EFFECT OF PHOSPHATIDE THERAPY ON BLOOD LIPIDS

BY

J. B. ENTICKNAP, M.D., D.C.P.

Consultant Pathologist, East Ham Memorial Hospital,
London

Subjects with ischaemic heart disease and other forms of arterial occlusion commonly have abnormal amounts of lipids in the blood. Numerous remedies have been proposed, and their efficiency has most often been assessed by following serial changes in, particularly, the level of total serum cholesterol. Emulsions of esters containing unsaturated fatty acids unquestionably lower the blood cholesterol, but the dose required is very large. There is considerable evidence that rats actually require certain fatty acids, particularly the highly unsaturated linoleic, linolenic, and arachidonic acids, which apparently they are unable to synthesize. These have been called the vitamin F acids. Although there is no direct evidence that human beings require them to be present in their diets it has been suggested that atherosclerosis is due to a relative deficiency of them (Sinclair, 1956). No final evidence in favour of this view has been produced, and Gresham and Howard (1961) claimed that some of the evidence suggests that linoleic acid is actually atherogenic. This is then still a live issue.

This communication records the results of a trial of a preparation of phospholipid ("lipostabil") claimed to be particularly rich in polyunsaturated fatty acids and to be effective in ordinary therapeutic doses of the order of 1 g. a day. It is claimed that lipostabil prevents the destruction of linoleic acid in the intestine and therefore promotes its absorption. Ten parameters of the serum lipids have been measured and the results followed by sequential analysis. This statistical method requires an identical number of test and control subjects and depends entirely upon differences in behaviour between them. The double-blind method was employed and allocation was initially by random numbers. I know of no other trial of this substance complying with these requirements.

Subjects and Methods

Lipostabil was used in doses varying from three to nine capsules a day. The majority of the subjects had six capsules a day for most of the time. Each capsule contains 200 mg. of a selected fraction of soya bean phospholipids (prepared by Nattermann, of Cologne, as "essentiale"), and 1.5 mg. of pyridoxine hydrochloride.

Blood tests were made initially at fortnightly intervals and later in the trial after one month, three months,

and six months of treatment. Thereafter tests were made at six-monthly intervals. The progress of the trial was followed by plotting sequential t-test lines for 10 of the lipid parameters (Armitage, 1953) planned to detect differences between groups of 20 subjects with a probability of 20:1 ($\delta=0.85$). The trial was stopped after two years and four months, when all the lines corresponding to six months of treatment in subjects with old infarcts had crossed the boundary. A total of 114 subjects were thus recruited in a trial lasting 28 months, divided into three groups as shown in Table I. There were no significant differences in age-and-sex composition of the groups.

The fractions of the fats measured were as follows: (1) Cholesterol: modified Liebermann-Burchard method (Enticknap, 1961). (2) Total esterified fatty acids: modified Stern and Shapiro method (Enticknap, 1961). (3) Phospholipids (King and Wooton, 1956). (4) Total lipids were approximately measured by addition of the above three fractions (Sinclair, 1958, personal communication). (5) Beta-lipoprotein: modified flocculation method of Burstein and Samaille (1956) (see Enticknap, 1960). (6) Pre-beta lipid (Dangerfield and Smith, 1955): electrophoresis and scanning with calculation of the proportion of the total beta-lipoprotein in advance of the front boundary of the beta-globulin. (7) Absolute estimates of pre-beta lipid were derived by multiplying this proportion by the units of beta-lipoprotein from method 5. (8) Another estimate was derived by the quantitative method of Smith (1957). (9) Alpha/beta-lipoprotein ratios were calculated from the scans. (10) Alpha/beta ratios were also calculated. The levels of serum gamma-globulin and transaminase, plasma accelerated clotting-time (Quick one-stage), and blood sedimentation rate (Wintrobe) were also measured, and in the first 20 subjects the serum fibrinolysin activity.

A questionnaire was also completed by a medical observer for every subject who completed three months' treatment. The patient's own assessment of changes in his general condition, pain, and mobility were recorded and the doctor's view of the dietetic history, the regularity of treatment, any objective changes in clinical condition, and of the overall value of the treatment were recorded.

Results

The values of the non-lipid substances measured showed no significant differences between the test and control groups either in composition or in progress, but they did show a considerable amount of variation. In particular the fibrinolysin levels in 20 subjects were closely similar before and after treatment.

