Feb. 4, 1969 M. CHVAPIL ETAL 3,425,418

ARTIFICIAL BLOOD VESSELS AND METHOD OF PREPARING THE SAME

Filed April 15, 1964

Fig. 1

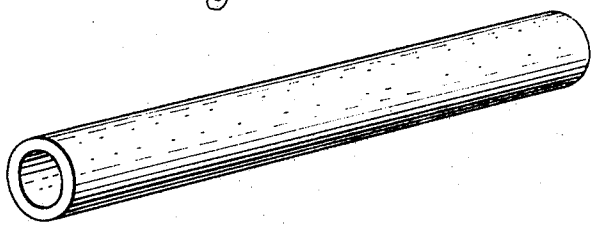


Fig. 2

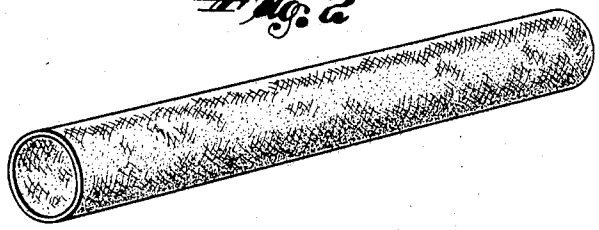


Fig. 3

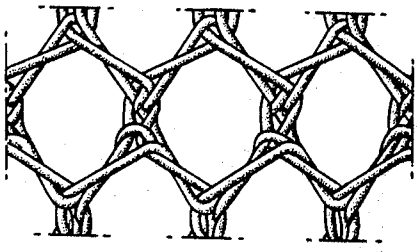


Fig. 4

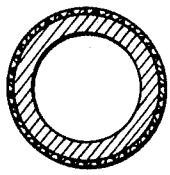


Fig. 5

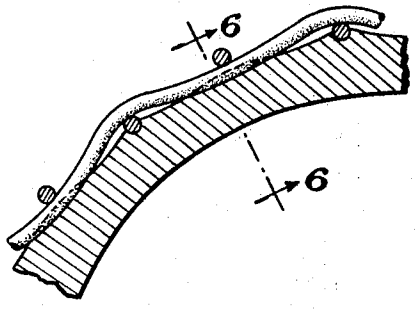
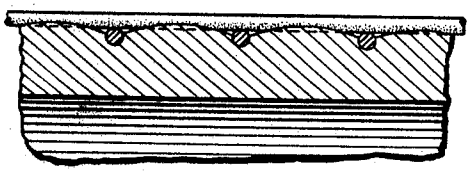


Fig. 6



INVENTORS.
Miloš Chvapil
Milan Krajíček
Miloslav Adam
Richard Chvapil
agent

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ARTIFICIAL BLOOD VESSELS AND METHOD OF PREPARING THE SAME

Milos Chvapil, Milan Krajicek and Miloslav Adam, Prague, Czechoslovakia, assignors to Spofa, Sdružení podniků pro zdravotnickou Vyrobu, Prague, Czechoslovakia

Continuation-in-part of application Ser. No. 330,221, Dec. 10, 1963. This application Apr. 15, 1964, Ser. No. 359,973
Claims priority, application Czechoslovakia, June 15, 1962, 5,644/62
U.S. Cl. 128—334 5 Claims
Int. Cl. A61b 17/04; A61f 1/24; A61l 17/00

ABSTRACT OF THE DISCLOSURE

An artificial blood vessel includes a tanned collagen tube strong enough to be self-supporting, and impregnated with an anti-coagulant which is covered with open mesh fabric secured to the tanned collagen by an outer wall portion of collagen in the native chemical state thin enough not to close the pores in the fabric.

This application is a continuation-in-part of our copending application Ser. No. 330,221, filed on Dec. 10, 1963, and now abandoned.

This invention relates to vascular grafts, that is, to artificial blood vessels, and to a method of preparing the same.

As described in more detail in a paper published by us and on our behalf in the Journal of Surgical Research (vol. 3, No. 7, pages 358 to 368, Sept. 1963), a vascular graft should be non-toxic, flexible, and porous. It should retain its strength permanently in intimate contact with body fluids, and should be readily accepted and incorporated into the surrounding tissue. The graft or prosthesis, moreover, should not unfavorably affect the flow of blood therethrough.

