ouabain), and provides the fastest digitalization possible with preparations now in use(1). The present demonstration of its positive inotropic action supports the continued use of acetyl-strophanthidin as one of the emergency measures in the treatment of acute congestive failure with pulmonary edema in the presence of normal sinus rhythm.

Summary and conclusions. 1. Papillary muscles from the right ventricle of the cat heart were stimulated in phosphate-buffered Krebs-Ringer solution until their force of contraction declined. 2. On addition of acetyl-strophanthidin, tryptamine-strophanthidin, or strophanthidin to the papillary muscles, their force of contraction improved, and the improvement persisted throughout the period of observation (average 4.3 hours). 3. It is concluded that these strophanthidin derivatives have a typical digitalis action on failing mammalian heart muscle.

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Poliomyelitis Viruses in Tissue Culture. III. Susceptibility of Testicular Tissue from Immune Monkeys.* (19805)

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Tissue culture technics have been applied in the study of poliomyelitis to the propagation of laboratory-adapted strains of the virus (1-8), to the quantitative estimation of virus and antibody(9-11), and to the isolation and immunologic identification of poliomyelitis viruses from various sources(12-14). The use of monkey testicular tissue by some investigators for these purposes raises a question of both theoretical and practical importance, *i.e.*, whether poliomyelitis viruses may be propagated in cultures of testicular tissue obtained from monkeys immune to these viruses. In an attempt to answer this, a comparison was made of the susceptibility of testicular tissue obtained from both immune and non-immune monkeys to poliomyelitis viruses. Observations of this nature also bear on the use of human tissues for the cultivation of poliomyelitis virus, for these are usually obtained from individuals whose immune status is unknown.

Materials and methods. Tissue cultures. Roller tube cultures were employed in these

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experiments. The technic and the nutrient medium used have been described in detail (11). Testes from immature rhesus (*Macaca mulatta* and cynomolgus (*Macaca irus*) monkeys were used as the source of tissue, for testes from both species have been found to be equally susceptible to poliomyelitis virus in roller tube tissue cultures(15). *Viruses.* These were used in the form of tissue culture fluid pools: (a) Y-SK (type 2) strain in its 14th passage in monkey testicular culture, having a tissue culture endpoint of 10^{-2}; (b) Leon (type 3) strain in its 8th culture passage, its titer being 10^{-2}, (c) Brunhilde (type 1) strain in its 9th culture passage, its titer being 10^{-3}. Virus titrations were performed using serial 10-fold dilutions. In these experiments, the tissue culture endpoint is the final dilution of virus producing unmistakable tissue degeneration in 4 days (11). *Antisera.* These were obtained from monkeys immunized to the 3 types of poliomyelitis virus: Brunhilde, Y-SK, and Leon. A 20% suspension of monkey cord was made, centrifuged at 2000 RPM for 10 minutes, and mixed with adjuvant(19) in the following proportions: 2 ml of Arlacel, 8 ml of Bayol F, and 10 ml of virus. Three weekly injections of 2 ml of the mixture were given intramuscularly. The monkeys were bled 30 days after the last injection. *Neutralization tests in tissue culture.* Various dilutions of unheated sera were mixed with a final amount of 100 tissue culture doses of virus, and the mixture incubated for one hour at room temperature. Neutralization titers were determined at the end of 4 days as described(11).

Experimental. Before attempting to determine whether multiplication of poliomyelitis viruses may occur in testicular tissue obtained from immune monkeys, it was necessary to determine the degree of susceptibility of tissue obtained from different non-immune monkeys. In 2 different experiments, testes were removed from a total of 10 "normal" monkeys, and roller tube cultures were prepared separately from the tissue of each. Serial 10-fold dilutions of the Y-SK virus pool were inoculated into a series of roller tube cultures prepared from the testes of each monkey. The tissue culture endpoint was read on the fourth day. At the end of 8 days, at which time virus growth reaches its maximum(11), the viral concentration of the fluid from the tubes inoculated with undiluted virus was determined by titration in roller tubes.

TABLE I. Susceptibility of Cultures of Testicular Tissue Obtained from 10 Different Monkeys to the Y-SK Strain of Poliomyelitis Virus.

	Monkey No.	Titration endpoints at 4 days: Tissue culture endpoint of Y-SK	Titration endpoints at 4 days: Virus recovery from tubes 8 days after inoculation with undiluted tissue culture virus
Exp. 1	Rh 5640	10^{-2}	100†
	5641	10^{-2}	100
	5642*	10^{-1}	10
	5643	10^{-2}	100
	5644	10^{0}	1
Exp. 2	Rh 5646	10^{-2}	100
	5647		
	5648		
	5649		
	5650		

* Monkey 5642 testicular tissue showed poor growth in culture.

† 100 tissue culture doses were recovered per .1 ml, *i.e.*, this fluid gave a titer of 10^{-2} on the 4th day in a new series of tubes.

