decrease in the renal concentration of ascorbic acid under the influence of ACTH. Increased urinary excretion of ascorbic acid, possibly as a result of a change in renal function, has been reported in human beings receiving ACTH(27). The administration of doses of cortisone adequate to suppress the growth of granulation tissue does not significantly alter the concentration of ascorbic acid in the plasma or muscles of rabbits(28). There is evidence suggesting that GSH serves to maintain ascorbic acid in the reduced state *in vitro*, and the amounts of ascorbic acid and GSH in the tissues may vary together under certain circumstances(18,29). Whether the reduction in renal nonprotein SH observed in this experiment had any causal relationship to the concomitant decrease in the concentration of ascorbic acid is not clear at this time.

Summary. Rats given ACTH for 3 to 10 days in doses sufficient to produce sustained eosinopenia when compared with controls, showed: (1) no change in the blood glutathione; (2) decreased nonprotein sulfhydryl contents of the kidneys; (3) increased nonprotein SH contents of the livers; (4) markedly decreased renal ascorbic acid; (5) no change in the ascorbic acid contents of the livers; and (6) no change in the protein sulfhydryl contents of the livers or kidneys.

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Propagation of Poliomyelitis Virus in Cultures of Monkey and Human Testicular Tissues.*† (18665)

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It was recently established that propagation in tissue culture of members of the poliomyelitis group of viruses can be affected in extraneural tissues(2-5) as well as in neural tissues(1-4). Human embryonic brain and cord were employed for the cultivation of the MV(1), Lansing(2-4), and Brunhilde(3) strains; a mixture of human embryonic skin, muscle and connective tissue for the Lansing(2-4) and Brunhilde(3) strains; human foreskin and subcutaneous tissues from patients ranging in age from 4 to 11 years for the Lansing strain(3); human embryonic intestine for the Lansing strain(2,4); human adult testicular tissue for the Lansing and Hof.

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strains(5). Dr. Evans kindly made known the results of this latter study before its publication(5). The importance of this discovery was obvious both from the practical point of view because of the ready availability of human testicular tissue, since patients commonly are subjected to castration for its hormonal palliative effect on inoperable carcinoma of the prostate, and because of the theoretical implication of this evidence that poliomyelitis virus can propagate in tissues devoid of neural elements. Accordingly, the efforts that had been currently under way in this laboratory to establish poliomyelitis virus in tissue culture were altered in an attempt to confirm the observations of Smith, Chambers, and Evans(5), and to extend their findings by employing the monkey as an even more readily available source of testicular tissue. Accordingly, 4 strains of poliomyelitis virus (murine-adapted Yale-SK, murine-adapted Lansing, Mahoney, and Minnesota, 1949) are under study in tissue cultures by employing for the maintenance of monkey testicular cells a technic described earlier(6). It is the purpose of the present communication to make known the evidence that establishes conclusively the propagation of poliomyelitis virus, Y-SK and Lansing strains, in monkey testicular tissues *in vitro*.

Materials and methods. Virus. The 2 strains of murine-adapted human poliomyelitis virus, Lansing and Yale-SK, were obtained from Dr. Joseph Melnick. The inoculum employed to initiate each experimental series in tissue culture was the supernatant fluid following centrifugation at 2500 r.p.m. of a mixture of mouse brain and cord in 10% suspension. At that time, the LD_{50} of the Lansing strain was $10^{-3.33}$ and that of the Y-SK strain was $10^{-3.1}$. *Tissue cells.* Testicular tissue, as whole testicle contained in its tunica vaginalis, was provided by castration of man or monkey. The human testicles were obtained from elderly men.§ Monkey testicles were obtained from fully mature *Macacus cynomolgus*, except for the first and third replacements, when rhesus monkeys provided the tissues. A newly removed testicle was employed for each tissue transfer. *Technic for tissue culture.* The technic employed was essentially similar to that utilized for the cultivation of the Minnesota strain of Coxsackie virus(6). Four flasks as a unit were made ready by inserting on the bottom of each a circular sheet of Beckley, OC design, perforated cellophane.‖

