does) the best long-term result is obtained with a homograft replacement of the whole of the bifurcation (if necessary up as far as the renal arteries), the two long ends of the bifurcation being brought down as low as is necessary to find good artery and anastomosed end to side. (Special Plate Fig. 1 well illustrates this principle.) Case 2 of Series I is a good example of how failure to observe this principle led to a poor result. A blocked external iliac artery was successfully resected and replaced by a graft. The patient remained symptom-free for a year, then the aortic bifurcation above the graft occluded, causing a return of symptoms. The aortic bifurcation was known to be extensively diseased at the time of the original operation, and for a permanent result the patient should have had a full bifurcation graft.

Finally, in aortic aneurysms we prefer a graft of orlon cloth. A number of such grafts tailored to all sorts of different sizes are kept in the theatre ready to hand for the occasion.

General Indications for Operation

On the basis of these results we now feel confident in recommending operation to any patient in the under-60 age group who has a major arterial occlusion and wants relief from his or her symptoms. If the lesion is a localized occlusion with reasonably good artery on either side, direct removal of the occluding thrombus from the artery—"rebore"—is a sound and safe operation. If the arterial disease is more widespread, a better long-term result will be obtained by radical resection of the whole aortic bifurcation and by-passing the diseased segments on each side with a stored homograft.

In the over-60 age group one's attitude is tempered by the increased likelihood of other arteriosclerotic lesions such as angina. However, even in these patients where a clear indication exists—such as pain and impending gangrene of a limb—the benefits of this type of surgery far outweigh the risks.

Aortic aneurysms tend to occur in the 60–80 age group, and here the indication is steadily increasing pain, which is the usual prelude to rupture.

Summary

A detailed follow-up study of 30 patients with either arteriosclerotic occlusion or aneurysm of the abdominal aorta and its major branches, who underwent direct arterial surgery during the last five years, is presented.

Three different types of direct arterial surgery have been used: (*a*) replacement with stored homograft; (*b*) replacement with plastic prosthesis; and (*c*) "endarterectomy" or "rebore" of the occluded segment.

The hospital mortality has been 7%. The delayed mortality (due directly or indirectly to the operation) has been a further 7%.

The specific indications for the use of these three types of procedure are given, based on the follow-up results reported.

The importance of performing an end-to-side large lumen type of anastomosis when using homografts for replacing or by-passing arteriosclerotic material is stressed, and the beneficial effect of dealing with the upper of two arterial blocks in the same limb is described.

On the basis of the follow-up results reported, and an analysis of these cases, general principles for recommending this type of surgery are suggested.

We would like to thank our many colleagues who have referred these cases. Also we record our grateful thanks to the many registrars and house-surgeons who have helped with these sometimes arduous operations, and the nursing staff of St. Thomas's Hospital and the Royal Waterloo Hospital, to whose care the low mortality in this series is largely due. We wish to thank Miss J. Dewe, our medical artist, for the illustrations.

ADDENDUM

Since this article was written one more polyvinyl graft has obliterated (Series II, Case 6). The patient has had slight increase of his claudication and symptoms in his left leg, but is in no immediate danger of amputation. This further strengthens the conclusions already mentioned above that polyvinyl alcohol sponge material is unsuitable for arterial grafting.

One further rebore of a common iliac artery and two further bifurcation grafts have been carried out successfully.

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GRAFT STERILIZATION

A BACTERIOLOGICAL AND HISTOLOGICAL STUDY OF THE RELATIVE MERITS OF ETHYLENE OXIDE AND β-PROPIOLACTONE AS TISSUE STERILIZING AGENTS, WITH SPECIAL REFERENCE TO ARTERIAL GRAFTS

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[WITH SPECIAL PLATE]

In discussing a method for the preparation of freeze-dried arterial grafts Flewett *et al.* (1955) drew attention to the obvious advantages of using a sterilizing agent, which, they suggested, would render asepsis unnecessary during collection and would allow the use of post-mortem material for grafts. Previous workers have used either ethylene oxide (Hufnagel *et al.*, 1953) or β-propiolactone (LoGrippo *et al.*, 1955; Rains *et al.*, 1956; Berkin *et al.*, 1957).

