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 spontaneous *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.

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

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Viscosity of Normal Human Synovial Fluid.

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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 present[2-4] 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 technics 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° ± 1°C. Total proteins were determined with a gradient tube method.[10] In this laboratory, it has been

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found that the gradient method has a good correlation with the Howe method in estimation of protein content of synovial fluid. It has also been shown that the specific gravity of a 1% solution of purified sodium hyaluronate* is below the scale of the gradient method employed and does not materially affect specific gravity estimation of synovial fluid protein.

Eight pathological joint fluids of varying viscosity were diluted with 0.9% sodium chloride, the relative viscosity at each dilution was plotted against dilution and curves were drawn (Fig. 1). It can be seen that the fluids with a very high initial viscosity show an abrupt fall on minimal dilution with

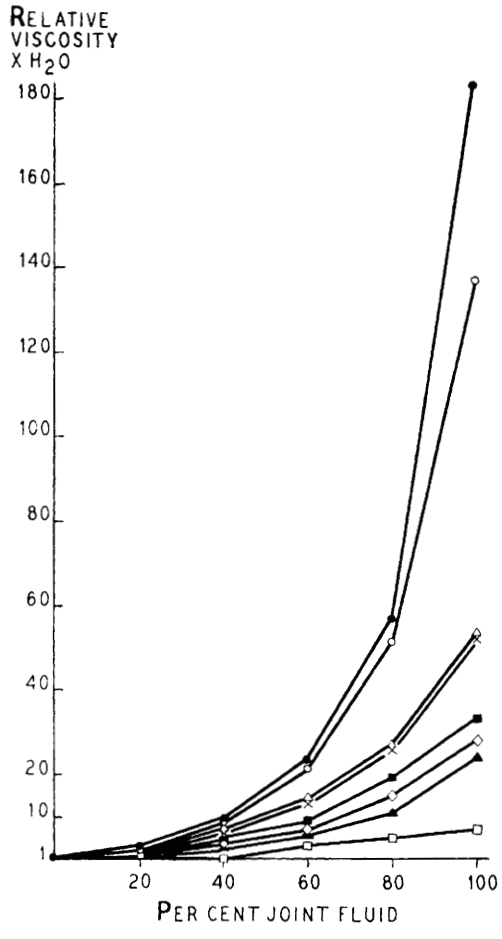

FIG. 1.
Curves of relative viscosity obtained by dilution of 8 pathological joint fluids with 0.9% NaCl.

* Obtained through the courtesy of Dr. K. Meyer.

TABLE I.
The Relative Viscosity and Total Protein Content of Normal Joint Fluids.

Pt.	Diagnosis	Peripheral oedema	Amt. removed both knees, cc	Relative viscosity: Dilution, %	Relative viscosity: Rel. visc. diluted sample	Relative viscosity: Relative visc. by extrapolation	Total protein
C.H.	Carcinoma vocal cords	0	2	40	11.8	>185	—
F.B.	Hypertensive cardiovascular disease	0	2.5	50	36	>185	1.3
R.D.	Lymphosarcoma	0	3	40	8.9	134-184	—
S.McC.	Hypertensive cardiovascular disease, mesenteric thrombosis	0	3	50	88.2	>185	1.5
E.T.	Hypertensive cardiovascular disease	0	3.6	50	16.8	185	2.3
E.C.	Intestinal obstruction	0	3.6	50	12.6	60-134	2.4
H.H.	Carcinoma stomach	0	4	50	12.8	60-134	1.8
F.L.	Coronary occlusion	0	1	20	4.4	>185	—
T.W.	Lung abscess with terminal hemorrhage	0	2	40	10.1	>185	2.0
C.N.	Hypertensive cardiovascular disease	+++	rt. 30	100	—	3.0	1.6
			lt. 20	100	—	7.2	1.7
T.J.	Hypertensive cardiovascular disease	++	rt. 15	100	—	47.0	2.2
			lt. 12	100	—	62.2	2.2
N.S.	Nephrosis	++++	not all removed	50	5.1	7-20	.94

TABLE II.
The Relative Viscosity and Total Protein Content of Pathological Joint Fluids.

Pt.	Diagnosis	Knee joint	Amt removed cc	Relative viscosity	Total protein
R.R	Rheumatoid arthritis—old	right	300	175	4.8
E. O'T.	" " —early	"	20	6.8	4.2
L.S.	Torn medial meniscus	left	15	13.8	4.6
H.T.	Rheumatoid arthritis—old	right	60	48.2	5.3
H.F.	" " "	"	40	16.8	4.8
P.R.	" " —early	"	20	5.6	5.9
T.B.	Gonococcal arthritis	"	20	8.4	4.4
A.N.	Arthritis one knee assoc. with lymphogranuloma inguinale	"	75	40.5	4.4
G.N.	Intermittent hydrarthrosis	left	40	11.2	5.9
M.P.	Gonococcal arthritis—old	"	40	8.8	6.0

an elliptical type of curve. This type curve may be superimposed on the dilution curve for pleural fluid reported by Meyer.[4]