In the surgical repair of blood vessels and body ducts, inert fabrics have found considerable use. The known inert fabric prostheses, however, do not become parts of the body tissues. They frequently remain surrounded by a pool of serum after the healing process is terminated. We have found that the relatively low porosity of the known prostheses accounts for the observed formation of fluid pockets, and prevents the growth of repair tissue through the fabric grafts.

The porosity of a fabric may be characterized by the volume of water in milliliters per minute that will pass through one square centimeter of the fabric under a pressure differential of 120 millimeters mercury and this definition of porosity is being employed in this specification and the appended claims. The vascular fabric prostheses that have found use in surgery heretofore have a porosity of less than 2,000 milliliters per minute.

It has now been discovered that healing will be greatly improved and the growth of repair tissue through the fabric promoted if the porosity of the fabric is much greater, that is, in the range of 10,000 to about 22,000 milliliters per minute. A vascular graft of the invention thus includes a fabric of the desired porosity. Such a fabric is not in itself impervious to blood.

In the improved prostheses of the present invention, the porous, non-absorbable fabric framework is rendered bloodtight by a collagen film or tube bonded to one side of the fabric. The fabric is exposed to the surrounding tissue thus permitting ingrowth of fibroblasts and endothelial cells which results in the attachment of the prosthesis to the host tisues. The collagen has considerable tensile strength, is non-antigenic, and is slowly absorbed at a rate which may be controlled by partial tanning so that there is no appreciable decrease in the strength of the vessel structure while the collagen is being replaced by new body tissue.

It has further been found that clotting of blood in vascular grafts of even very small internal diameter (three millimeters and less) can be prevented if the interior collagen surface of a vascular prosthesis of the invention contains an anti-coagulant such as heparin. The anticoagulant properties of natural blood vessels are ascribed to the presence of mucopolysaccharides, particularly chondroitin sulfate B and heparitin sulfate. We may therefore add these or other anticoagulants to the collagen structure as a whole, or we incorporate an anticoagulant only in the inner wall portion of the collagen tube to decrease the thrombotic effects of the graft. We have found that heparin is quickly adsorbed from aqueous solutions by a partly tanned collagen wall, and cannot readily be removed from the wall under ordinary physiological conditions. A collagen wall containing heparin has permanent anticoagulant properties.

While the present invention is not limited to any particular theory of action, it is believed that heparin and other anticoagulants are reversibly bound to the collagen through polar groups present in the anticoagulants and in the collagen, and that the anticoagulant is slowly released into the bloodstream over a period of days as the collagen is absorbed by the body. Thus, the probability of clotting is sharply reduced during the first few days after the implantation of the graft when the patient is most susceptible to this danger, and an open lumen is maintained during the deposition and organization of the fibrin lining surrounding the graft.

Added mucopolysaccharides also increase the hydrophilic properties of the collagen, and they improve the physicochemical stability of the collagen by the presumed link between the mucopolysaccharides and the collagen molecules. The hydrophilic properties of the absorbable tube are also greatly enhanced by the admixture of glycerin which ensures elasticity of the tube even after tanning.

The artificial blood vessels of the inveniton may be sterilized by radiation or by chemical means in a known manner. They may be stored over extended periods without loss of their desirable properties. Small amounts of antibiotics added to the collagen during the preparation of the vessel and contained in the adsorbable walls thereof further ensure safe storage and have the expected beneficial results after implantation.

An outer wall portion of the collagen layer adjacent the fabric layer is preferably kept untanned, that is, in the native chemical state and amorphous. The untanned outer wall portion is adhered to the fabric layer, but does not obstruct access from the outside to the pores of the fabric.

Fabrics well suited for the vascular grafts of the invention include those made from polyester fibers such as polyethylene terephthalate which are sold under the registered trademarks Dacron and Terylene. Polytetrafluoroethylene fibers, such as those known under the trademark Teflon also may be employed, but are less resilient and are not so conveniently fabricated as the polyester fabrics. The polytetrafluoroethylene fabrics, however, have superior healing properties and produce thinner, more compact capsules.

