

6. The pH of the medium was shown to influence the toxicity effects.

7. When Nile Blue Sulfate, Neutral Red and Bismark Brown are used as vital dyes, the factors of concentration, length of ex-

posure and stage of development must be considered to avoid induced abnormalities in development, most of which begin at the time of gastrulation.

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A Method for Producing Experimental Venous Thrombosis.*

EDWARD HIRSCH AND LEO LOEWE. (Introduced by B. Kramer.)
(With the technical assistance of Florence Kashdan.)

From the Thromboembolic Disease Unit, Jewish Hospital of Brooklyn, N.Y.

In our attempts to study the genesis and functional pathology of intravascular thrombosis, and to determine the *in vivo* action of heparin on preformed clot, it was found necessary to devise a method of experimental clot formation in animals which produced consistent, predictable results. This report deals briefly with such a method successfully employed in rabbits.

Experimental venous thrombosis has hitherto been accomplished by chemical and mechanical means.¹⁻⁴ The introduction of an extraneous factor in chemically-induced thrombosis unnecessarily complicates the proper evaluation of the effect of heparin. Accordingly, mechanical methods of inducing thrombosis are preferable. Vein crushing over an intraluminal silk thread,⁵ although reported to be 80% successful, did not give us consistently good results. Similar-

ly, we found stretching of the vein to induce intimal damage⁶ successful in only 20% of instances.

The elaboration of a thrombus depends on (a) stagnation of blood, (b) injury to the intima, (c) local release of considerable amounts of thrombokinase. A method utilizing these 3 factors is herewith described. Three kilogram adult rabbits are anesthetized with ether and a midline cervical incision is made. The jugular veins on either side are exposed. Both veins are treated alike. A 3-cm segment of vein is dissected free and the most proximal portion securely tied with a silk ligature. A flat, narrow strip of metal, such as a ribbon retractor, is placed under the vein distal to the ligature and acts as an anvil. The vein is then given 15 to 30 sharp taps with the handle of a Mayo scissors. Brisk bleeding will occur which is readily controlled by gauze pressure. Care is taken not to fracture the vein completely across. When bleeding has ceased, usually in about 2 minutes, a palpable and visible thrombus appears. If clotting does not occur, the procedure is again repeated. Clotting invariably is present after the second series of strokes. All animals are reexamined after 48 hours to reaffirm the presence of clots. Examination and study of these clots, in the gross and in microscopic sec-

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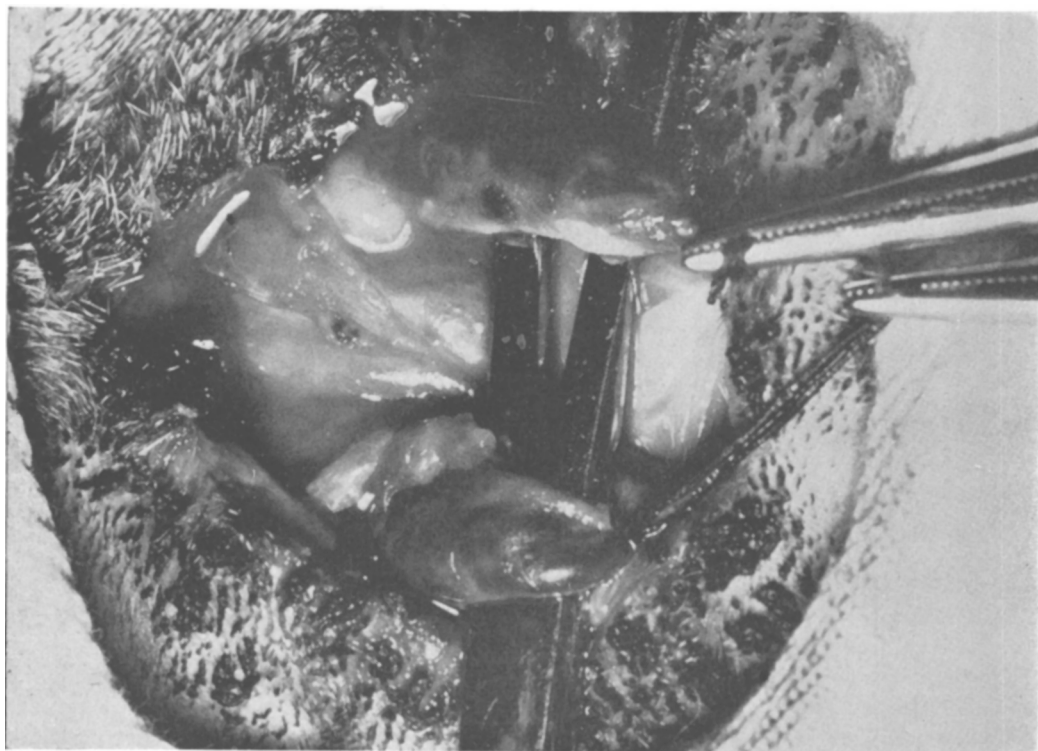