From the results listed in Table I, it is evident that the titration endpoint of Y-SK virus was uniformly reproducible in the individual tissues of 8 out of the 10 monkeys tested. The tissue of monkey 5642, in contrast to the others tested under the same conditions, showed poor growth in culture, and yielded a decreased amount of virus. The tissue of monkey 5644 definitely gave a decreased titer using the cytopathic endpoint, and the yield of virus was also low. Since this finding could not be explained by the poor growth of the tissue, the question arose whether the possible presence of antibody may have been the cause. Therefore, experiments were next designed in which the serum of each monkey whose tissue was to be tested for susceptibility to the Y-SK virus was also examined for the presence of antibody.

In 2 separate experiments, testes were removed from a total of 8 "normal" monkeys, and roller tube cultures were prepared separately from the tissue of each monkey. Serial

TABLE II. Susceptibility of Cultures of Testicular Tissue Obtained from 8 Different Monkeys to the Y-SK Strain of Poliomyelitis Virus.

	Monkey No.		Tissue culture endpoint at 4 days	Serum antibody
Exp. 1	Rh	6095	10^{-2}	0
		6097	10^{-2}	0
		6101	10^{-2}	0
		6103	10^{-3}	0
Exp. 2	Rh	5115	10^{-2}	0
		5116	10^{-2}	0
	Cyno	5796	10^{0}	0
		5798	10^{-2}	0

10-fold dilutions of the same Y-SK virus pool, as used in the previous experiments, were inoculated into a series of tubes prepared from the testes of each monkey. The tissue culture endpoint was read in 4 days. At the time of removal of the testes, the monkeys were bled. Undiluted serum was tested for neutralizing activity against Y-SK virus. The results are given in Table II. Of the 8 monkeys tested for susceptibility to the Y-SK virus, 6 showed a tissue culture endpoint of 10^{-2}. The tissue of Cyno 5796 grew well but it poorly supported virus growth. Since this monkey had no serum antibody at the time the tissue was removed, other factors must be considered responsible. Whether the tissue of Rh 6103, which gave an endpoint of the virus of 10^{-3}, represents an especially susceptible monkey is open to speculation. These experiments are representative of a large series of separate experiments.

Experiments were then planned to determine whether testicular tissue obtained from immune monkeys would support the growth of poliomyelitis viruses. A series of immune monkeys were selected and tested for their susceptibility to the viruses with which they had been immunized, and in certain instances to a heterotypic virus as well. Four experiments were designed:

Exp. 1. Testes were removed from 4 Y-SK immune monkeys, and roller tubes were prepared separately. Serial 10-fold dilutions of Y-SK virus were added to 5-day growing tissue, and the endpoint was read in 4 days. The sera of the monkeys were tested with the same virus, Y-SK, using the following final serum dilutions: 1:10, 1:100, 1:1000, and 1:10000.

Exp. 2. In the regular procedure employed in making roller tubes the tissue is washed twice before being used. The washing was intentionally omitted in the experiment. The tissue was minced, suspended in 5 ml of salt solution, and roller tube cultures immediately prepared. In addition to allowing the tissue to incubate for 5 days before inoculating the virus, dilutions of the virus were added to the tubes at 0 time of incubation. Considerable dilution of the antibody might take place in 5 days, due to 2 renewals of the nutrient medium before virus inoculation in the regular procedure(11). The tubes inoculated at 0 time of incubation were read 9 days after inoculation of virus, at the same time when the tubes, inoculated after 5 days of tissue incubation, were examined for degeneration 4 days after inoculation of the virus. The tissues of 5 Y-SK immune monkeys were tested, and the same strain was used in testing their susceptibility in culture and in the neutralization tests.

Exp. 3. Two Leon immune monkeys were used, and their testicular tissue and sera were tested with the homologous strain.

Exp. 4. The testicular tissue and sera of 3 Brunhilde immune, and 2 Y-SK immune monkeys were tested with both Brunhilde and Y-SK viruses, according to the procedure of Exp. 1.

The results are given in Table III. The testicular tissues of 9 Y-SK immune monkeys, whose serum antibody titers ranged from 1:10 to 1:1000, were tested for susceptibility to the Y-SK virus. In all cases. the tissue culture endpoint of the virus was found to be 10^{-2}, the titer which was obtained in tissue from non-immune monkeys. Even though the regular procedure of washing the tissue before the preparation of roller tubes was omitted and the tubes inoculated immediately with virus, the tissue culture endpoint was still found to be 10^{-2}. Of the 2 Leon-immune monkeys tested, the testicular tissue of both proved equally susceptible to the action of the virus. The tissue culture endpoint was found to be 10^{-2}, the titer obtained in tissues from non-

TABLE III. Susceptibility of Testicular Tissue of Different Immune Monkeys to Poliomyelitis Virus.

