Double blind controlled trials and improved methods of assessment of the patient's condition are very time-consuming; they are nevertheless essential in studying the effects of drugs on psychiatric patients in order to circumvent the capacity for unwitting self-deception which both psychiatrists and their patients display.

We thank Mr. N. W. Blacow, chief pharmacist of the General Infirmary at Leeds, for supervising the coding and dispensing of the substances under trial; and Glaxo Laboratories Ltd. for the gift of "suavitil" brand benactyzine and the matched inert tablets used in the trial.

REFERENCES

- Cameron, D. E. (1944). *Amer. J. Psychiat.*, 101, 36.
 Davies, E. B. (1956). *British Medical Journal*, 1, 480.
 Jacobsen, E., Kjetil, H., Larsen, V., Munkvad, I., and Skinhoj, K. (1955). *Acta psychiat. neurol. scand.*, 30, 627.
 — and Skaarup, Y. (1955a). *Acta pharmacol. (Kbh.)*, 11, 117.
 — (1955b). *Ibid.*, 11, 125.
 — and Sonne, E. (1955). *Ibid.*, 11, 135.
 Jensen, O. Ø. (1955). *Dan. med. Bull.*, 2, 140.
 Ostentfeld, I. (1955). *Nord. Med.*, 54, 1523.
 Raymond, M. J., and Lucas, C. J. (1956). *British Medical Journal*, 1, 952.
 Seager, C. P., and Leitch, A. (1956). *Ibid.*, 2, 1407.

ANTILIPAEMIC AGENT WITHOUT ANTICOAGULANT ACTION

BY

E. M. M. BESTERMAN, M.D., M.R.C.P.

AND

JOHN EVANS, M.D., D.Phil.

Institute of Cardiology, London

There is now ample evidence that heparin influences the transport or metabolism of fats in man. When injected after a fatty meal it accelerates the disappearance of visible lipaemia (Anderson and Fawcett, 1950; Oliver and Boyd, 1953). Following heparin administration, analysis of serum lipoproteins by the ultracentrifuge shows a redistribution of the low-density fractions with reduction of the Sf 12-20 and increase of the Sf 6-12 group (Graham *et al.*, 1951). In 1953 Nikkilä reported comparable changes in the electrophoretic pattern of serum lipids after heparin, with diminution of the beta- and increase of the alpha-lipoproteins. However, this quantitative change in the electrophoretic pattern has not been found by all observers. The most constant change is an increase in mobility of both beta- and alpha-lipoproteins with an absolute decrease of the neutral fats and apparent diminution of the beta-lipoprotein (Herbst *et al.*, 1955; Kuo *et al.*, 1955; Besterman, 1957).

Thus heparin tends to rectify the abnormal lipaemia and Sf distribution associated with atheroma. There is as yet no convincing proof that human atherosclerosis will regress with heparin therapy, and to obtain such evidence a prolonged course of treatment would be required. Daily intravenous injection is impracticable, and the anticoagulant action of heparin makes repeated subcutaneous or intramuscular therapy hazardous.

To overcome this difficulty a substance is required which retains the antilipaemic properties of heparin but has no anticoagulant action. Constantinides *et al.* (1954) reported that certain sulphated polysaccharides with negligible anticoagulant action would clear turbid serum in rats, thereby establishing that the antilipaemic and anticoagulant effects were independent properties. Subsequently at the Institute of Seaweed Research Black and

Dewar (1954) showed that laminarin, a relatively homogeneous polysaccharide from the seaweed *Laminaria cloustoni*, could be sulphated to varying degrees. The highly sulphated compounds have an anticoagulant action, whereas the laminarins with few sulphate groups have little or no effect on blood-clotting. The object of the present study was to determine whether either of two lower-sulphated polysaccharides exhibited antilipaemic properties similar to those of heparin as judged by their effect on the electrophoretic pattern of the serum lipoproteins.

Method

The preparations used in this study were two samples of laminarin sulphate, M and N, with a low sulphate content—namely, 0.62 and 0.37 sulphate groups per glucose unit respectively. The anticoagulant activities of these preparations as tested *in vitro* were 1.4 and less than 1.3 international units per mg. respectively. Previous work had shown that laminarin sulphate M and N had very low acute toxicities in mice and guinea-pigs, in contrast to laminarin sulphate K, a preparation with high sulphate content which has been shown to be very toxic to guinea-pigs (Adams and Thorpe, 1957). Toxicity data are shown in the Table.