The material proved not to be highly active in modifying the blood lipids, and so one of the six-monthly lines required as many as 19 points and two

TABLE I.—Distribution of 114 Subjects in the Trial

	Group O. (Subjects who suffered myocardial necrosis for more than two months prior to starting treatment)			Group R. (Subjects who suffered acute myocardial necrosis within two months of starting treatment)			Group A. (Subjects suffering from other non-cardiac forms of clinical arterial disease)			Total All Groups
	Test	Control	Total	Test	Control	Total	Test	Control	Total	
Registered during trial	24	24	48	19	19	38	9	9	18	104
Paired subjects completing six months' treatment	20	20	40	12	12	24	8	8	16	80
Died	3	3	6	5	4	9	2	1	3	18
Stopped treatment	3	2	5	3	3	6	4	2	6	17
Pretreatment test only: not registered			3			6			3	12

others 18 points each to acquire significance. Two of the other seven lines had crossed their boundaries by the ninth (minimum possible) pair point. Twenty-six of the 36 sequential t-tests on changes in nine fractions during four periods of up to six months in the sera of patients seen shortly after an acute attack of myocardial necrosis were also completed by this time. The numbers receiving 12 months' treatment were rather smaller, but grouping together all subjects who had not recently suffered myocardial necrosis (groups O and A) enabled eight of the nine completed lines to be obtained for a group of arteriosclerotics with abnormal lipids. In all these t-tests only three results indicating significant differences between test and control groups were obtained. Two of these show changes in opposite directions in beta-lipoproteins in consecutive three-monthly periods and so represent a natural fluctuation. The other is the only significant result suggesting that the drug has a desirable effect—the pre-beta-lipid measured in Smith (1957) units did not rise as fast in the first three months of treatment as it did in untreated subjects. Nevertheless it did not fall in this period, and no significant differences were seen in the pre-beta-lipid values of the two groups when measured by scanning or computation from flocculation units.

Mean values have been calculated for all the blood constituents analysed for periods up to 18 months of treatment separately for test and control groups in each of the three diagnoses. Nearly all results showed higher mean values after a year or more and none showed a large fall. Table II shows the course of the mean cholesterol value, the total esterified fatty acids, and the beta- and pre-beta-lipoproteins in the group of

TABLE II.—*Course of Some of the Mean Values of Some of the Lipid Fractions Analysed. Subjects had Suffered Myocardial Necrosis More than Two Months Before Beginning Treatment*

	Initial Level			After 3 Months			After 6 Months		
	No.	Mean	S.D.	No.	Mean	S.D.	No.	Mean	S.D.
Whole blood cholesterol (mg./100 ml.):									
Treated subjects ..	23	282	47	23	328	65	20	320	50
Untreated ..	21	273	52	20	283	45	18	303	38
Total esterified fatty acids (mEq/l.):									
Treated subjects ..	21	13.9	2.6	21	13.6	5.1	19	11.7	4.1
Untreated ..	21	13.9	2.8	21	11.6	4.9	19	13.3	3.5
Beta-lipoprotein (units/100 ml.) (Burstin and Samaille, 1956):									
Treated subjects ..	23	11.6	2.2	23	10.1	3.5	20	12.2	3.0
Untreated ..	22	10.1	2.5	19	11.3	3.5	18	10.4	2.5
Pre-beta-lipid in Smith (1957) units:									
Treated ..	23	1.4	0.6	23	1.4	1.0	20	1.2	0.7
Untreated ..	20	1.2	1.0	18	1.8	0.8	18	2.0	1.0

subjects with old infarcts. None of the non-lipid analyses indicate any differences in selection of test and control groups, for in all cases the initial and subsequent mean levels in the two groups are similar.

Changes in mean lipid levels in the subjects with recent infarcts are rather erratic. The control subjects do, however, show the characteristic rise in every fraction over the first month. Similar rises are seen in the treated subjects, excepting only the total esterified fatty acids, and the difference in response in the two groups of 22 subjects is not significant. The mean level of these actually fell by 0.9 mEq/l. (13.1–12.2), but in the small number of cases treated for 18 months the final mean is actually higher than in their controls (14.9 and 13.5 mEq/l.).