The non-absorbable fabric that reinforces the collagen may be prepared in tubular form by braiding, weaving, or knitting. Braided grafts lack dimensional stability and are not widely used, whereas, both woven and knitted grafts have been used clinically. Woven grafts must be tightly

constructed or fraying and fiber slippage become problems at implantation. It is for this reason that woven grafts have comparatively low porosities. Knitting permits variations in the tightness or porosity without commensurate penalties in fray or slip qualities, and is the preferred method of construction.

Other features and many of the attendant advantages of this invention will be readily appreciated as the same becomes better understood by reference to the following detailed description when considered in connection with the accompanying drawing in which:

FIG. 1 is a perspective view of an extruded collagen tube;

FIG. 2 is a perspective view of a non-absorbable fabric tube;

FIG. 3 is a greatly enlarged view of the nonabsorbable knitted fabric that forms the reinforcing structure illustrated by FIG. 2;

FIG. 4 is a greatly enlarged view of a section of a vascular prosthesis constructed in accordance with the present invention;

FIG. 5 is a fragment of FIG. 4, enlarged; and

FIG. 6 is a view along the line **6—6** of FIG. 5.

Referring now to FIGS. 1, 2, and 4, a vascular graft may be constructed in accordance with the present invention by slipping a collagen tube inside of a fabric tube. The collagen tube is softened by soaking in water or a dilute aqueous acid solution and is then expanded against the external fabric tube. A convenient way to accomplish this is to increase the pressure of the air within the tube, and thereby force the collagen into contact with the inner wall of the fabric. In so doing the collagen is forced against the fabric and into the interstices between the non-absorbable fibers. The structure is then air-dried whereby the inner collagen tube is stabilized in the form illustrated in FIGS. 4, 5, and 6.

The non-absorbable fabrics may be knitted, crocheted, woven, or braided in the shape of the desired prosthesis, as a film, tube, Y-tube, etc. While the drawings illustrate a tubular structure suitable for use as a vascular graft, it will be understood that other shapes in which collagen is bonded to one side of a non-absorbable fabric characterized by a porosity not less than 10,000 milliliters per minute and not substantially more than 22,000 milliliters per minute offer similar advantages.

It will be understood that the collagen present in the prostheses of the present invention may be treated with tanning agents such as formaldehyde, pyrogallol, chromium, etc., by methods well known in the art to obtain increased strength, and to control the rate at which the collagen will be absorbed.

A combination fabric-collagen vascular graft may also be manufactured by constructing the tubular fabric framework of Dacron or Teflon to the desired diameter. The fabric framework is then coated on both sides with a collagen mass obtained by swelling collagen fibrils in an aqueous acid solution. The swollen collagen fibrils are then frozen in position and deswollen by dehydration in an organic solvent. Finally the collagen that lines the interior wall of the composite tube is treated with heparin.

In this type of construction, it is important that the mesh of the non-absorbable fabric be sufficiently open to permit the collagen fibrils to extend into and through the interstices of the fabric. These collagen fibrils that pass through the fabric cohere to the collagen fibrils on the other side of the fabric framework and form a unitary structure that resists delamination. It is preferred that only the interior wall of the graft be treated with heparin as the ingrowth of fibroblasts and endothelial cells appears retarded by the presence of heparin on the exterior surface of the graft.

The improved prostheses of the present invention may also be constructed by weaving, knitting, crocheting, or braiding together an inert non-absorbable thread or yarn with collagen yarn or collagen multifilament. Again the interior surface of this tubular structure is treated with heparin.

In applying heparin to the interior collagen wall an aqueous solution containing from 0.25% to 1% heparin may be used. The contact time of this solution with the collagen should be sufficient to permit the heparin to penetrate about 70% of the distance through the wall of the tube. For the reasons indicated above it is preferred that there be no heparin on the exterior wall of the tube.

The present invention is more fully described and exemplified in the following examples. It is to be understood, however, that our invention is not to be limited to any specific form of materials or conditions set forth in the examples, but is limited solely by the appended claims. Throughout the examples which follow, all quantities are expressed in parts by weight.

Example I

A rigid, self-supporting collagen tube having an internal diameter of about 10 millimeters and a wall thickness of about 1 millimeter was extruded by the procedure described in the Becker Patent No. 2,246,236, using a plastic collagen mass containing about 30% collagen by weight. The extruding mechanism was that described in the Becker Patent No. 2,115,607 and resulted in orientation of the collagen fibers to give excellent strength characteristics.