FIG. 1.
Jugular veins of rabbit 6 days after ligation and hammering.

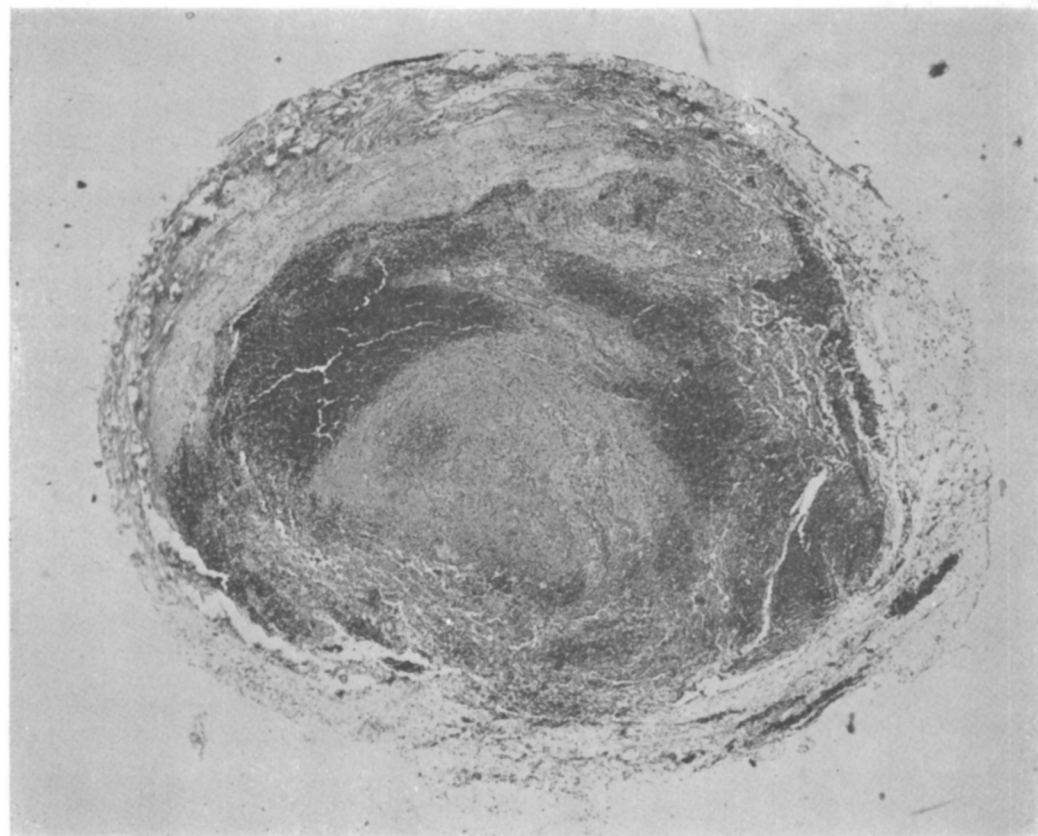


FIG. 2.
Microscopic section of Fig. 1 ($38\times$) showing organization of clot and beginning
recanalization.