Small fragments of testicular tissue were excised, minced finely to a maximal diameter of from 1 to 1.5 mm, and from 6 to 10 pieces were introduced beneath the cellophane membrane of 3 of the 4 flasks. The fourth flask in each unit, by receiving all material except tissue fragments, served as a control to test for the survival of virus in the absence of cells. Successive steps thereafter consisted of the introduction into each of the four flasks of 0.1 ml of the test virus suspension, 5 ml of nutrient fluid consisting of one part Simms' Ox Serum Ultrafiltrate¶ and two parts Simms' 3-X6, or 3-X7 salt solution, 0.1 ml sodium or potassium penicillin (50,000 units per ml) and 0.1 ml of streptomycin or di-hydrostreptomycin (50 mg per ml). Incubation was carried out at 36°C. On the third and sixth day thereafter, the maximal amount of overlying fluid was removed from above the cellophane membrane and replaced by 5 ml of fresh nutrient fluid; on the ninth day, the supernatant fluid from each of the three flasks containing tissue was pooled, and 0.1 ml was removed for transfer to each of the 3 test flasks containing fresh testicular tissue. This routine was maintained throughout except for Series C in which both human and monkey testicular tissues were employed in the propagation of Yale-SK virus. In this series, human testicular tissue was employed to initiate the series, but when it was not available after 12 days of culture, fresh monkey testicular tissue was used thereafter. A second exception in the routine procedure occurred on the sixth tissue passage after 6 days in culture when the replacement

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§ Testicular tissue was kindly supplied by Dr. C. D. Creevy, University Hospitals, Minneapolis, and by Drs. T. H. Sweetser and W. H. Card, Minneapolis General Hospital.

‖ As approved by the Tissue Culture Commission.

¶ Purchased from Microbiological Associates, Coral Gables, Fla.

TABLE I. Results of the Propagation of the Lansing Strain in Tissue Culture, Series A.

GROWTH OF POLIOMYELITIS VIRUS (LANSING) IN CULTURES OF TESTICULAR TISSUE FROM MONKEY

FLUID FROM	RESULTS IN MICE	LOG OF DILUTION OF ORIGINAL VIRUS BY FLUID REPLACEMENT							
		2.7 3.6 4.5	6.2 7.1 8.0	9.7 10.6 11.5	13.2 14.1 15.0	16.7 17.6 18.5	20.2 21.1 22.0	23.7 24.6 25.5	27.2 28.1 29.0
TISSUE FLASKS	PARALYSIS†	3/6*	1/6	1/6 2/6 2/6	1/6 1/6 3/6	0/6 2/6 2/6	1/6 0/6 4/6	0/6 2/6 3/6	1/6 2/6 1/6
	DEATH†	4/6	3/6	2/6 2/6 6/6	3/6 5/6 5/6	2/6 4/6 6/6	4/6 4/6 5/6	2/6 6/6 6/6	2/6 3/6 1/6
CONTROL FLASKS	PARALYSIS	0/5	0/6		0/5				
	DEATH	0/5	0/6		0/5				
		2.7	4.4	6.1	7.8	9.5	11.2	12.9	14.6
		LOG OF DILUTION OF ORIGINAL VIRUS BY TISSUE REPLACEMENT							

* Denominator signifies No. of mice inj. intracer., .03 ml. Numerator signifies No. that died.
† "Paralysis" signifies No. of mice paralyzed (one observation per 24 hr). Paralysis within 24 hr of inoculation was not included. "Death" signifies No. of mice dead, excluding those dying within 48 hr of inoculation.

of monkey testicular tissue was effected, instead of observing the customary 9-day interval between the replacement of tissue fragments. The utilization of the especially designed tissue culture flask and of Mohr clamps for closure made it possible to pass daily over the content of each flask a gaseous mixture made up either of 5% carbon dioxide, 21% oxygen and 74% nitrogen, or of 5% carbon dioxide and 95% air. The control series of flasks was treated similarly, but independently. This control series of flasks was employed to learn the survival of virus in the absence of tissue. A second control, which was irregularly employed, consisted of a flask containing tissue, nutrient fluid, penicillin and streptomycin, but no virus.

Measurement of results. The presence of virus in tissue cultures was determined routinely in 15- to 25-day-old Swiss albino mice, CFW strain, by the injection intracerebrally, 0.03 ml, of undiluted, or successive 10-fold dilutions of pooled supernatant fluid which had been harvested from the 3 test tissue flasks, or from the control flask, respectively. Centrifugation at 5,000 r.p.m. for from 15 to 30 minutes was employed when fluid was titrated for virus content, or when neutralization tests were carried out. Simms' 3-X6 balanced salt solution¶ was the diluent utilized in titration and in neutralization tests.

Paralysis and death of the recipient mice were accepted as evidence for the presence of virus. When titrations were carried out to assess the extent of multiplication in successive tissue culture transfers, the Reed and Muench method was used to calculate the 50% endpoints. The virus under propagation in each tissue culture series was tested at least once for its pathogenicity for monkeys.

TABLE II. Results of the Propagation of the Yale-SK Strain in Tissue Culture, Series B.