Acute Intravenous Toxicities of Laminarin Sulphates to Mice and Guinea-pigs

Animal	Substance	I.V. Dose (mg./kg.)	Deaths
Mice	Laminarin sulphate K	1,000	6/10
	" " "	1,500	9/10
	" " M	4,000	2/12
	" " N	2,800	1/9
	" " "	3,600	1/5
	" " "	4,000	5/8
Guinea-pigs	Heparin (100 I.U./mg.)	1,500	2/10
	" " "	2,500	5/5
	" " "	20	8/11
	Laminarin sulphate K	40	3/5
	" " M	200	0/5
	" " N	200	0/5
	Heparin (100 I.U./mg.)	250	0/5

These compounds, comparable to heparin in their low acute toxicity, were considered to be suitable for short-term clinical investigations.

Twelve patients with ischaemic heart disease were given an intravenous injection of 10,000 units of heparin and in 11 the electrophoretic pattern of the serum lipids was significantly altered. Only these 11 patients were subjected to further study. Nine of these patients subsequently received a single intravenous injection of 100 mg. of laminarin sulphate; six were given laminarin sulphate M and three laminarin sulphate N. The remaining two patients were given 100 mg. of laminarin sulphate M by intramuscular injection.

Samples of venous blood were taken from each patient before and at varying times from 10 minutes to 24 hours after injection. Paper electrophoresis of the serum was carried out as described by Dangerfield and Smith (1955). Each strip of filter-paper was divided longitudinally, half being stained for proteins with "light green" and half for lipids with "Sudan black B."

Two of the patients who received laminarin sulphate M had been given a fatty meal four hours before the intravenous injection. The visible turbidity of the serum and the electrophoretic pattern of the lipids were observed. To determine whether laminarin sulphate M or N altered the turbidity or electrophoretic pattern of the serum *in vitro*, 1 ml. of lipaemic serum was incubated at 37° C for one hour with 1 mg. of each of the polysaccharides.

The clotting-time of whole blood was estimated by the method of Lee and White before and after injection in five patients who received laminarin sulphate M and in two who received laminarin sulphate N.

Observations

Heparin.—The alterations in the electrophoretic pattern of the serum lipids after heparin consisted of increased mobility of the beta-lipoproteins and less consistently of the alpha-lipoproteins, reduced intensity of staining of the beta-lipoproteins, and clearing of the neutral fat zone (Fig. 1). Changes of variable degree were noted in 11 of the 12 patients. They were fully developed within 10 minutes of the injection and persisted for 6 to 24 hours. The serum proteins were unchanged after heparin save for the appearance of a band ahead of the albumin corresponding in position to the alpha-lipoproteins.

Laminarin Sulphate M.—After an intravenous injection of laminarin sulphate M in six patients, the electrophoretic pattern in all cases showed similar changes to those observed following intravenous heparin (Figs. 2 and 4). Increased mobility of the beta-lipoproteins and, to a lesser extent, of the

FIG. 1.—Electrophoretic pattern of serum proteins and lipids before and at varying times after an intravenous injection of 10,000 units heparin. NF=neutral fat; α-L=α-lipoprotein; β-L=β-lipoprotein; A=albumin; α₂=α₂-globulin; β=β-globulin. γ=γ-globulin. Arrow marks, point of application of serum.

FIG. 2.—Electrophoretic pattern of serum proteins and lipids before and at varying times after an intravenous injection of 100 mg. of laminarin sulphate M. (For symbols see Fig. 1.)

