hog duodenal mucosa, has been studied in 22 patients. Thirteen patients who had previously been treated satisfactorily with parenteral vitamin B₁₂ for at least one year, received biopar therapy for up to five years. After 3½ years the level of vitamin B₁₂ in the serum was abnormally low in most patients, and haematological relapse occurred in two patients after four to five years.

Nine patients with previously untreated pernicious anaemia showed a satisfactory initial response to biopar therapy, but one relapsed after 2½ years and in four others the vitamin B₁₂ in the serum did not reach a satisfactory level. The level of vitamin B₁₂ in the serum reached the normal range in the remaining four, in two of whom normal values were still found after treatment for five years.

Skin tests performed with an extract of hog duodenal mucosa did not show any relationship between skin sensitivity to the extract and refractoriness to biopar treatment.

The results of the investigation show that this oral treatment is not reliable for maintenance of patients with pernicious anaemia.

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REFERENCES

- Bastrup-Madsen, P. (1957). *Acta med. scand.*, **159**, 323.
 Berlin, R., Berlin, H., Brante, G., and Sjöberg, S.-G. (1958). *Ibid.*, **161**, 143.
 Blackburn, E. K., Cohen, H., and Wilson, G. M. (1955). *Brit. med. J.*, **2**, 461.
 Chalmers, J. N. M., and Shinton, N. K. (1958). *Lancet*, **2**, 1298.
 Della Vida, B. L., and Dyke, S. C. (1942). *Ibid.*, **2**, 275.
 Estren, S., and Wasserman, L. R. (1956). *Proc. Soc. exp. Biol. (N.Y.)*, **91**, 499.
 Killander, A. (1957). *Lancet*, **1**, 1041.
 — (1958). *Acta med. scand.*, **160**, 339.
 Kristensen, H. P. Ø., Lund, J., Ohlsen, A. S., and Pedersen, J. (1957). *Lancet*, **1**, 1266.
 Lowenstein, L., Brunton, L., Shapiro, L., De Leeuw, N., and Dufresne, M. (1957). *Canad. med. Ass. J.*, **77**, 923.
 Mollin, D. L., and Ross, G. I. M. (1954). *Proc. roy. Soc. Med.*, **47**, 428.
 Schwartz, M. (1958). *Lancet*, **2**, 61.
 — Lous, P., and Meulengracht, E. (1957). *Ibid.*, **1**, 751.
 Spray, G. H. (1955). *Clin. Sci.*, **14**, 661.
 — and Witts, L. J. (1958). *Brit. med. J.*, **1**, 295.
 Stokes, J. B., and Pitney, W. R. (1958). *Ibid.*, **1**, 322.
 Taylor, K. B., and Morton, J. A. (1958). *Lancet*, **1**, 29.
 Wilkinson, J. F. (1949). *Ibid.*, **1**, 249.

The polio vaccination campaign among young people is being intensified in two of the L.C.C.'s health divisions in north London. Dr. J. A. SCOTT, the L.C.C.'s Medical Officer of Health, has written to nearly 10,000 firms, coffee bars, dance halls, hairdressers, gramophone record shops, and others in Finsbury, Hackney, Holborn, Islington, Shoreditch, and Stoke Newington, to ask for their help. In Finsbury, Holborn, and Islington the council will use a mobile clinic to take "the jab to the job," parking in or near the factories and offices where the young people work. Up to September this year there have been 84 confirmed cases of polio in these six north London boroughs, six of them fatal. But Dr. Scott points out that quite three-quarters of young Londoners between the ages of 15 and 26 have not yet sought protection: this despite the fact that the chances of being attacked by polio are about five times greater for the unvaccinated than for the vaccinated.

PLACENTAL IMPLANTATION FOR PERIPHERAL VASCULAR DISEASE

BY

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Since Filatov (1935) reported the use of various tissue implants as an aid in corneal grafting, numerous papers have been published from the continent of Europe advocating placental tissue or placental extract in the treatment of many diseases. Sneepe (1955) from Holland, and Piccoli (1954) and Cordaro (1957) from Italy, have recommended this therapy for advancing the healing of wounds and ulcers, while in the German literature several reports have appeared on the use of placental extract in the disturbances of the climacteric (Branger and Krattiger-Müller, 1957). The extract has also been used in skin wounds by Bromilski (1956), of Poland, and in intestinal fistula by Junod (1954), of France. In this country Troensegaard-Hansen (1956) has used amnion both as an implant and as a surface application for ulcers.

