susceptible humans. Regardless of the possible virulence of the present BKTC virus, propagation of measles virus in this tissue culture system may prove valuable as a means of further modification. It would also permit economical production of large quantities of measles virus for use in either modified or killed vaccines. Bovine embryonic tissue would be superior to monkey tissue since it is more readily available and, to our knowledge, contains no latent viruses; it further would be superior to chick embryo tissues to which many people are already sensitive.

Summary. 1. The Edmonston strain of measles virus was propagated in BKTC for 20 passages, and produced a CPE typical of measles, characterized by multinucleated giant cells and eosinophilic intranuclear inclusion. 2. Identification of this cytopathogenic agent was confirmed by neutralization test with known measles immune serum. 3. BKTC-propagated virus was inoculated intramuscularly into susceptible cynomolgus monkeys without causing clinical illness, but the virus could be recovered from throat and blood. All inoculated monkeys developed complement-fixing and neutralizing measles antibody. The significance of these results is discussed.

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Failure to Transfer Delayed Tuberculin Sensitivity from Human to Animal Species.* (25373)

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Transfer of delayed cutaneous sensitivity to tuberculin and to other materials with leucocytes obtained from specifically sensitive donors has been reported by Chase in the guinea pig and by Lawrence in man(1). In animals, the transfer of sensitivity is successful only when intact leucocytes are employed. However, in man, it has been shown that the factor in sensitive leucocytes concerned with transfer of delayed sensitivity retains its activity following leucocyte disruption and enzymatic treatment(2). This finding has been confirmed recently by Freedman, Fisher and Cooke(3). A valuable approach to the study of transfer factor would be provided by transfer of delayed sensitivity from man to animals —particularly if leucocyte extracts could be used. Lawrence, as well as Rammelkamp, and Thomas and Stetson(4) have repeatedly attempted to transfer tuberculin sensitivity to guinea pigs and rabbits with intact as well as disrupted human leucocytes obtained from sensitive donors. Their results have been consistently negative. The present study is based on the assumption that species barrier in transfer of sensitivity might be crossed more readily within a closer evolutionary relationship, as exists between man and the monkey. This report describes a series of attempts to

* The opinions or assertions contained herein are the private ones of the authors, and are not to be construed as official, or reflecting the views of the Navy Department or of the Naval Service at large.

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transfer delayed tuberculin sensitivity from man to monkey, and from man to rabbit by means of peripheral blood leucocytes obtained from strongly tuberculin-sensitive human donors.