Monkey No.	Immune to:	Tested vs.:	T.C. endpoint of virus at 4 days — Tissue inoc. at — 0 days	5 days	Serum neutralization titer in T.C.*
Exp. 1					
Cyno 5255	Y-SK	Y-SK		10^{-2}	1:1000
5464				10^{-2}	1:1000
5806				10^{-2}	1:10
5728				10^{-2}	1:100
Exp. 2†					
Rh 6043	Y-SK	Y-SK	10^{-2}‡	10^{-2}	1:10
6045			10^{-2}	10^{-2}	1:10
6048			10^{-2}	10^{-2}	1:10
6049			10^{-2}	10^{-2}	1:10
6050			10^{-2}	10^{-2}	1:10
Exp. 3					
Rh 6052	Leon	Leon		10^{-2}	1:1000
6054				10^{-2}	1:1000
Exp. 4					
Rh 6055	Brunhilde	Brunhilde		10^{0}	1:100
		Y-SK		10^{0}	0
6056	"	Brunhilde		10^{-3}	1:1000
		Y-SK		10^{-2}	0
6057	"	Brunhilde		10^{-3}	1:1000
		Y-SK		10^{-2}	0
6042	Y-SK	Brunhilde		10^{-3}	0
		Y-SK		10^{-2}	1:10
6044	"	Brunhilde		10^{-3}	0
		Y-SK		10^{-2}	1:10

* The serum neutralization titer was determined with the virus used to obtain the tissue culture endpoint.
† Tissue was not washed as in the routine procedure.
‡ Tissue culture endpoint of virus read at 9 days.

immune monkeys. The serum antibody titer of both monkeys was 1:1000.

In Exp. 4 in Table III, the testicular tissues of 3 Brunhilde immune monkeys and 2 Y-SK immune monkeys were tested for susceptibility to both the Brunhilde and Y-SK poliomyelitis viruses. While the tissue of 2 of the Brunhilde-immune monkeys gave a titer of 10^{-3} with the Brunhilde strain, the tissue of the third Brunhilde-immune monkey, Rh 6055, proved to be "incompletely" susceptible to this virus. The tissue culture endpoint obtained was only 10°. That this result was not due to the presence of Brunhilde antibodies is indicated by the fact that the tissue culture endpoint obtained with the Y-SK virus was also 10°. The tissue of the other 2 Brunhilde-immune monkeys gave a tissue culture endpoint of 10^{-2} with Y-SK. In like fashion, 2 Y-SK-immune monkeys, with no Brunhilde antibodies, gave a titer of 10^{-3}, and of 10^{-2}, with the Brunhilde and Y-SK viruses, respectively. The serum antibody titers of the above monkeys given in Table III, indicate the specificity of the antibody response.

Discussion. It is apparent from these experiments that the presence of antibody in the serum of a monkey, whose testicular tissue is used for setting up roller tube cultures, plays little part in determining the susceptibility, or resistance, of that tissue to poliomyelitis virus. Testicular tissue obtained from immune monkeys supported growth of poliomyelitis viruses equally as well as tissue obtained from non-immune monkeys, under the experimental conditions used. Other investigators have also found that growth of virus in tissue culture may occur in tissues taken from an immune host. Andrewes found that herpes virus(16) and virus III(17) grew in testes

obtained from immune rabbits. Growth of herpes virus occurred, even with no preliminary washing of the tissue. In the experiments reported above, growth of Y-SK (type 2) poliomyelitis virus also occurred in testicular tissue which had received no preliminary washing. However, each of the sera in the last mentioned experiment had a low antibody titer of 1:10. Had the titer been high, virus propagation might have been inhibited.

The testicular tissue obtained from different monkeys possesses fairly uniform susceptibility to infection with the poliomyelitis viruses. Of a total of 34 monkeys tested above, both immune and non-immune, the tissue of only 3 has proved to be "incompletely" susceptible. The cause of the "incomplete" susceptibility of certain monkey testicular tissues is not understood. A nutritional deficiency of the monkey may contribute to this state. The possible presence of an interfering, latent virus should also be considered, and stock monkeys are known to be infected at times with B virus. Last year, a B-like virus was actually isolated from a rhesus monkey in the Yale colony(18). Or cellular susceptibility or insusceptibility may be a genetically controlled character. An "incompletely" susceptible state may be a contributing factor in the failure to propagate poliomyelitis virus in tissue culture, if tissue from a single insusceptible monkey is used. If virus isolations from various sources are to be made, a pool of tissue from more than one monkey is desirable. Such a factor may also influence experimental conclusions dealing with the comparison of tissue culture and animal pathogenicity of various poliomyelitis strains, if tissue from a single monkey is used.

Summary. The titration endpoints of Brunhilde (type 1), Y-SK (type 2), and Leon (type 3) poliomyelitis viruses were uniformly reproducible in roller tube cultures of testicular tissue from 31 of the 34 monkeys tested. The tissue of approximately 10% of the monkeys tested was "incompletely" susceptible to the poliomyelitis viruses. This insusceptible state could not be attributed to the presence of serum antibody. Testicular tissue obtained from immune monkeys supported growth of all 3 antigenic types of poliomyelitis virus comparable to that obtained in tissue of non-immune monkeys.

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