In 1956 D. Le Grand (personal communication) stated that implantation of the cotyledon surface of the placenta gave spectacular results in persistent intermittent claudication, and the present paper is a favourable preliminary report of a clinical trial of this method of treatment.

Method

Selection of Patients.—Every patient reporting to the clinic on account of severe intermittent claudication was admitted for investigation and included in the first part of this trial. Later, the method was attempted on an out-patient basis. These patients had previously had Buerger's exercises, and in many cases had received vasodilator drugs. A few had had a previous sympathectomy. Each patient was warned that two implants might be required, so that a control series could be arranged. If the control method of treatment failed, the patient received an implant of placenta.

Assessment of the Patients.—A full history and medical examination revealed the general state of the patient. Examination of the cardiovascular system was supplemented in most cases by an electrocardiogram and ophthalmoscopy. The peripheral circulation was assessed by inspection of the skin, palpation of the pulses, estimation of venous filling time, and exercise tolerance. The actual lesion was identified by arteriography, which also gave an indication of the collateral circulation and general state of the vessels. The capacity of the arteries to dilate was assessed by a reflex heating test and the blood flow in the limbs by oscillometry. This assessment enabled the patient to be placed in one of three groups suitable for: (1) conservative treatment, (2) sympathectomy, or (3) arterial graft. Patients in all three groups received placental implants as the first method of treatment.

Preparation of Placenta.—A placenta was delivered straight from the maternity unit in a sterile container. The blood group of mother and placenta was

established. Under full aseptic precautions a number of 1-cm. cubes of the cotyledon surface were excised, and each cube was placed in a universal container with 1 mega unit of penicillin dissolved in 4 ml. of normal saline. These containers were incubated at 4° C. for five days, when the tissue was considered ready for implantation. Three 1-cm. cubes were implanted subcutaneously in the thigh of the maximally affected limb, through small separate incisions.

Control Series.—The patients were divided by random selection into five groups, only one of the authors knowing to which group the patients belonged. Group 1 received an implant of placenta. Group 2 received an "implant" of penicillin and saline to act as controls for the trauma of incision and for the use of preparation fluid. Group 3 received an "implant" of the preparation fluid after it had been incubated with the placental tissue for five days. This method produced a saline extract of the placenta. Group 4 received an "implant" of similarly prepared human protein, thereby acting as a foreign-body protein. Group 5 received an "implant" of 150 mg. of ethinyloestradiol.

Assessment of Results.—The patients were examined at intervals in the immediate post-operative period, and, in particular, the reflex heating test and oscillometry were repeated five days after implant.

After discharge from hospital the patients were seen regularly by various members of the staff.

Clinical Features

TABLE I.—Age and Sex Incidence

	Age in Years					
	40–	50–	60–	70–	80–	90–
Males	3	9	10	6	1	1
Females	2			5		

Age and Sex Incidence (Table I).—62% of the patients were over 60, and the sex ratio was approximately three males to every female.

Symptoms

Intermittent Claudication (Table II).—This was the main symptom, and the severity was graded as: severe=less than 100 yards; moderate=less than 440 yards;

TABLE II.—Pain

Side	Duration	Severity
Left 6 } 14	< 6 months .. 8	Severe 27
L > R 8 }	6/12–1 year .. 4	Moderate .. 9
	1–2 years .. 2	Mild 1
Right 8 } 18	2–5 " .. 13	
R > L 10 }	5–10 " .. 5	
L = R 5	10–20 " .. 5	

mild=more than 440 yards. The majority of the patients were in the severe grade: 60% had a history of claudication for over two years. It was interesting to note that the right leg was affected more frequently than the left.

Associated Symptoms (Table III).—Evidence of coronary arterial disease was common, six patients subsequently developing coronary thrombosis and four dying with myocardial infarction.

TABLE III.—Associated Symptoms

Angina of effort	12
Dyspnoea on exertion	11
Previous coronary thrombosis	4
Varicose veins	4
Near gangrene	5
Necrotic ulcer	5
Diabetes mellitus	2

Signs

Peripheral Pulses (Table IV).—The right popliteal pulse was absent more often than the left, which agreed with the frequency of the complaint of pain in the right side.

TABLE IV.—Pulses Palpable

Artery	Right	Left
Femoral	35	36
Popliteal	17	23
Anterior tibial	12	11
Posterior	11	10
Dorsalis pedis	12	7

Severe Trophic Changes.—In two cases there were chronic ulcers on the leg, which failed to respond to conservative therapy and were thought to be due to inadequate arterial circulation. Four cases had incipient gangrene, and three of these eventually required amputation.