GROWTH OF POLIOMYELITIS VIRUS (YALE-SK) IN CULTURES OF TESTICULAR TISSUE FROM MONKEY

FLUID FROM	RESULTS IN MICE	LOG OF DILUTION OF ORIGINAL VIRUS BY FLUID REPLACEMENT									
		2.7 3.6 4.5	6.2 7.1 8.0	9.7 10.6 11.5	13.2 14.1 15.0	16.7 17.6 18.5	20.2 21.1 22.0	23.7 24.6 25.5	27.2 28.1 29.0	30.7 31.6 32.5	34.2 35.1 36.0
TISSUE FLASKS	PARALYSIS†	3/6*	4/8 4/6	1/6 2/6 5/7	1/7 2/8 4/7	0/5 3/6 3/6	0/6 3/7 3/6	3/6 4/6 3/6	0/6 3/6 5/6	0/4 3/6 1/6	2/5 2/6
	DEATH†	5/6	7/8 6/6	2/6 5/6 6/7	5/7 6/8 5/7	4/5 5/6 6/6	4/6 6/7 5/6	5/6 6/6 5/6	4/6 5/6 6/6	0/4 6/6 5/6	5/5 5/6
CONTROL FLASKS	PARALYSIS	0/6	0/4 0/6			0/6					
	DEATH	1/6	0/4 1/6			0/6					
		2.7	4.4	6.1	7.8	9.5	11.2	12.9	14.6	16.3	18.0
		LOG OF DILUTION OF ORIGINAL VIRUS BY TISSUE REPLACEMENT									

* Denominator signifies No. of mice inj. intracer., .03 ml. Numerator signifies No. that died.
† "Paralysis" signifies No. of mice paralyzed (one observation per 24 hr). Paralysis within 24 hr of inoculation was not included. "Death" signifies No. of mice dead, excluding those dying within 48 hr of inoculation.

Results. The 3 passage series, Series A (Lansing-monkey testicular tissues), Series B (Yale-SK monkey testicular tissue), and Series C (Yale-SK virus in human or monkey testicular tissue), had been carried through from 8 to 10 successive tissue changes and from 24 to 30 fluid changes when this report was written. The results of 2 of these 3 passage transfer series are made known in Tables I and II. The data, as presented, will be made easily understandable by a few comments. Evidence for the multiplication of virus necessarily must be determined by relating at any given passage transfer the dilution factor and the infectious titer, as measured by the LD_{50}. Accordingly, the presence of virus was measured at successive stages in each experimental series by the incidence of paralysis and death in the test mice. For interpretation of the results, the inoculum on transfer to each of the first set of flasks was diluted $10^{2.7}$, when one part by weight of virus-containing brain and cord was added to 529 parts of the mixture that made up the balance of flask content. Thereafter, the viral content was diluted at each subsequent tissue replacement by 1:53, or $10^{1.7}$. On the other hand, the calculation of the viral dilution that resulted from each replacement of supernatant fluid was not absolute, simply because of the mechanical difficulty of removing all fluid from beneath the cellophane membrane. However, when actual measurements were made of the residual fluid, the mean quantity was established as 0.7 ml. Accordingly, it was concluded that each fluid change by the addition of 5.0 ml of fresh nutrient fluid resulted in a dilution of 1:8, or $10^{0.9}$. However that may be, it can be seen from the data presented in Tables I and II that unequivocal evidence

TABLE III. Summary of Experimental Findings Showing Propagation of Poliomyelitis Virus in Tissue Culture.

Tissue culture series	Virus and cells employed	Tissue No.	Fluid No.	Dilution of original virus by: Tissue replacement	Dilution of original virus by: Fluid replacement	LD_{50}	Neutralization test
A	Lansing-monkey testicle	7	20	$10^{12.9}$	$10^{24.6}$	$10^{-1.5}$	
	Lansing-monkey testicle	7	21	$10^{12.9}$	$10^{25.5}$	$10^{-1.6}$	+
B	Yale-SK monkey testicle	7	20	$10^{12.9}$	$10^{24.6}$	$10^{-3.0}$	
	Yale-SK monkey testicle	7	21	$10^{12.9}$	$10^{25.5}$	$10^{-2.1}$	+
	Yale-SK monkey testicle	10	29	$10^{18.0}$	$10^{35.1}$	$10^{-1.8}$	
C	Yale-SK human-monkey testicle	8	23	$10^{14.6}$	$10^{28.1}$	$10^{2.4}$	

for the propagation of virus was established for each of the three experimental series, whether calculated by dilution from successive replacements of tissues, or from successive replacements of fluid. In contrast to this evidence for viral multiplication, the virus employed as inoculum quickly disappeared from the control flasks.

It can be seen from Table I that the calculated dilution of the original inoculum of the Lansing strain of virus was $10^{29.0}$ after 24 fluid replacements over a period of at least 120 days, or $10^{14.6}$ when calculated after 8 tissue replacements. Table II makes it known for Series B that the calculated dilution of the original inoculum of Yale-SK strain of virus, after 30 fluid changes, was $10^{36.0}$, or 10^{18} after 10 tissue changes. The third passage series employed the Yale-SK strain of virus, as in Series B, but differed by utilizing testicular tissues derived both from man and monkey. The results after 30 fluid changes and 10 tissue changes were in complete agreement with the findings which are presented in Table II for Series B.