The tube so obtained was placed within a woven Dacron tube having a porosity of 22,000 milliliters per minute and the assembly was immersed in water until the collagen became plastic. One end of the prosthesis was then closed and sufficient air pressure was applied to the other end of the prosthesis to force the collagen against the inner wall of the fabric. The fabric-collagen tube was then air-dried in an oven at 45° C. overnight without damage. The product so obtained is illustrated in FIGS. 4, 5, and 6.

One end of the collagen-Dacron prosthesis was again closed and the tube was filled with an aqueous solution containing 1.0% of heparin. The heparin used, standardized by the sheep plasma coagulation method, was found to contain 100 units of anticoagulant activity per milligram of dry material. After the heparin solution had penetrated about three-fourths of the distance through the wall of the collagen tube, the prosthesis was emptied of the heparin solution and air-dried overnight.

The collagen-Dacron prosthesis was next tanned by immersing it for 30 seconds in a solution of 0.4 part of pyrogallol, 0.1 part tetrasodium ethylenediamine tetraacetic acid, and 99.5 parts of water adjusted to pH 8.3 with ammonium hydroxide. It was then re-dried in an oven at 50° C. for 6 hours.

The prosthesis was next immersed for 30 seconds in a solution of chromium (III) sulfate comprising 0.8 part of chromium as chromic oxide, 0.5 part of lactic acid (85%), 0.24 part of formaldehyde, and 98.46 parts of water, adjusted to pH 2.7 with sodium hydroxide. The prosthesis was again dried in an oven at 50° C. overnight. The finished prosthesis was sterilized by irradiation.

Example II

Fresh steer hides were washed with cold water at 13° C. or less in a rotating drum for 10 to 24 hours. After washing, the hides were defleshed with a scraping machine, and the hair and epidermis were cut off with a horizontal band knife. This preliminary cleaning was accomplished with standard tannery equipment.

The remaining hair and poorly cleaned sections were cut off by hand and compositions were prepared from five hides. The hide composites were then cut into ½ to 4 square inch sections and reduced to pulp by three passes through a meat grinder, each pass being a finer grind. The first and second passes were through 18 and 8 millimeter holes, respectively. The final grind was through holes 1.5 millimeters in diameter. Special care was taken during

the grinding process to keep the pulp below 20° C. This was done by adding crushed ice to the hide sections as they were fed to the grinder.

The ground pulp was next diluted with tap water at 16° C. to give a smooth slurry containing 25% dry solids. To this slurry was then added lactic acid, and the mixture was kneaded to form a homogeneous mass of swollen collagen fibers. The mixture so obtained contained about 25% by weight hide solids.

The collagen mass was extruded as described in Example I to form a collagen tube having a diameter of about 8 millimeters and a wall thickness of about 0.4 millimeter.

This extruded tube was placed within a Teflon fabric tube of the type illustrated in FIG. 2, characterized by a porosity of 10,000 milliliters, and expanded against the interior wall of the fabric as described in Example I.

The vascular prosthesis so formed was tanned by immersing it in an aqueous solution containing 2% trimethylolmelamine (Solarpret), and dried in an oven at 45° C. overnight. It was further treated with heparin as in Example I.

Example III

The deep flexor tendon of cattle was cleaned of fat, superficial non-collagenous protein and other extraneous. matter and was sliced on an electric meat-slicing machine (rotary knife) in the frozen condition. The tendon sections were sliced perpendicularly to their longitudinal axis to a thickness of about 11 mils. An aliquot sample of the tendon slices was analyzed; the dry solids amounted to 36.97%.

The sliced tendon was next treated with an enzyme solution to dissolve elastin. This enzyme solution was prepared by dissolving 0.15 part of ficin and 3.75 parts of ethylene diamine tetrasodium tetraacetate in 750 parts of water. Seventy-five parts of the sliced tendon was immersed in this solution which was stored at room temperature overnight. Then 2.25 parts of 30% hydrogen peroxide were added to destroy any residual ficin.

To this mixture of tendon slices in about 750 parts of water were added an additional 2244 parts of water and 5.87 parts of cyanoacetic acid. The swelling solution was cooled to below 25° C. This mixture was stirred in a dispersion kettle at about 60 r.p.m. The remaining steps in the process were carried out at a temperature below about 25° C. and the temperature of the collagen dispersion was not allowed to exceed this temperature.