Materials and methods. 1. *Selection of leucocyte donors and recipients.* The staff of the Naval Biological Laboratory were screened with a tuberculin patch (Vollmer). The most sensitive reactors, each giving a raised, vesicular and intensely erythematous reaction at test site 48 hours after application of the patch, were selected as donors. These were subjects *H, T, C,* and *D.* The recipients included 10 monkeys (*Macacus mulatta*), weighing 2-7 kg each, and 8 white male albino rabbits weighing 0.8-1.0 kg each. All animals gave a negative reaction to 0.1 ml of diluent solution containing 0.005 mg PPD (Purified Protein Derivative)(4) intradermally. 2. *Isolation of leucocytes.* Each blood donation (400 ml) is collected in a plastic Fenwal bag to which are added 100 ml of aqueous solution containing 0.05 g/ml of bovine fibrinogen (Fraction I, Armour). The bag contents are mixed, and the bag suspended in incubator at 37°C for 45 minutes. Sedimented erythrocytes are discarded, and the supernatant plasma suspension of leucocytes is collected in graduated conical centrifuge tubes, and spun at 2000 g 15 minutes. Volume of packed leucocytes in each tube is recorded, the supernatant plasma is discarded, and the leucocytes pooled and resuspended in sterile pyrogen-free normal saline. 3. *Methods of transfer.* Three routes of administration were employed to transfer tuberculin sensitivity with leucocytes collected from 4 human donors. Several methods of leucocyte preparation were used: a) *Intradermal and subcutaneous route. First method of leucocyte preparation*: 0.84 ml of leucocytes from donor *H* were suspended in 4 ml of normal saline, and injected in 0.3 ml aliquots into the left thigh of recipient monkey (R1). A second monkey recipient (R2) received a similar volume of *H* plasma. *Second method*: 0.375 ml of leucocytes from donor *D,* suspended in 2 ml of normal saline solution, were incubated at 37°C for 30 minutes with 1 ml of normal saline solution containing 50 γ of PPD. 0.30 ml of resulting mixture (containing approximately 0.04 ml leucocytes) were injected into the next 3 monkey recipients (R3, R4, R5). *Third method*: 0.375 ml of leucocytes obtained from donor *D,* suspended in 2 ml of normal saline solution, were subjected to 7 cycles of rapid alternate freezing (95% alcohol-dry ice mixture) and thawing (37°C water bath). One ml of normal saline solution containing 50 γ of PPD was added to the disrupted leucocyte suspension, and the resulting mixture was incubated at 37°C for 30 minutes. Following incubation, 0.30 ml of this material was injected intradermally into 2 additional monkeys (R6 and R7). b) *Intraperitoneal route.* 0.72 ml of leucocytes obtained from donor *T* were suspended in 13 ml of normal saline solution, and injected intraperitoneally into monkey recipient (R8); another monkey (R9) received a similar volume of *T* plasma intraperitoneally. c) *Intravenous route.* 1.07 ml of leucocytes obtained from donor *C* were suspended in 12 ml of normal saline solution, and administered intravenously to final monkey (R10). No untoward reaction was noted to any of the methods of administration of human leucocytes employed, with or without added PPD. 4. *Concurrent study with rabbits.* Two groups of 4 rabbits each were included in this study. The first group (Rabbits 1-4) each received intradermal injections of 0.30 ml of *D* whole leucocyte-PPD mixture described in section 3A. The second group (Rabbits 5-8) each received intradermal injections of 0.30 ml of disrupted *D* leucocytes-PPD mixture described in section 3A.

Results. Monkeys receiving whole leucocytes, either intradermally, intraperitoneally, or intravenously (R1, R8 and R10), as well as control running mates (R2 and R9) were tested intradermally with 0.005 mg PPD in 0.1 ml diluent solution, at 24 hours, and thereafter at weekly intervals for 5 weeks following cell transfer. Neither test nor control animals developed tuberculin sensitivity during this period.

Monkeys which had receivd intradermal injections of mixtures of intact leucocytes and PPD (R3, R4 and R5), as well as the group which had received intradermal injections of mixtures of leucocyte extract and PPD (R6

TABLE I. Attempted Transfer of Tuberculin Sensitivity to Monkeys with Human Leucocytes.

Subject	Material used for transfer	Route of administration	Skin reactivity to tuberculin after transfer, at: 24 hr	1 wk	2 wk	3 wk	4 wk	5 wk
R 1	.84 ml intact leucocytes	Intrad. & subcut.	0	0	0	0	0	0
R 2	Plasma from same donor	*Idem*	0	0	0	0	0	0
R 3	.04 ml intact leucocytes incubated with tuberculin	"	—	0	0	—	—	—
R 4	*Idem*	"	—	0	0	—	—	—
R 5	"	"	—	0	0	—	—	—
R 6	.04 ml disrupted leucocytes incubated with tuberculin	"	—	0	0	—	—	—
R 7	*Idem*	"	—	0	0	—	—	—
R 8	.72 ml intact leucocytes	Intraper.	0	0	0	0	0	0
R 9	Plasma from same donor	"	0	0	0	0	0	0
R10	1.07 ml intact leucocytes	Intrav.	0	0	0	0	0	0

and R7), were similarly skin tested one and 2 weeks after transfer. None of the animals developed tuberculin sensitivity. The results of this experiment are summarized in Table I.

Rabbits which had received intradermal injections of either mixtures of intact leucocytes and PPD (Rabbits 1-4) or mixtures of leucocyte extract and PPD (Rabbits 5-8) were also skin tested with the same concentration of PPD, one and 2 weeks after cell transfer. No animals in this group developed reactivity to tuberculin during this period. The results of this experiment are summarized in Table II.

