widely in the lungs of rabbits, less so in guinea pigs, but are not as consistent as has been reported for rats. The extracellular water in the 3 human lung specimens was remarkably constant.

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Use of Trypsin in Preparing Subcultures of Monkey Testicular Tissue.* (20106)

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The use of trypsin as a method of obtaining tissue culture cells for transplanting purposes was introduced by Rous and Jones(1) and elaborated by Vogelaar and Erlichman(2). A detailed study of the stimulating effect of various proteolytic enzymes on tissue growth was reported by Simms and Stillman(3). Several interesting observations were made by these various authors: a) Contact with 3% crude trypsin for 1 hour at 37°C did not injure rodent or avian cells, but 5% did(1); b) Following solution of the plasma clot, repeated subculture was possible(1,2); c) Cell growth was rapid and at times explosive (1,3); d) The lag period for adult cells in tissue culture was shortened(3); e) Similar stimulation was obtained with both crude and crystalline trypsin, chymo-trypsin, and papain, suggesting that proteolysis of growth inhibitory substances was an essential factor (3). The purpose of this report is to present a simple tryptic digest method of transplanting roller tube cultures of monkey testicle with the objective of increasing the yield of usable explants for the propagation of poliomyelitis and other viruses.

Methods. Propagation of original tissue. Freshly obtained rhesus monkey testicles were minced in Simms-Hanks solution (1 part ox serum ultrafiltrate and 3 parts Hanks' balanced salt). Six to 8 fragments per tube were planted in chicken plasma which was then clotted with 50% chick embryo extract. The nutrient fluid consisted of 1.5 ml of a medium containing 10% chick embryo extract (1:1), 78.75% Simms-Hanks solution, 10% unheated horse serum, and 1.25% human albumin. Penicillin and streptomycin were added in a final concentration of 100 units and 100 μg per ml, respectively. The tubes were placed in the roller drum at 37°C and incubated until well developed fibroblast outgrowth was obtained (2 weeks). The cells were maintained thereafter by adding fresh medium at weekly intervals.

Subculture. The nutrient fluid and the original tissue fragments were removed leaving the outgrowth intact. To each tube was

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added 1 ml of 0.5% trypsin in Ringer's solution that had been freshly prepared from a filtered 2% crude trypsin stock(2,3). The culture tubes containing trypsin were returned to the roller drum and incubated for 1 hour at 37°C, at which time dissolution of the clot was usually complete. Simms-Hanks mixture (10-12 ml) was then added and the tubes were rotated in an angle centrifuge at 2500 rpm for 5 minutes. The supernate was discarded and the cells were washed once again in the same manner and recentrifuged. The sediment was suspended in 1.5 ml of 50% chick embryo extract and the cell clumps dispersed by gentle suction back and forth through a small bore capillary pipette. With the same pipette 2 drops of the cell suspension were spread lengthwise over the inner surface of a tube which had previously been coated with chicken plasma. When clotting was complete, 1.5 ml of nutrient fluid was added and the tubes were placed in the roller drum.

Results. Initial proliferation of fibroblasts was unusually vigorous and distinct growth originating from small clumps and single cells was observed in 24 hours. By 6 to 7 days all the tubes containing transplants were suitable for virus inoculation. The yield from one tube of original culture averaged 15 subcultures, which required an estimated working time of approximately 30 minutes for the entire procedure. A stock of primary cultures may be prepared when the testicle is first obtained and at selected intervals subcultures can be made thus insuring a continuous supply of young fibroblasts over a period of a month or longer. In a few experiments with human tissue, successful subcultures have been propagated from material kept in the roller drum for as long as 3 months.

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Effect of Hyaluronidase on Inflammation.* (20107)

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Investigations with hyaluronidase have shown that the spreading properties of the enzyme permit more effective application of certain drugs(1). The effect of this enzyme on local inflammation due to irritating drugs, however, was not known; and these experiments were designed to study this aspect and to evaluate the applicability of hyaluronidase as an adjuvant in therapy with irritating agents.

Two strong irritants were used: the oil-soluble allylisothiocyanate and the water-soluble paraphenylenediamine. Gross and microscopic observations on animals treated with these irritants showed that in the presence of hyaluronidase, the local inflammatory

TABLE I. Effect of Intradermal Paraphenylenediamine in the Presence and Absence of Hyaluronidase (End of 3 Hr). 6 rabbits per exp.

Treatment*	Reaction area† No. reacting	Reaction area† Avg area of dye (mm² × 10²)
.9% NaCl + .9% NaCl	1	.02
.9% NaCl + H	5	.23
4% P + .9% NaCl	6	11.98
4% P + H	6	21.96

*H = Hyaluronidase. P = Paraphenylenediamine.

† None of the rabbits showed petechiae. Only the rabbits (6) inj. with 4% P + .9% NaCl showed edema.

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