Stirring was continued for about 3 hours, during which time the individual collagen slices were swollen. The dispersion was homogenized by repeated passes through series-connected jets having orifices of 50 mils and 40 mils respectively. The dispersion was then forced through a leaf filter containing three screens of #316 stainless steel. These screens were separated by ⅛-inch spacers and decreased in mesh size so that the dispersion first passed a 14-mil screen, then a 9-mil screen, and finally a 4-mil screen. The dispersion of swollen solvated collagen fibrils so obtained analyzed 1.09% solids and had a pH of 2.52.

A glass tube having an inside diameter of about ¾-inch was fitted with a one-hole stopper of rubber through which a 5/16-inch glas rod was placed so that the glass rod extended coaxially within the glass tube. Before placing the glass rod and rubber stopper in position, the glass rod was covered with a piece of rubber tubing, and a cylindrical tube of open-mesh woven Dacron about ⅝-inch in diameter was slipped over the glass rod and rubber tube. The glass tube and glass rod were assembled in an upright position with the bottom of the Dacron fabric tube resting on the rubber stopper. The dispersion of swollen collagen fibrils, prepared as described above, was poured into the glass tube while maintaining the fabric tube in a coaxial position and equally spaced between the rubber tube and glass tube so that both sides of the fabric were coated with the collagen dispersion. This mold with the dispersion and fabric in place was frozen in the vertical position for at least 4 hours at −20° C.

The mold was then placed in a static coagulation bath consisting of 2 liters of isopropanol, 30 cubic centimeters of concentrated ammonia (25%) and 10 cubic centimeters of formaldehyde (37% solution) at room temperature, and the mold was maintained in the solution for 16 hours. The glass rod covered with the rubber tube and the formed collagen tube were then removed and placed in a dehydrating bath consisting of 2 liters of isoproponal. The collagen tube was left in this bath for an additional 16 hours to complete the dehydration.

After dehydration, the rubber tube with the collagen tube on it was very carefully slid from the glass rod, and the rubber tube was removed from the interior of the collagen tube by pulling on both ends of the rubber tube, thereby stretching the rubber tube and reducing its diameter. After the collagen tube was removed from the rubber tubing, it was plasticized in a bath consisting of 2 liters of 90% isopropanol (10% water) containing 5% glycerine. This plasticizing operation is optional. After 24 hours in the plasticizing bath, the collagen tube was supported on a glass rod and air-dried.

One end of the collagen-Dacron prosthesis so obtained was closed and the tube was filled with an aqueous solution containing 0.25% heparin. When the heparin solution had penetrated three-fourths of the way through the collagen-Dacron tube, the tube was emptied and air-dried.

Example IV

Hide sections of beef cattle were comminuted and homogenized together with a dilute solution of sodium hydroxide for 24 hours, 60 milliliters 1/100 N NaOH being added per 100 grams of the hide material. The homogenized mass was left standing in a refrigerator for 24 hours at a temperature between 0° and 4° C. whereupon it congealed into a soft gel.

During homogenizing, there were added 0.5 percent hyaluronic acid (a mucopolysaccharide), 3 percent glycerin, and 1 gram chlorotetracyclin per 100 grams of hide material.

The gel was extruded in the manner described in Example I to form a tube of 9 millimeter external diameter and a wall thickness of 0.5 millimeter. Air was simultaneously blown into the axial cavity of the extruded tube at a pressure which was controlled between 40 and 150 mm. water column to prevent collapse of the extrudate.

The extruded tube was dried to constant temperature at ordinary room temperature (15 to 25° C.) in a gentle stream of air. A very thin layer of amorphous unmodified collagen was applied to the dry outer surface by spraying from a gun, and a loosely knit tube of smooth, non-crimped Terylene was slipped over the tube in contact with the freshly deposited collagen layer.

The fabric, of conventional weft knit construction, had a permeability to water of 18,000 ml./cm.^{2}/min. at 120 mm. Hg. It was elastically stretchable about 15% longitudinally as well as transversely. While some of its threads were adhered to the collagen tube by the freshly deposited collagen layer, the porosity of the fabric was virtually unaffected by its engagement with the resorbable material.