Blood Pressure.—Most of the patients were hypertensive.

Venous Filling Time (Table V).—This test confirmed the diminution of flow in the more affected leg and was considerably prolonged in three-quarters of the group.

TABLE V.—Venous Filling Time

	< 15 Seconds	< 30 Seconds	> 30 Seconds
Better leg	7	11	3
Worse "	5	6	10

Electrocardiography.—Eleven out of 33 patients had evidence of a damaged myocardium, which confirmed the clinical evidence of a history of previous infarction or angina pectoris.

Reflex Heating Test.—This was done on 34 patients and only eight showed a good response to the test. It was interesting to note the flat curves of patients who had previously had a sympathectomy. In these cases the initial temperature level was higher on the sympathectomized side.

Oscillometry.—This was carried out in the first 28 patients investigated. Only six had normal readings; the remainder showed evidence of severely diminished blood flow in the affected limb. The test was not used routinely for the rest of the series.

Arteriography.—Fourteen of the initial series showed a localized block, and in seven the disease was found to be a diffuse atheroma.

Blood Group.—The blood-group distribution was almost identical with figures for the South-Eastern Region of Scotland, and was insignificant in this series.

Results

Group 1, Placental Implant (Table VI)

TABLE VI.—Results

	Subjective	Oscillometry	Reflex Heat
Group 1:			
Initial series ..	19/28	2	7
Total	27/39		
Group 2	0/5	0	0
" 3	3/5	1	1
" 4	0/3	0	0
" 5	3/8	0	1

Thirty-nine patients received placental implants, and of these 27 felt a noticeable effect. Following implant, there was a definite pattern of response. Within six hours the patient usually experienced a feeling of

tingling and heat in both lower limbs. Subsequently, the exercise tolerance increased, as measured by the claudication distance.

In the follow-up period, some of the patients who had responded relapsed after some months and had a

peripheral pulses. In four patients, two with early infective necrosis of toes and two with long-standing ulcers over the medial malleolus, otherwise resistant lesions healed and remained healed.

Investigation showed an improvement in the reflex heating response in 7 out of 28 cases, but an improvement in the oscillometry readings was noted in only 2 of the 28.

Groups 2-5 Controls (Fig. 2)

Patients in group 2 and group 4 showed no change, but three of the five cases in group 3 had a temporary improvement in their claudication distance. Group 5 cases also showed some improvement for a short time, three out of eight responding to the implantation of oestrogen hormone.

Discussion

This investigation into the effect of placental implantation in patients with peripheral vascular disease was planned to discover — (a) if it caused any improvement in the patient's symptoms, (b) if the improvement was subjective or if objective evidence of improvement could be obtained, and (c) if an obvious cause could be found for improvement when it occurred.

From the results obtained it was clear that most patients did obtain some relief of their symptoms, especially as judged by their claudication distance. It is realized that the estimation of walking distance without carefully controlled treadmill conditions may be misleading, yet the effect was so striking and appeared in such a short time that it seemed to be convincing.

The subjective feeling of increased warmth in the limbs noted by all patients who were in the improved group was accompanied by a rise in the skin temperature, with a better response to the reflex heating test in 7 out of 28 cases. Despite this, however, oscillometry showed an improvement in only 2 out of 28 cases, and in no case was any alteration in pulses noted.

FIG. 1.—Duration of improvement in symptoms.

repeat implantation. These patients again experienced improvement in their symptoms. Fig. 1 indicates the duration of the improvement and the fact that in over two-thirds of the patients it was prolonged over six months.

Clinical examination revealed an apparent increased skin temperature, but there was no change in the

GROUP 2			
	1	2	3
T.B. []	Reimplant	Improved	
AM'S [X]	Reimplant	Failed	
E.B. []	Reimplant	Improved	
GG. [X]	Reimplant	Improved	
R.M.F. []	Reimplant	Improved	

GROUP 4		
F.F. [X]	Reimplant	Improved
A.B. [X]	Reimplant	Improved
M.B. []	Reimplant	Improved
R.S. []	Reimplant	Improved

GROUP 3								
	1	2	3	4	5	6	7	8
D.K. []								
A.C. [X]								
J.N. []								
J.H. []								
A.B. [X]								

GROUP 5		
J.C. []	Reimplant	Improved
C.C. [X]		
D.C. [X]		
M.T. [X]		
J.M.C. [X]		
M.T. []	Developed Cerebral Thrombosis	
G.C. [X]		
V.M.K. [X]	Reimplant	Temporary 4/12 → Symp.