It has been possible to approximate the viscosity of normal joint fluid by dilution and extrapolation on these curves This has been done by dilution with 0.9% NaCl and extrapolation on the curves in Fig. 1 (Table I). In normal joints, one factor which is very important is the amount of fluid which can be obtained at tapping. In this small series this seems to be directly related to the amount of peripheral edema present and thus the joint space can be looked upon, as Bauer[11] has felt, as an extracellular space.† Where no more than 5 cc was obtained on tapping both knees, the viscosity of the joint fluid was greater than or equal to the viscosity of our most viscous pathological joint fluid. In 2 instances, with rightsided heart failure and marked pretibial edema, but with apparently normal knee joints, a larger amount was withdrawn from the knees (Pt. C.N. and T.J. in Table I), and the viscosity of these was less than that seen in normal fluids. One patient on the ward with generalized anasarca in the nephrotic stage of chronic glomerulonephritis was tapped and 2.5 cc of fluid was removed from his right knee. This was not viscous and on dilution and extrapolation gave a viscosity of 7 to 20 times water. As shown in Table I, in the absence of peripheral edema, the calculated viscosity of normal joint fluid was very great compared to the viscosity of the joint fluid obtained from a random selection of pathological joints shown in Table II. The protein content of fluid from normal joints as measured by a specific gravity method was below 3.0%, while in pathological joints, the protein content of the fluid was above 3.0%. (Table I and Table II).

Discussion. The viscosity of synovial fluid from knee joints of patients without symptoms or signs of joint disease is equal to or in most instances greater than the viscosity of joint fluids obtained from pathological knee joints. Because of the small amount of fluid obtained, the amount of hyaluronic acid present in these normal joint fluids cannot at the present time be determined by chemical means. However, the high viscosity would indicate that these fluids contain either a large amount of hyaluronic acid per unit volume or that the polysaccharide is in a highly polymerized state or in a state of high aggregation. On standing at icebox temperature over a period of weeks in a sterile state, the viscosity of the pathological fluids does not change.[12] Thus, if a specific enzyme is present, it is in an inactive state when removed from the body.

Conclusions. 1. The viscosity of normal human knee joint fluid is greater than that of fluid found in most pathological conditions. 2. The decreased viscosity of joint fluids found in pathological states may be

[11] Bauer, W., Ropes, M. W., and Waine, H., *Physiol. Rev.*, 1940, **20**, 272.

† We would like to visualize this space as the interfibrillar tissue space. In this sense it is extracellular.

[12] Robertson, W. VanB., Ropes, M. W., and Bauer, W., *J. Biol. Chem.*, 1940, **133**, 261.

due to one of two factors or to both, (a) dilution with extracellular water, (b) depolymerization or dissociation of a highly polymerized hyaluronic acid by hyaluronidase.

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Inactivation of Poliomyelitis Virus in Relation to Gastric and Intestinal Digestion.*

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Whether or to what extent gastric and intestinal digestion inactivates poliomyelitis virus is a problem that has an important bearing on intestinal entry of infection and on the source of virus in the intestinal contents and stools. This problem has not been studied as closely as might be desired in its relation to the conditions of normal digestion, although such studies as are available indicate that the virus displays a considerable degree of resistance to inactivation in the gastrointestinal tract. Thus, Flexner, Clark and Dochez[1] found active virus in the intestine 2 hours after administration by stomach tube; and by the same method of administration Clark, Schindler and Preston;[2] Clark, Roberts and Preston;[3] and Levaditi, Kling and Lepine[4] found active virus in the stools within 48 hours. The effects of H-ion concentration upon virus in the range with which we are here concerned has been studied by Loring and Schwerdt[5] but their experiments were limited to periods of 1-2 hours and were done at room temperature. So far as we know no studies on the inactivating effects of individual digestive enzymes on poliomyelitis virus have been recorded.

In the present experiments the test conditions were intended to approximate those obtaining in the human stomach in respect to temperature, time, pH range, and presence of pepsin. In children over one year of age (the group most susceptible to poliomyelitis), Cutter[6] has shown that the fasting gastric contents 10 minutes after stimulation with histamine display an average acidity of pH 1.5 and occasionally reach pH 1.2. In young adults, the level is pH 1.0-1.2.[7] Hammon and Izumi[8] showed that poliomyelitis virus is not inactivated at levels of pH 4.0 and up. Gastric evacuation begins a few minutes after ingestion.[9] At 4 hours, after ingestion of various types of food, the stomach is nearly empty.[9] On the basis of such data, we have selected for our studies a range of pH 1.0-4.0 and exposure periods of 5 minutes to 4 hours. All observations were made at 37°C, or approximate body temperature.

The Lansing (Armstrong) mouse-adapted strain of poliomyelitis virus was used. Stock 10% and 20% suspensions of infected mouse cord in physiological NaCl solution were clarified at 18,000 r.p.m. for ½ hour, then

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