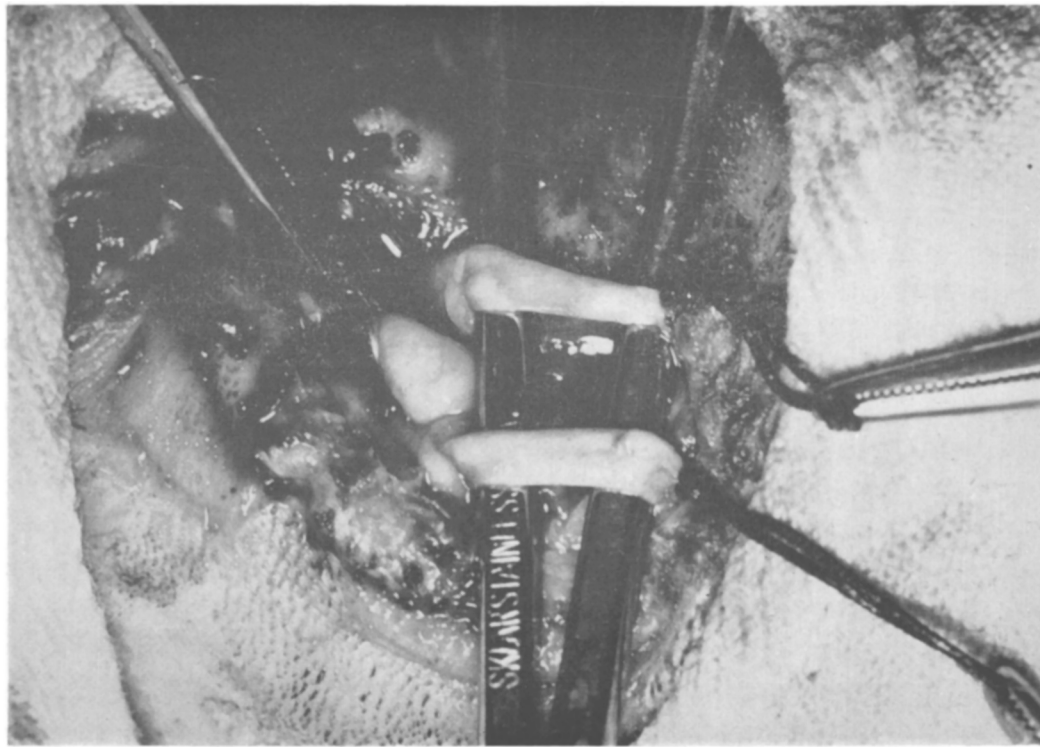


FIG. 3. Jugular veins of rabbit 14 days after ligation and hammering. The veins are thickened and fibrotic.