FIG. 2.—Results in groups 2-5. Key as Fig. 2.

That the collateral circulation is not obviously improved is suggested by the fact that of five patients who had deteriorated despite previous lumbar sympathectomy placental implantation gave a good result in only two, while of three patients who failed to respond to placental implant sympathectomy relieved only one patient, the other two showing no improvement from either form of treatment.

The possibility that any effect is due to a non-specific reaction to foreign protein introduced in the placental tissue seems unlikely, since patients in Group 2 showed no beneficial response of any duration.

The fact that the injection of the fluid in which the placental tissue had been stored for five days gave a satisfactory, if temporary, response suggests that there is some substance in placenta which is responsible for the effect, and that this effect lasts longer when active placental tissue is implanted. The nature of the substance has not yet been determined, but from the effect of 150 mg. of ethinyloestradiol it is tempting to associate it with this hormone.

Urinary gonadotrophin excretion was assayed in one patient who gave a good response to the implant of placenta, and this showed no change in levels.

In two cases the placental implant was removed after amputation and had remained viable after seven days. In cases with remission of symptoms it was noted that the longer the implants remained palpable the longer was the relief of symptoms.

In five patients who had a good effect from the first placental implant a second implant was made, and in three the satisfactory response was repeated (Fig. 3). This again suggests that an active principle is secreted by or liberated from the placental tissue which is responsible for the effect.

In this investigation arteriograms were carried out in each patient, and 14 showed a localized block in the worst-affected limb. Nine of these patients were on general grounds unfit for any major surgical procedure, a tenth had additional gross atheroma, while a further patient had a block in the popliteal fossa, with no filling of the collateral vessels. Thus only three of this series of patients could be regarded as suitable for arterial grafting. The remainder of the series showed generalized atherosclerosis, and since this investigation was begun two years ago six have died as a result of myocardial infarcts or cerebrovascular accidents.

Sympathectomy was considered in all cases in which placental implant failed to improve the condition of the skin, but only 8 out of 34 patients tested by reflex heating showed a good response, and, of these, only three were regarded as good operative risks.

In conclusion, this investigation seems to have established that placental implant causes subjective improvement in the patient's symptoms and has a useful place in the treatment of peripheral vascular disease. The active principle is not yet known, and is a matter for further investigation.

Summary

A controlled investigation into the effect of placental implant in peripheral vascular disease was carried out.

Placenta, placental extract, and implantation of ethinyloestradiol produced relief of symptoms for varying periods.

Foreign protein, or saline and penicillin, inserted under identical conditions, produced no remission of symptoms.

There is some indication that the favourable effects are due to placental hormones, possibly those of the oestrogen group.

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REFERENCES

- Branger, F., and Krattiger-Müller, D. (1957). *Praxis*, **46**, 216.
Bromilski, J. (1956). *Pediat. pol.*, **31**, 1341.
Cordaro, M. (1957). *Transplant. Bull.*, **4**, 13.
Filatov, V. P. (1935). *Arch. Ophthal. (Chicago)*, **13**, 321.
Junod, J. M. (1954). *Praxis*, **43**, 1102.
Piccoli, B. (1954). *Folia med. (Napoli)*, **37**, 1083.
Sneep, A. J. (1955). *Ned. T. Geneesk.*, **99**, 850.
Troensegaard-Hansen, E. (1956). *Brit. med. J.*, **2**, 262.

FIG. 3.—Results from placental implant. Key as Fig. 2.

An international panel of experts, convened by the International Atomic Energy Agency to study the best methods of collecting and measuring trace amounts of radioactive substances present in the biosphere (man's surroundings), met in Vienna from September 7 to 12. The rapidly increasing use of radioactive materials for peaceful purposes is confronting many countries with the necessity of introducing a system of collection and accurate measurement of radioactivity, both natural and artificial, in the biosphere. This will serve as a means of checking whether or not small amounts of radioactive substances from reactors, for instance, or from industrial processes, are reaching the soil, rivers, or the atmosphere. Such systematic measurement could be used as the basis for controls to forestall any dangers to health and property. The I.A.E.A. panel hopes to issue recommendations as to the best methods of collecting samples and of measuring their radioactivity. (International Atomic Energy Agency, Vienna.)