alpha-lipoproteins, was noted in each case. The beta-lipoprotein band was decreased and the neutral fat zone, when present, was cleared. As with heparin, the magnitude of the changes varied from patient to patient. These effects were most marked in the serum taken 10 minutes after injection and tended to disappear within four to six hours. The serum proteins appeared unchanged. Once again a band beyond the albumin fraction was seen in cases with greatly increased migration of the alpha-lipoproteins (Fig. 4). The distribution and mobility of the lipoproteins was not significantly altered in either of these patients, who received 100 mg. of laminarin sulphate M by intramuscular injection. No discomfort was noted locally at the site of injection. The clotting-time of whole blood was slightly prolonged after intravenous laminarin sulphate M in each of the five patients in whom it was measured. The maximum increase was from 9 minutes before injection to 13 minutes after injection. The visible turbidity of the serum was reduced in both patients

given laminarin sulphate M after a fatty meal (Fig. 3). These observations were confirmed on electrophoresis (Fig. 4). The sample taken 10 minutes after injection showed clearing of the heavy zone of neutral fat in addition to marked changes in the lipoproteins. Within four hours neutral fat had reaccumulated and the lipoproteins had reverted to the control pattern.

Laminarin Sulphate N.—No changes were observed in the electrophoretic pattern of the serum lipoproteins in any of the three patients given an intravenous injection of laminarin sulphate N (Fig. 5). The clotting-time of whole blood was not prolonged in either of the patients in whom it was measured.

FIG. 3.—Visible turbidity of serum following a fatty meal, before and at varying times after an intravenous injection of 100 mg. of laminarin sulphate M.

FIG. 4.—Electrophoretic pattern of serum proteins and lipids before and at varying times after an intravenous injection of 100 mg. of laminarin sulphate M in a patient who had been given a fatty meal. (For symbols see Fig. 1.)

FIG. 5.—Electrophoretic pattern of serum proteins and lipids before and at varying times after an intravenous injection of 100 mg. laminarin sulphate N. (For symbols see Fig. 1.)

In-Vitro Studies.—The addition of either laminarin sulphate M or N *in vitro* to lipaemic serum caused neither visible clearing of the serum nor any significant alteration in the electrophoretic distribution of neutral fats and lipoproteins.

Discussion

As only small amounts of the two laminarins were available for clinical trial, it was particularly useful that laminarin sulphate N proved to be inert and thus acted as a control for the positive findings following injection of laminarin sulphate M.

A single intravenous injection of the lower-sulphated polysaccharide laminarin sulphate M cleared lipaemic serum, increased the electrophoretic mobility of lipoproteins, and altered the distribution of lipoproteins in the same way as heparin. The changes could be demonstrated almost immediately after injection, and persisted for four to six hours. They were not reproduced by the addition of laminarin sulphate M to serum *in vitro*. It is evident, therefore, that the antilipaemic properties of laminarin sulphate M closely parallel those of heparin, except for the shorter duration of action of the dose used in this study. On the other hand, laminarin sulphate N, a closely related polysaccharide, showed no demonstrable effect on the electrophoretic mobility or distribution of lipoproteins.

No effect was noted when 100 mg. of laminarin sulphate M was injected intramuscularly. It is possible, however, that with larger doses changes may be seen following both intramuscular and subcutaneous injections, and that the duration of the changes will be prolonged by slow release from the tissues at the site of injection. No local reaction resulted from intramuscular injection of the polysaccharide, and no reactions were observed in any patient following intravenous laminarin.

Since laminarin sulphate M possesses the antilipaemic properties of heparin without the hazards of its anticoagulant action, it would appear to be a suitable substance with which to study the effect of prolonged administration of "lipaemia-clearing" substances on the course of atherosclerosis in man. Following the completion of chronic toxicity trials in animals and further information on the optimal dose and most convenient method of administration, a long-term investigation of patients with ischaemic heart disease will be undertaken.

Conclusion

A lower-sulphated polysaccharide of laminarin is described that has antilipaemic properties similar to those of heparin without significant anticoagulant activity. It would appear to be a suitable agent to assess the effect of "lipaemia-clearing" substances on the course of atherosclerosis in man.

We are indebted to Dr. Woodward and his staff at the Institute of Seaweed Research for advice and supplies of laminarin sulphate, and to the Research Department of Boots Pure Drug Company Ltd. for carrying out toxicity trials and the preparation of the material for clinical use. We would in particular like to acknowledge the co-operation and assistance of Drs. E. V. B. Morton, Cobb, and Adams, of Boots. Dr. Paul Wood, Director of the Institute of Cardiology, has throughout provided us with invaluable advice and encouragement, and we are grateful to him and to the other physicians of the National Heart Hospital for their kind co-operation. For untiring technical assistance we are indebted to Miss B. Hall.