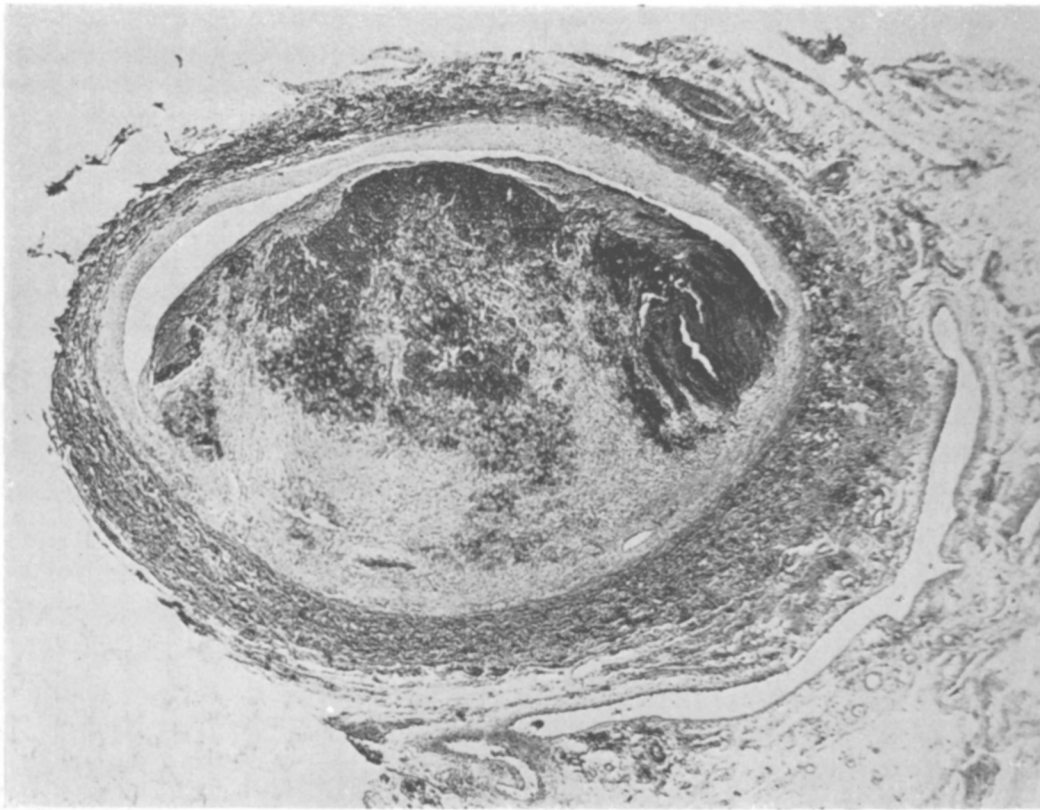


FIG. 4. Microscopic section of Fig. 3 (30 $\times$) showing organization and recanalization.

tion,[†] reveals that they differ in no way from the spontaneous *in vivo* clot seen in aseptic thrombophlebitis. The progressive morphologic changes leading to organization and recanalization are also similar to spon-

[†] We wish to thank Dr. David M. Grayzel for his interpretation of the microscopic sections.

taneous *in vivo* clots (Fig. 1, 2, 3, 4). In the last 140 veins, the procedure has been successful in every case.

Conclusion. An easily controlled, readily available method of experimental clot production is described. It has been uniformly successful in our hands.

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Viscosity of Normal Human Synovial Fluid.

CHARLES RAGAN. (Introduced by W. W. Palmer.)
(With technical assistance of Audrey F. Grubin.)

From the Edward Daniels Faulkner Arthritis Clinic of the Presbyterian Hospital and the Department of Medicine, College of Physicians and Surgeons, Columbia University, New York.

Synovial fluid is a viscous solution containing a longchain mucopolysaccharide (hyaluronic acid), protein and electrolytes.¹ The relative viscosity of such fluid is related to the amount of hyaluronic acid present²⁻⁴ and to the state of polymerization and aggregation of the polysaccharide.⁵ When joint fluid is incubated with an hyaluronidase,⁶ the viscosity approaches but does not quite reach the viscosity of water.⁶ The slight residual increase in viscosity may be due to the proteins present which are unaffected by purified preparations of hyaluronidase. (Isolated pure hyaluronic acid never attains the high viscosity found in native (human or animal) synovial fluids, presumably because the procedures used in isolation disaggregate the polysaccharide as it occurs in the native form.⁵ The human knee joint is functionally far different from the 4-legged animal's astragalo-tibial joint from which most specimens of so-called normal joint fluid have

been obtained.^{3,7} It seemed of interest to determine, if possible, the characteristics of normal human synovial fluid, to compare with pathological fluids which can readily be obtained in large quantity. Reports on the viscosity of normal human synovial fluid have yielded wide variations and the techniques of viscosimetry are open to some criticism.^{8,9} When an apparently normal human knee joint is tapped at the postmortem table, one can rarely withdraw more than 1 or 2 cc of joint fluid. Thus, 2 to 4 cc of human knee joint synovial fluid can be obtained from the 2 knees of an individual, which is less than the capacity of the standard 5 cc Ostwald viscosimeter. On casual inspection, it was found that the fluid obtained was very viscous, and it was probable that a viscosimeter of less than 5 cc capacity would give an inaccurate reading with such viscous material.

Methods. Viscosities were measured in 5 cc Ostwald viscosimeters in a water bath at temperature of $21^{\circ} \pm 1^{\circ}\text{C}$. Total proteins were determined with a gradient tube method.¹⁰ In this laboratory, it has been

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