Two questions arise if chemical sterilizing agents are used: (1) Can such agents effectively sterilize necropsy material intended for use as grafts? (2) Do such agents give rise to gross histological changes in the arterial wall? In this paper we compare ethylene oxide and β-propiolactone for sterilizing power and for failure to produce gross histological changes in graft material.

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Methods

Bacteriological Investigations

Source of Material.—A total of 136 arterial and 289 bone grafts were collected for preservation. All the bone grafts and 54 of the arterial grafts were obtained by dissection under aseptic conditions. The remainder of the arterial grafts (82) were removed in the post-mortem room during routine necropsies.

Sterilization.—Ethylene oxide: a 1% solution (v/v) was used as previously described (Flewett *et al.*, 1955). β-Propiolactone: a liquid and relatively innocuous, although a concentrated solution may damage the skin and a mist is injurious to the eyes and respiratory tract. If it is carefully handled, and especially if dropping it on the skin is avoided, this agent is safe to use and has the added advantage that it is soon hydrolysed to the acid in aqueous solution. The procedure used, with almost unfailing success in our hands, is as follows. To the graft completely immersed in physiological saline and contained in a sterile tube of standard bore (Flewett *et al.*, 1955) is added sufficient β-propiolactone to give a final concentration of 1% (v/v). The tube is tightly stoppered with a sterile rubber bung and the contents are thoroughly mixed by tilting. The rubber bung is replaced with a sterile gauze-covered cotton-wool plug, and after incubation at 37° C. for two hours the fluid is poured off. The tube is then left inverted for about 10 minutes to allow surplus fluid to drain away from the graft. Any residual β-propiolactone either breaks down, is sublimed during the freeze-drying process, or is washed out during reconstruction, making any further attempts at elimination as described by LoGrippo *et al.* (1955) unnecessary.

Sampling.—Before sterilization a small portion of a number of the arterial grafts was taken for histology. Immediately after completion of either of the sterilization procedures outlined above the contents of each tube were sampled for sterility and for histological change. The sterility tests were carried out in nutrient broth for aerobic growth and in Robertson's meat medium for anaerobic growth, incubation extending over at least five days. The cultures in Robertson's meat medium were incubated in the normal manner for the first 24 hours (allowing recognition of the less strict anaerobes, in particular *Clostridium welchii*) and then in a McIntosh and Fildes anaerobic jar for a further five days (for isolation of *Cl. tetani*). After the graft material had been reconstituted in physiological saline containing 100 units penicillin, 100 μg. streptomycin, and 50 units heparin per ml., any fragments left unused were examined bacteriologically and histologically.

Since sterilized freeze-dried bone grafts are completely assimilated, as shown radiologically (Berkin *et al.*, 1957), only bacteriological examination of these was deemed necessary.

Histological Investigations

Material.—This consisted of 16 specimens of aorta or iliac artery prepared as freeze-dried grafts by the method of Flewett *et al.* (1955). The ages in years of the donors were respectively 8, 13, 17, 21, 23, 26, 29, 30, 31 (2), 32, 33 (2), 34, 36, and 37. Ten specimens were sterilized with ethylene oxide and six with β-propiolactone.

Technique.—Samples from vessels were fixed in formol-saline (*a*) before treatment; (*b*) after snap-freezing and freeze-drying, and reconstitution, but without chemical sterilization; (*c*) after sterilization alone; and (*d*) after sterilization, snap-freezing and freeze-drying, and reconstitution. Blocks from all but one of the samples of vessels after reconstitution and a few blocks from the other samples were cut as frozen sections. Blocks were taken from parts of the samples showing early atheroma or lipid flecking, whenever these lesions were apparent. Paraffin sections were stained with Ehrlich's haematoxylin–eosin, Verhoeff's elastic stain counterstained with Van Gieson's mixture, toluidine blue, and the periodic-acid–Schiff (P.A.S.) procedure. Frozen sections were stained with scarlet-red.