Most of the subjects who stopped treatment defaulted, but some developed untoward symptoms. These were sickness in a single case receiving the active preparation, and sickness (two), leg pains, diarrhoea, the onset of nephrosis, and the development of carcinoma of the stomach respectively in the six control subjects.

A clinical analysis and questionnaire of the patients with recent necrosis showed no significant differences between test and control groups. About a quarter of each group were given dietetic instructions, and a similar number lost weight. On the other hand, significantly more frequent improvement was seen in the general condition, pain, and mobility of the treated subjects when all patients with disease of more than two months' duration were grouped together. Thus the general condition of 79% of the subjects improved with treatment but of only 40% of the controls. The S.E. of this difference of 39% is 13.7%. For pain the difference is 35% and the S.E. 14.5%, and for mobility 28% with S.E. 13.3%. Further, the doctors thought the active treatment useful in 75% of cases and the placebo capsules in 40% (S.E. of diff. 14.5%), although they were unable to show any difference in objective responses.

Discussion

These results show that administration of this particular phospholipid preparation in the doses recommended produced no useful objective effects when test series are compared with carefully matched controls. The total daily dose of polyunsaturated fatty acid (about 0.5 g.) is very small compared with the amounts of olive oil required to lower the blood cholesterol. Schettler (1956) claims that the favourable effect of lipostabil on serum lipids has been definitely established, but these results do not confirm his view. On the other hand, the preparation used in Germany also contains theophylline and potassium thiocyanate, although it is unlikely that these drugs have any effect of their own on the blood lipids. It is also claimed that the favourable changes are seen only in patients who are first treated some time after an attack of myocardial necrosis. This trial was planned specifically to test this view and has failed to show any real differences between the various diagnostic groups when treated cases are compared with controls in a similar clinical state. Finally, F. Leupold (1960, personal communication) suggested that good results will be seen only when the patient is dieted as well as treated. In this series no specific dietetic policy was followed, but those who were dieted did not obviously respond differently from those who were not.

The sequential method of analysis has proved valuable in this trial. The degree of probability required and the nominal size of the groups between which differences of this order must appear after losses from default and death have been made good must be predetermined for this analytical method. During the time needed for these criteria to be satisfied for patients with old infarcts receiving six months' treatment not only were a large number of incomplete individual protocols collected—a quite usual finding in all series requiring precise observations at specified times—but smaller groups of patients in other diagnostic groups and completing other periods of treatment were also studied. In fact, a complete set of 20 pairs was available at the end of this period for only one investigation, one which needed micro-amounts of serum. Nevertheless a sufficiently large number of pairs in this diagnostic group were

available for all the other lipid fractions to yield results of a degree of significance identical to that which would have obtained if groups of 20 test and 20 control subjects had been conventionally analysed. Furthermore, some significant results, albeit negative, were obtained for 12 months of treatment.

Similarly, although only 12 pairs of subjects who were treated soon after acute myocardial necrosis survived three months during the same period and 11 pairs for six months, a majority of the t-tests were completed, with the same probability. In short the sequential method has, as well as giving interim data and determining the trial, nearly doubled the amount of information obtained and therefore approximately halved the cost. Since there are no objective responses the differences between the replies of treated and untreated patients to questions on their well-being are probably explained by non-specific stimulating effects perhaps due to the pyridoxine content (Célice *et al.*, 1958) of the test capsules. Further, survival rates in both groups are comparable. The period of the trial is, however, as clearly shown by McMichael and Parry (1960), too short for significant conclusions on mortality to be drawn.

In view of the large amount of negative data here presented the trial has been stopped.

Summary

A total of 114 patients in three clinical groups, all of whom were suffering from results of atherosclerotic arterial occlusion, have been treated with a phospholipid preparation (lipostabil) for periods up to two years. Ten fractions of the serum lipids were unaffected by it.

The lipostabil was kindly supplied by Aspro-Nicholas of Slough, Bucks, who also made a grant towards the cost of the investigation. Many of the members of the staff of the East Ham Memorial Hospital, as well as local general medical practitioners, have co-operated willingly, and without their help the trial would not have been possible.