The tube was filled with an aqueous 1% solution of 2,4,6-trimethoxytriazine, a commercial tanning agent commonly sold under the name Solapret. The tanning solution was removed after ten minutes, and the tube was washed out with water to remove residual unreacted tanning agent. The treatment was then repeated with aqueous 1% heparin solution which was left in contact with the inner wall of the tube for five minutes. The heparin penetrated about ¾ of the collagen wall. The tube was emptied and air-dried overnight.

The completed artificial blood vessel was sealed in a glass ampoule and was sterilized by exposure over five hours to gamma radiation of 2×10^6 roentgen.

The method outlined above may be modified in many respects without departing from the spirit and scope of this invention. It is not necessary fully to dry the collagen tube prior to covering the same with fabric. Actually, it is possible to encase a freshly extruded wet collagen tube in a tubular fabric envelope, and to adhere the fabric to the collagenous material by expanding the tube under internal air pressure. The pressure has to be carefully matched to the mechanical strength of the collagen tube to avoid embedding the fabric material in collagen to an extent which would make the pores of the fabric unavailable or not readily available. The use of a sprayed or brushed outer coating of unmodified collagen may be dispensed with in such a case, but it may also be omitted where a fabric layer is applied to dried collagen, and high mechanical strength of the vascular graft is not required.

The composition of the collagen tube may be varied as to the minor admixtures. Hyaluronic acid thus may be replaced by other mucopolysaccharides or mixtures thereof, such as those derived in a conventional manner from the corpus vitreum or cartilage of beef cattle. The mucopolysaccharides have distinctly noticeable effects on the stability of the gel when present in amounts of as little as 0.5 percent. We have not observed further desirable changes when more than 3 percent of the mucopolysaccharides were added to those naturally present in the collagen material after processing.

Glycerin is beneficial in amounts of at least 0.5 percent, and may be increased to five percent with corresponding improvement of elasticity and plasticity of the collagenous material, even when partially tanned.

The effects and dosages of antibiotics are well known from the literature on the use of implants of a similar nature. Merely by way of example, it may be stated that suitable antibiotics include chlortetracycline in amounts of 1 to 5 grams per 100 grams of the collagen mass. 0.5 to 5 grams neomycin, or 10,000 to 50,000 units bacitracin. The quantitative limits indicated are not critical nor is this list complete.

While it is critically important that the resorption and swelling rates of the collagen be reduced by hardening, the hardening reagent and the hardening method may be freely chosen from the tanner's art. Synthetic tanning agents are preferred because of the very close reproducibility of their results, but we have successfully employed natural tanning extracts of Chinese gallnuts, oak, pine, quebracho, sumach, mimosa, and valonia.

Many other modifications and variations of the present invention are possible in the light of the above teachings. It is therefore to be understood that, within the scope of the appended claims, the invention may be practiced otherwise than as specifically described.

This application is a continuation-in-part of our copending application Ser. No. 330,221 filed on Dec. 10, 1963.

We claim:

1. An artificial blood vessel of tubular laminar structure comprising, in combination:

(a) an exposed outer tubular layer of a physiologically inert fabric resistant to resorption by body fluids,

(1) said outer layer having a permeability to water of not less than 10,000 milliliters per square centimeter per minute at a pressure differential of 120 millimeters mercury column; and

(b) a tubular composite layer essentially consisting of two laminae of collagen,

(1) one of said laminae of collagen being tanned and constituting an inner and self-supporting wall of said vessel,

(2) said inner wall containing an amount of an anti-coagulant effective to prevent coagulation of blood in contact with said inner wall, and

(3) the other of said collagen laminae being essentially in the native chemical state and being bonded to said outer layer, said outer layer and said other lamina constituting an outer wall of said vessel.

2. A vessel as set forth in claim 1, wherein said other lamina of collagen is of a thickness sufficient to adhere said fabric to said inner wall without closing the pores of the fabric.

3. A vessel as set forth in claim 1, wherein said anticoagulant includes a mucopolysaccharide admixed to said collagen.

4. A vessel as set forth in claim 1, wherein said inner wall further includes glycerin admixed to said collagen.

5. A vessel as set forth in claim 1, wherein said inner wall further includes an antibiotic admixed to said collagen.

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DALTON L. TRULUCK, *Primary Examiner.*

U.S. Cl. X.R.

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