Results

Bacteriological

In all, 425 grafts have been sterilized and freeze-dried. The earlier ones (79 specimens) were sterilized with 1% ethylene oxide; of these, eight (10%) were still contaminated and five were discarded. The remaining three were successfully re-sterilized with 1% β-propiolactone when we became aware of its advantages. The later grafts (346) were all treated with 1% β-propiolactone, and four (1.1%) of these yielded some growth. All the failures in both series occurred with arterial grafts.

Table I shows that β-propiolactone is somewhat superior to ethylene oxide as a sterilizing agent. Furthermore, of all the grafts tested for sterility after use in the operating theatre, only 3% of both the ethylene-oxide-treated and of the β-propiolactone-treated bone grafts proved to be contaminated, and then merely with non-pathogenic cocci. This observation indicates that the process of freeze-drying as carried out in Leeds (Flewett *et al.*, 1955; Berkin *et al.*, 1957) is bacteriologically sound, and that the contamination observed may well have occurred in the operating theatre after the tube containing the graft was opened. The persisting sterility of arterial grafts following reconstitution and use in the operating theatre is probably due to the addition of penicillin and streptomycin to the reconstituting fluid.

TABLE I.—*Comparison of the Sterilization of Arterial and Bone Grafts With 1% (v/v) of Ethylene Oxide and of β-Propiolactone*

Graft	Ethylene Oxide				β-Propiolactone			
	No. Treated	No. Sterile after Treatment	No. Used and Examined Bacteriologically	No. Sterile after Use	No. Treated	No. Sterile after Treatment	No. Used and Examined Bacteriologically	No. Sterile after Use
Arterial	33	25 (76%)	3	3 (100%)	103	99 (96%)	23	23 (100%)
Bone ..	46	46 (100%)	35	34 (97%)	243	243 (100%)	113	110 (97%)

Table II shows that the failure rate for sterilization of arterial grafts taken at necropsy is much lower with β-propiolactone than with ethylene oxide, although the ethylene oxide series is rather small. It must be pointed out, however, that wastage of some valuable arterial grafts because of persisting contamination after sterilization with ethylene oxide led us to abandon this technique as soon as we were convinced that β-propiolactone was superior. Although dissection in the operating theatre reduces the failure rate with ethylene oxide, the results barely equal those obtained for post-mortem material with β-propiolactone as a sterilizing agent.

TABLE II.—*Comparison of Results of Sterilization of Arterial Grafts Obtained from Routine Necropsies With Those Taken Under Aseptic Conditions*

Source of Material	Sterilizing Agent	Number Treated	Number Sterile after Treatment
Post-mortem room	Ethylene oxide	15	9 (60%)
	β-Propiolactone	67	64 (91%)
Operating theatre	Ethylene oxide	18	16 (89%)
	β-Propiolactone	36	35 (97%)

Histological

Samples from vessels in groups *a*, *b*, and *c* were examined as controls.

(a) Microscopical Appearances Before Treatment.—There were no changes to be seen other than slight atheroma.

(b) Microscopical Appearances after Snap-freezing and Freeze-drying and Reconstitution, but Without Chemical Sterilization.—Sections were available from six specimens of aorta. The intima shows no more than slight fragmentation. The changes in the media are similar to those seen in samples after sterilization, snap-freezing and freeze-drying, and reconstitution (see under (*d*)).

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(c) Microscopical Appearances After Sterilization Alone.—The first group comprised seven samples of aorta treated with ethylene oxide. One specimen also included part of one common iliac artery. Microscopic changes are slight and inconstant. The elastica seems slightly fragmented in the media of two specimens of aorta. Occasionally small ovoid spaces are present in the intima and media.

In the second group there were four samples of aorta and four samples of common iliac or femoral artery after treatment with β-propiolactone. In general, microscopical examination shows a very slight fragmentation of the medial elastica and a loss of metachromasia with toluidine blue. Occasional disrupted patches are seen in the intima. The changes are no more pronounced than in the specimens treated with ethylene oxide. One specimen of aorta was also stained with scarlet-red after frozen section, and this showed lipid in the intima without disruption.