REFERENCES

- Armitage, P. (1953). *Quart. J. Med.*, **23**, 255.
 Burstein, M., and Samaille, J. (1956). *C.R. Acad. Sci. (Paris)*, **243**, 2185.
 Célice, J., Plas, F., Duchesnay, G., and Mme. Lemonnier (1958). *Presse méd.*, **66**, 389.
 Dangerfield, W. G., and Smith, E. B. (1955). *J. clin. Path.*, **8**, 132.
 Enticknap, J. B. (1960). *J. forensic Med.*, **7**, 135.
 — (1961). *J. clin. Path.*, **14**, 496.
 Gresham, G. A., and Howard, A. N. (1961). *Lancet*, **1**, 285.
 King, E. J., and Wooton, I. D. P. (1956). *Micro-analysis in Medical Biochemistry*, 3rd ed., p. 79 (method B). Churchill, London.
 McMichael, J., and Parry, E. H. O. (1960). *Lancet*, **2**, 991.
 Schettler, G. (1956). *Disch. med. Wschr.*, **81**, 1623.
 Sinclair, H. M. (1956). *Lancet*, **1**, 381.
 Smith, E. B. (1957). *Ibid.*, **2**, 910.

The Hong Kong Government plans to open a new welfare centre on the island to house 600 old and disabled people in about two years' time. It will replace the North Point camp, which was first opened in 1938 to house refugees of the Sino-Japanese War. The proposed welfare centre will occupy an area of 188,500 square feet and will consist of eleven buildings, mostly of two or three stories, providing accommodation for 600 people. It will serve as a transit centre for old people awaiting admission to a home for the aged and as a place of refuge for destitutes and disabled people pending a more permanent solution to their problems. There will also be sheltered workshops for those less capable of open employment.

SERUM GLYCOLYTIC ENZYMES AND ACID PHOSPHATASES IN MAMMARY CARCINOMATOSIS

BY

G. F. JOPLIN, M.B., M.R.C.P.

AND

K. A. JEGATHEESAN,* Ph.D., F.R.I.C.

From the Departments of Medicine and Chemical Pathology, Postgraduate Medical School of London, Hammersmith

Assessment of the effect of endocrine treatment on the progress of breast cancer is sometimes difficult, and an addition to the usual parameters would be clinically valuable. Bodansky (1954a) has shown that serum phosphoglucose isomerase is a good index of tumour growth in metastatic breast cancer, and Lemon and Reynolds (1956) have reported a correlation between the fall in serum copper-resistant acid phosphatase and regression of osseous metastases in oophorectomized or elderly (>70 years) women with advanced breast cancer treated with cortisone.

In the present study the clinical effects of pituitary destruction on breast carcinomatosis were followed and compared with serial measurements of the following serum enzymes: total (T.A.P.), formol-stable (F.S.A.P.), and tartrate-labile (T.L.A.P.) acid phosphatases, phosphoglucose isomerase (P.G.I.), and phosphoglucomutase (P.G.M.). A few determinations were made of serum aldolase (ALD) and lactic dehydrogenase (L.D.H.). Enzyme determinations were also made in patients who had not been treated by pituitary destruction. From these data an assessment of the clinical value of these serum enzymes as an index of cancer growth has been made. In an accompanying paper (Jegatheesan and Joplin, 1962) elevations of the different serum enzymes have been correlated with sites of metastases.

Patients Studied

Serial enzyme determinations were made in 17 women with advanced mammary carcinomatosis who were treated by pituitary destruction by needle implantation of yttrium-90 seeds (Fraser *et al.*, 1959). The age range was 36 to 72, with a mean of 49 years. Serum levels of the various enzymes were measured just before pituitary destruction and followed for 6 weeks to 18 months thereafter.

The effect of this treatment on the carcinoma was assessed independently by measurement of visible masses, radiology of bone metastases, study of serum and urinary calcium, serum alkaline phosphatase, and haematology, as well as the general progress of the patient, thus enabling classification of the patients into three groups, according to their clinical response to pituitary destruction: (1) *responders*—those in whom a clear remission in the above parameters was obtained (followed in some cases right into terminal relapse); (2) *non-responders*—those showing no clinical response; and (3) *"mixed" responders*—those whose improvement

*Part of this work is from a thesis submitted by K. A. J. (present address: Biochemistry Laboratory, General Hospital, Colombo, Ceylon) for the Doctorate of Philosophy of the University of London.