Discussion. Our results suggest that, within the limitations of the experimental approach employed, it is not possible to transfer tuberculin sensitivity from man to monkey or from man to rabbit, by means of leucocytes obtained from peripheral blood of specifically sensitive donors. Despite variations in route of administration and in method of treatment of the leucocytes, all recipients remained tuberculin negative upon repeated subsequent skin testing.

The significance of these results may be weakened by the absence of specific information concerning whether delayed sensitivity can be transferred from monkey to monkey by this method. However, as monkeys are known to have the capacity to develop cutaneous tuberculin sensitivity(5), it is not likely that this species would differ markedly from guinea pig, rabbit, or man, in their response to cellular transfer of sensitivity performed within the species.

Summary. This report describes attempts to transfer delayed sensitivity to tuberculin to monkeys and to rabbits by means of peripheral blood leucocytes obtained from sensitive human donors. No such transfers were obtained.

TABLE II. Attempted Transfer of Tuberculin Sensitivity to Rabbits with Human Leucocytes.

Subject	Material used for transfer	Route of admin.	Skin reactivity to tuberculin after transfer at: 1 wk	2 wk
1	.04 ml intact leucocytes incubated with tuberculin	Intrad. & subcut.	0	0
2	*Idem*	*Idem*	0	0
3	"	"	0	0
4	"	"	0	0
5	.04 ml disrupted leucocytes incubated with tuberculin	"	0	0
6	*Idem*	"	0	0
7	"	"	0	0
8	"	"	0	0

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Mechanism of Clot Lysis by Streptokinase and Effects of Epsilon-Aminocaproic Acid.* (25374)

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In past years considerable experimental work, initiated in laboratories of W. S. Tillet (1), was undertaken to investigate the possibility of dissolving intravascular thrombi in humans by intravenous administration of streptokinase (SK). These and other investigators employed plasmin, prepared by activation of human plasminogen with SK, as the lytic agent, and recently reviewed by Sherry *et al.*(2). It is recognized that either form of treatment may cause certain undesirable side-effects(2) from induced hyperplasminemia, among which may be listed (a) destruction of fibrinogen and plasminogen, (b) destruction of some components involved in blood coagulation, (c) destruction of other physiologically active polypeptides and (d) elevation of antithrombin levels. Obviously it would be desirable to prevent or minimize the untoward systemic effects induced by hyperplasminemia, without interfering with dissolution of the thrombus. Epsilon-aminocaproic acid (ε-ACA), first reported to be a plasmin inhibitor (3), was recently shown by Ablondi and Hagan(4) to be a far more potent inhibitor of plasminogen activation. Subsequent studies confirmed and extended this observation to show that ε-ACA inhibited both SK and urokinase activation of plasminogen(5,6). Our more recent studies demonstrated that ε-ACA does not adsorb to a fibrin clot, a situation analogous to that found by Johnson, *vide infra,* for humoral inhibitors of fibrinolysis. It seemed worthwhile, therefore, to determine (a) the effect of ε-ACA on dissolution of a clot suspended in plasma and (b) its simultaneous effect on plasminogen activation in plasma. Our purpose here is to present data which demonstrate that, under certain conditions *in vitro,* ε-ACA can effectively inhibit activation of plasminogen by SK in plasma without markedly inhibiting the lysis of a clot immersed in such plasma.

Methods. I^{131}-labeled clots fortified with plasminogen were prepared as follows: Twelve cc of 0.9% saline were added to 1 cc aliquots of human plasma followed by 0.1 cc of a solution of I^{131}-labeled fibrinogen prepared by the method of Mihalyi and Laki(7). One-tenth cc human plasminogen containing 4000 units of proactivator(8), and prepared by the method of Kline(9), was then added. Clotting was effected at pH 7.4 by addition of 1.25 units of thrombin (Parke-Davis) in 0.1 cc volume. As clots formed they were collected by carefully winding onto glass capillary tubes (melting point tubes) to which they became tightly fixed. As much fluid as possible was squeezed from the adhered clot by pressing against inner wall of test-tube. The radioactive content of each clot was determined in a well type scintillation counter

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