(d) Microscopical Appearances After Sterilization, Snap-freezing and Freeze-drying, and Reconstitution.—Changes in the media were insignificant. Small oval spaces were formed in the media of the aorta as well as in the common iliac and femoral arteries. In specimens sterilized with ethylene oxide these spaces contain material staining metachromatically with toluidine blue, like mucin or acid mucopolysaccharide. Often this mucinoid material is limited to the margin of the oval spaces and is deeply staining. The mucinoid material does not stain with P.A.S. In specimens sterilized with β-propiolactone the space is devoid of mucinoid material, although the mucinoid material is present in other parts of the arterial wall. The elastic laminae were slightly fragmented and showed some crowding near the oval spaces. Most of these changes have been depicted by Hyatt *et al.* (1952).

Lipid in the intima, staining with scarlet-red, was present in eight out of 16 specimens subjected to frozen section. This finding emphasizes the frequency of early atheroma or "superficial fatty streaking" (Duguid, 1954) in specimens of aorta and large arteries from young subjects. The lipid was present in variable amount, but in six specimens it was moderately abundant (Special Plate, Figs. 1 and 2). The presence of lipid might be expected to lead to disruption of the intima during preparation of the graft. On the whole, however, disruption was slight, and in a graft from a man aged 34 a substantial infiltration of lipid was separated from the lumen by a layer of intact intima. In four specimens considerable infiltration was associated with only the slightest intimal disruption. In other specimens lipid infiltration was sometimes associated with the formation of oval spaces (Plate, Fig. 1) and, strikingly seen in a sample of a graft from a woman of 23, a crenellated intimal surface (Plate, Fig. 2). Often the lipid is distributed as if still contained in macrophages.

Discussion

Hufnagel *et al.* (1953) reported that 1% ethylene oxide sterilizes cultures of *Staphylococcus aureus, Clostridium welchii, Escherichia coli,* and *Bacillus subtilis* in culture media, of heterografts taken without aseptic precautions, and of such grafts deliberately contaminated with these organisms. Similarly, 1% β-propiolactone has been shown to kill *Staph. aureus* and members of the normal flora of the intestinal tract (Hartman *et al.*, 1951; Rains *et al.*, 1956) and a number of pathogenic fungi (Bernheim and Gale, 1952).

Of the two sterilizing agents examined, ethylene oxide is the more difficult to handle. It is volatile at room temperature, toxic, and explosive. These properties alone make it less suitable for use than β-propiolactone. Moreover, the results of this investigation show that it is less effective as a sterilizing agent for arterial grafts. The greater the degree of contamination the less effective is its action (see Table II). β-Propiolactone, on the other hand, is almost equally effective whether the grafts are obtained under aseptic conditions in the operating theatre or at necropsy.

When dealing with bone grafts no difference between the two sterilizing agents was apparent. This might be misleading, for in the collection of all bone tissue an aseptic "no-touch" technique has been employed hitherto (Berkin *et al.*, 1957).

Histological changes in the vessels, although slight, involve both intima and media. The most interesting change seems to have been the result of freeze-drying when pre-existing lipid was present in intimal plaques and streaks. Neither chemical sterilizing agent appears to have caused any significant structural changes.

The observations of DeBakey *et al.* (1954) suggest that the intima, after grafting, is lost or replaced by a layer of fibrin. The long-term fate of atheromatous changes in grafts remains to be determined, and such lipid accumulations must be studied by microscopical examination of grafts removed at necropsy from recipients. Duguid (1954) has emphasized that fatty lesions are almost constantly present in the aorta from childhood onwards, and are known to be compatible with long life. DeBakey *et al.* (1954) stressed that the elastica persists microscopically unchanged after grafting, and seems to support the graft during the period of organization in the host. Fragmentation of the medial elastica, seen in a few of our specimens of prepared grafts, seems too slight to cause any significant weakening of the arterial wall. Such fragmentation may have occurred during the preparation of the microscopical sections, as it is also present in a proportion of grafts treated chemically with or without subsequent freeze-drying and reconstitution.

