>
<td>+6.3</td>
<td>−21.8</td>
<td>−28.2</td>
<td>−32.3</td>
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<tr>
<td></td>
<td></td>
<td>.05 &lt; p</td>
<td>.01</td>
<td>.01 &lt; p</td>
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</tr>
<tr>
<td>6 hr. controls</td>
<td>8</td>
<td>+1.6</td>
<td>+2.4</td>
<td>−2.9</td>
<td>+4.1</td>
</tr>
</tbody>
</table>

n = number of observations.

p = probability that the observed difference is due to chance as obtained by Student’s "t" test.

* Controls, untreated, taken from diurnal variation study.

**Discussions and Conclusions**

Within the limitations of the present study, a diurnal constancy of serum Sf 12–20 lipoproteins and cholesterol was found. Exceptions to this conclusion were indicated by the presence of high initial levels of one or both of these lipid components. This limitation indicated that if therapeutic trials are to be conducted with subjects or groups which by design or accident have high serum lipid levels then the biologic variability of the group must be measured in suitable preliminary studies in order to assure a valid basis for comparison of the effect of treatment. Alternatively, but probably less satisfactorily, the data secured for evaluation should be compared with control data obtained from a group matched for initial levels and studied under similar circumstances.

During a single day there was less stability in the Sf 20–100 class of lipoproteins than in the Sf 12–20 class. The changes observed throughout the day in both classes of lipoproteins and in cholesterol showed no pattern that might be related to meals or the day's activities.

The striking reduction, reported by Graham and associates in all the measured lipoproteins, without change in cholesterol, as a result of intravenous heparin was confirmed. The degree of decrease and the time required to accomplish this varied in different individuals in the present study. All subjects observed within six hours after treatment with parenteral heparin have demonstrated a response in the same direction. This effect of 50 mg. of heparin injected intravenously had disappeared by six hours.
for the Sf 20–100 class of lipoproteins and by 12 hours for the Sf 12–20 class.

Anfinsen and his collaborators have traced the mechanism of this action of heparin to a system involving substances separated mainly with the globulin fractions of plasma, using Cohn’s method of fractionation. Graham, by ultracentrifugal methods, also found that the clearing factor fell in the globulin fraction. It is interesting that the cofactor in plasma which is influenced by heparin to strong antithrombin activity is associated mainly with serum albumen. This suggested that the lipoprotein-altering effects of heparin or heparin-like substances may be dissociated from the anticoagulant effect. Should the initial reports of dramatically beneficial results of heparin in clinical atherosclerotic disease be confirmed in subsequent investigation, and attributable to this action on serum lipoproteins, it would be desirable to find a substance with the same lipoprotein effect yet devoid of potential dangers such as hemorrhage and also one that could be taken conveniently by mouth.

The effect on lipoproteins of transmucosally absorbed Treburon was similar to that of parenteral heparin in that the concentration of the Sf 20–100 class of lipoproteins was significantly reduced at three and five hours after treatment. No reduction of the Sf 12–20 class was demonstrated. It is possible that a more prolonged experiment, to 8 or 10 hours, would show such a change. The rate and degree of response with buccal Treburon were much less than with intravenous heparin, suggesting an attenuation of effect as a result of mode of administration, low dosage, or both. On the other hand, the late and moderate response of the Sf 20–100 molecules and the absence of effect on Sf 12–20 lipoproteins together with the novel effect in lowering the serum cholesterol and phospholipid concentrations suggest that an alternative explanation may be applicable. The only pharmacologic agent known to decrease acutely the serum cholesterol is adrenocorticotropic hormone (ACTH), as mentioned previously in the work of Mann and White. It is conceivable that some factor of absorbed Treburon operates through a mechanism related to that of corticotropin or involving release of this substance. Since the majority of the cholesterol and phospholipid content of serum is in materials of density less than that corresponding to a migration rate of Sf 20, it follows that further study of the effects of Treburon probably will reveal changes in the concentrations of other lipoproteins or perhaps in their composition. This probability re-emphasizes the importance of a more decisive segregation and description of the atherogenic potentialities of the various classes of serum lipoproteins.

**Summary**

1. The fluctuations observed in the Sf 12-20 class of lipoproteins and total serum cholesterol levels during 24-hour periods were no greater than those attributable to measurement error alone. No diurnal trends were observed.

2. The diurnal variation observed in the Sf 20–100 class of lipoproteins was greater than could be attributed to technical error, showed no consistent pattern, and tended to increase with higher mean levels.

3. Fifty milligrams of heparin given intravenously resulted in marked reductions in all lipoproteins from Sf 100 through Sf 12 with maximal effect in the Sf 20–100 class within 20 minutes and in the Sf 12–20 class at about six hours. This effect had disappeared by 12 hours after the heparin administration.

4. The effect of heparin on lipoproteins was not accompanied by serum cholesterol changes.

5. Heparin, in dosage of 250 mg. by transmucosal absorption, had no effect on lipoprotein levels, serum cholesterol, or blood clotting times.

6. Treburon, in a dosage of 1.0 Gm. given by buccal absorption, had a definite effect in decreasing serum Sf 20–100 lipoproteins after three and five hours. This effect had decreased but persisted to seven hours.

7. A significant reduction in serum cholesterol and serum phospholipids was observed five and seven hours after administration of transmucosal Treburon.

8. Treburon given under these conditions caused no appreciable changes in blood clotting time.
CHANDLER, LAWRY, POTE AND MANN

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SUMARIO ESPAÑOL

La variación diurna de algunos grupos de lipoproteínas del suero y colesterol se ha determinado en sujetos humanos. La clase S₄ 12–20 de lipoproteínas y el colesterol se encontraron estables en referencia a la limitación técnica del método, pero la clase S₄ 20–100 mostró variación significativa aunque sin evidencia de curso o tendencia. El efecto del tratamiento con heparina intravenosa se describe. La administración de heparina oral fue ineffectiva. La substancia heparinoide Treburon produjo un decremento en el material S₄ 20–100 a las tres y cinco horas y un decremento en colesterol total del suero y fosfolipina a las cinco y siete horas cuando se administró oralmente.

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7. Unpublished data.


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H. L. CHANDLER, E. Y. LAWRY, K. G. POTEE and G. V. MANN

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