REFERENCES

- Adams, S. S., and Thorpe, H. M. (1957). To be published.
Anderson, N. G., and Fawcett, B. (1950). *Proc. Soc. exp. Biol. (N.Y.)*, **74**, 768.
Besterman, E. M. M. (1957). To be published.
Black, W. A. P., and Dewar, E. T. (1954). *J. Sci. Food Agric.*, **5**, 176.
Constantinides, P., Cairns, A., and Werner, A. (1954). *Arch. int. Pharmacodyn.*, **99**, 334.
Dangerfield, W. G., and Smith, E. B. (1955). *J. clin. Path.*, **8**, 132.
Graham, D. M., Lyon, T. P., Gofman, J. W., Jones, H. B., Yankley, A., Simonton, J., and White, S. (1951). *Circulation*, **4**, 666.
Herbst, F. S. M., Lever, W. F., Lyons, M. E., and Hurley, N. A. (1955). *J. clin. Invest.*, **34**, 581.
Kuo, P. T., Joyner, C. R., and Reinhold, J. G. (1955). *Circulation*, **12**, 492.
Nilkiilä, E. (1953). *Scand. J. clin. Lab. Invest.*, Suppl. 8.
Oliver, M. F., and Boyd, G. S. (1953). *Clin. Sci.*, **12**, 293.

DIFFUSION OF RADIOACTIVE SODIUM IN NORMOTENSIVE AND PRE-ECLAMPTIC PREGNANCIES

BY

TREVOR JOHNSON, M.D., F.F.A. R.C.S.
M.R.C.O.G.

AND

C. G. CLAYTON, M.Sc.

From the Department of Obstetrics and Gynaecology, King's College, University of Durham, and the Department of Radiotherapy, Royal Victoria Infirmary, Newcastle upon Tyne

The diminished placental transfer of radioactive sodium which is known to occur in pre-eclampsia (Browne and Veall, 1953; Cox and Chalmers, 1953; Johnson and Clayton, 1955; Clayton *et al.*, 1956) may be due either to a change within the placenta itself or to a reduction in its blood supply from the uterine decidua. This latter possibility has been investigated by Morris *et al.* (1955), who developed a simple method for measuring the effective blood flow in the pregnant uterus. It is based on a determination of the clearance rate of radioactive sodium ions following their injection into the anterior wall of the uterus in the final stages of pregnancy.

It is claimed that the physiological half-life of the injected radiosodium is almost constant in normotensive subjects, whereas in patients suffering from pre-eclampsia there is a very significant reduction in the clearance rate of the isotope. The actual values recorded are dependent upon the clinical severity of the condition as judged by the magnitude of the maternal diastolic blood pressure.

The significance of these results in relation to the possible use of the method in the identification of pre-eclampsia, and to a better understanding of this syndrome, is clear.

We have made similar investigations and have measured the radiosodium clearance rates in normotensive patients, pre-eclamptics, and a group under treatment with a hypotensive drug.

Method

(a) Uterine Muscle

Twenty microcuries of $^{24}\text{NaCl}$ contained in exactly 0.4 ml. of isotonic saline is injected into the antero-lateral aspect of the pregnant uterus between the 34th and 43rd weeks of pregnancy, in the manner described by Morris *et al.* (1955).

The abdominal skin in the region of McBurney's point is infiltrated with a small quantity of 1% procaine, and a hypodermic needle 2 in. (5 cm.) long is introduced into the subjacent myometrium. Localization within the uterine wall is confirmed by asking the patient to inspire, when the needle mount is depressed downwards as the abdominal wall slides over the uterus. Subsequent aspiration with a sterile syringe confirms that the needle point has not punctured the amniotic sac or a large vessel. Experience shows that very careful attention to these details of technique is essential.

After injecting the radiosodium, a gamma-ray G.-M. counter, with the end window screened, held in a delicately balanced support, is located over the injection site. It is kept in position with a thin strip of adhesive plaster. Pressure of only a few grammes is exerted on the skin, and the balancing mechanism enables the counter to ride any abdominal movement which may take place, so that a constant relationship with the injected site is maintained.