Muscular arteries can be successfully grafted as elastic arteries, and therefore, if the views of DeBakey *et al.* (1954) are correct, it seems that the slender internal and external elastic laminae are sufficient to support the vessel wall.

Small oval spaces in the media have also been observed by Creech *et al.* (1954), who consider that they are probably the result of the formation of ice crystals during freeze-drying. These spaces appear too small and sparse to cause any significant weakening.

Summary

A technique for the sterilization of tissue grafts with 1% β-propiolactone is described.

A comparative study shows that β-propiolactone is superior to ethylene oxide as a sterilizing agent for artery and bone grafts; it has greater sterilizing powers and is much easier and safer to use.

Freeze-drying, but not sterilization with either agent, leads to the appearance of small spaces within the intima and media of the arterial wall. We are of the opinion that the spaces are too small and sparse to cause any significant weakening of the arterial wall.

We wish to thank Professor C. L. Oakley, F.R.S., for the laboratory facilities necessary to the freeze-drying process. We gratefully acknowledge a grant towards expenses given by the Board of Leeds United Hospitals. We are indebted to Mr. F. Dexter, A.I.S.T., and Mr. D. F. High for technical assistance.

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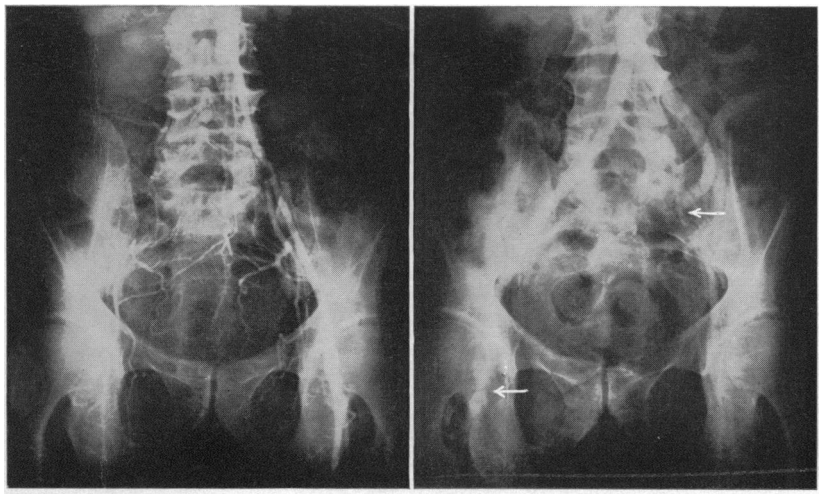

FIG. 1A FIG. 1B

FIG. 1.—Case 5, Series I. A: Pre-operative aortogram, showing widespread obliterative disease of aortic bifurcation and left common iliac artery with complete occlusion of right common iliac. B: Aortogram taken six months after operation, showing complete replacement by large homograft. The sites of the two peripheral end-to-side anastomoses are shown by arrows.