The pathogenicity for monkeys of the products of tissue culture growth was tested for Series A and Series B after 6 tissue changes, and for Series C after 7 tissue changes, by the injection intracerebrally, 0.5 ml of supernatant fluid which had been pooled for 3 tissue flasks. The development in each of the recipient monkeys of fever, paralysis, and the histological alterations in the central nervous system of poliomyelitis was accepted as evidence that a virus of the mouse encephalomyelitis group had not been picked up inadvertently.

A summary of the findings at the 7th and 8th tissue replacement is presented in Table III.

Table III makes known the results (a) of LD_{50} determinations, and (b) of the neutralization tests which established the identity of the virus after 7 tissue passages in each culture series. The samples of specific antiserum were kindly supplied by Dr. Jonas Salk. Specific identification of the Lansing virus was established by monkey serum labeled "Lans. Antisera Pool A-300. 20 June 1949"; the Yale-SK virus was identified by monkey antiserum labeled "YSK Aliquot Pool B-532—6/15/50."

Summary. In experiments in which monkey or human testicular cells in tissue culture were infected with poliomyelitis virus, Lansing and Yale-SK strains, proof was obtained that the virus in 3 passage series had been propagated. At the time this report was written it had been established for these 3 passage series that the minimal dilution factor based on tissue replacements ranged from $10^{14.6}$ to 10^{18} and, when assessed by fluid replacement, from 10^{29} to 10^{36}. The LD_{50} of each strain of virus was determined on successive transfers, and the identity of each strain of virus was established by neutralization tests and histopathological findings in monkeys dead from the injection of tissue culture virus. Control experiments and other tests made known

that propagation of poliomyelitis virus did not occur in the absence of viable testicular cells and that an extraneous virus was not inadvertently acquired during the course of these studies. It is plain that the results of the present investigation substantiate the observations of other workers that poliomyelitis virus can propagate in extraneural tissues. From these findings it is apparent that the assumption of an obligate neurotrophism for poliomyelitis virus no longer is tenable. This observation and the demonstration regularly of the natural occurrence in poliomyelitis of virus in the oro-intestinal tract emphasize the need for a renewal of investigative studies of the factors that make possible invasion of the central nervous system to result in the overt and residual manifestations of poliomyelitis.

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Further Studies on Experimental Parasystole and Extrasystoles in Groups.* (18666)

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In parasystole a center in one chamber of the heart forms rhythmic stimuli, undisturbed by the basic cardiac rhythm or by any other stimulus spreading over the heart. This leads to a regular interference of the basic and the ectopic rhythm. An experimental study of this interesting arrhythmia, which is not too rare in man, was impossible because no method was available to provoke it regularly.

Recently, we reported that the subepicardial application of veratrine alkaloids regularly leads to the appearance of parasystole, lasting for a few minutes, and showing all its characteristic features(1). In this paper another method will be reported for eliciting parasystole with ease. It consists of applying mechanical or electrical stimuli to the ventricles of the dog's heart after the intravenous administration of acetylcholine and complete atropinization.

Method. All 18 experiments were performed on dogs with the same technic as reported in previous investigations of the senior author in this journal. The electrocardiograms were taken in lead 2. A fresh solution of acetylcholine chloride "Roche" was used in all experiments. The amount of 0.05 g of acetylcholine was injected intravenously in 3-4 divided doses within 10 minutes and 0.2 mg per kg of atropine sulfate was given intravenously shortly afterwards. When acetylcholine was administered without atropinization, mechanical or electrical stimulation of the ventricles initiated ventricular fibrillation(2).

Results. Following the injection of acetylcholine and atropine, the mechanical stimulation of the ventricles by a stroke with a blunt instrument or the application of a few induction shocks provoke a heteropic ventricular tachycardia originating in the stimulated area. In 15 out of 18 experiments a parasystole was observed; in 3 experiments a regular ectopic tachycardia appeared, which persisted for 45 to 160 seconds and then reverted to regular sinus rhythm.

Fig. 1 shows a representative electrocardiogram exhibiting parasystole. A short part of the tracing between Fig. 1a and Fig. 1b is omitted. Fig. 1b and Fig. 1c are continuous. The dog had received acetylcholine and atropine in the doses mentioned above. The stormy changes caused by the acetylcholine, with the primary inhibition of the heart and the secondary increase of rate and motility had subsided. At the beginning of Fig. 1a a regular sinus tachycardia exists with a rate of

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