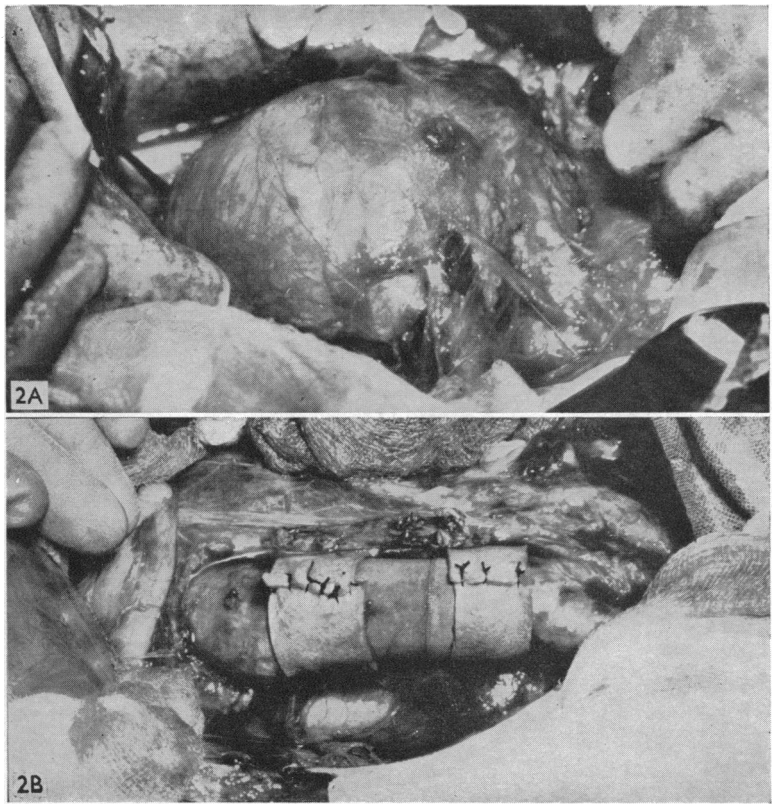

FIG. 2.—Aneurysm of abdominal aorta: (A) before and (B) after replacement by an "orlon" tube. Note method of reinforcement of anastomosis by two polyvinyl cuffs.

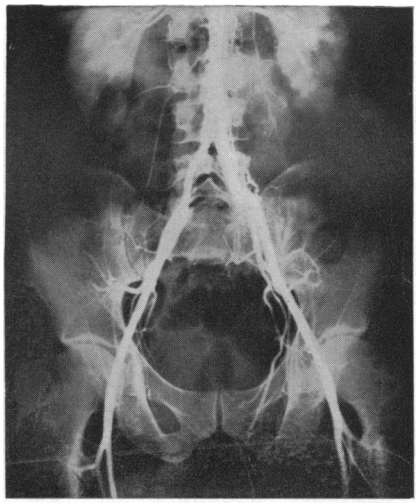

FIG. 3A

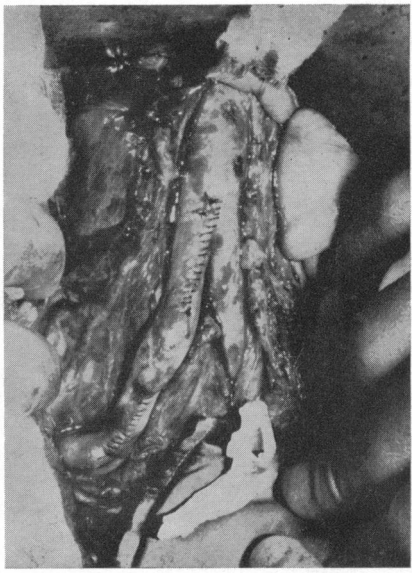

FIG. 3B

FIG. 3.—Case 4, Series III. A: Aortogram showing long narrowed segment of right common iliac artery. B: Operative photograph showing obstructing thrombus, which was removed from artery through two small arteriotomy incisions. (Thrombus is white object at bottom centre.)

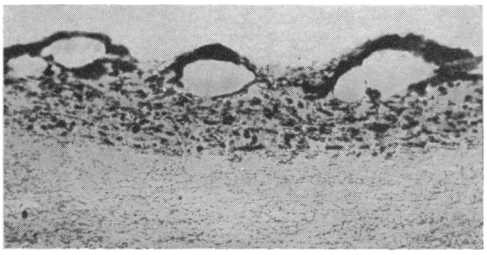

FIG. 1

FIG. 1.—Intimal lipid (black in figure) in prepared graft from donor aged 17. Lipid infiltration is here associated with formation of oval spaces. (Frozen section, stained scarlet-red. ×75.)

FIG. 2.—Intimal lipid (black in figure) in prepared graft from donor aged 23. Note crenellated intimal surface. (Frozen section, stained scarlet-red. ×20.)

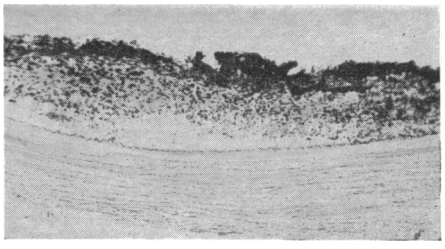

FIG. 2

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