TABLE II. Some Properties of the Anti-SGI Factor in Trypsin Powder.

Trypsin powder ≈ 5% (see text for details)	Rat growth g/day ± SE g gain/g food consumed — Soybean oil meal — Autoclaved	Raw	Raw autoclaved, %
Autoclaved	3.84 ± .24 (.50)	3.88 ± .18 (.44)	101
Autolysate soluble	3.37 ± .29 (.49)	3.44 ± .14 (.51)	102.1
pH 3, 60% ethanol soluble	3.40 ± .14 (.46)	3.46 ± .11 (.45)	101.7
n-butanol insoluble	3.39 ± .19 (.46)	3.43 ± .18 (.43)	101.2
acetone ppt.	3.83 ± .15 (.45)	2.31 ± .08 (.28)	60.3
ammonium sulfate ppt.	2.99 ± .08 (.40)	2.16 ± .14 (.26)	72.3
dialysis residue	3.94 ± .13 (.49)	3.43 ± .15 (.42)	87.1

activity is not essential to the anti-SGI activity.

The importance of the anti-SGI factor of crude trypsin powder in animal nutrition is difficult to outline at present. Nutritional effects have been ascribed to preparations from pancreas, particularly lipocaic. Also, no untoward effects, that is deficiency symptoms other than depressed growth, have been reported as a result of feeding raw soybean oil meal. The relation of the anti-SGI factor of crude trypsin powder to other nutritional factors as well as to the normal nutrition picture requires further investigation.

Summary. Growth of rats fed a ration containing 25% raw soybean oil meal was improved to the same rate of gain as rats fed autoclaved meal when 5% crude trypsin powder was added to each ration. The ability of the crude trypsin powder to counteract the soybean growth inhibitor (SGI) has established the existence of an anti-SGI. That the anti-SGI activity of crude trypsin powder was not dependent upon trypsin was established by heat stability.

Received July 17, 1951. P.S.E.B.M., 1951, v78.

Differences in Cellular Pathogenicity of Two Immunologically Related Poliomyelitis Viruses as Revealed in Tissue Culture.* (18982)

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With the exception of antigenicity, biological differences within the group of poliomyelitis viruses have not been intensively investigated. This may be due in part to the fact that convenient technics are not available for such a study, as is the case with the influenza viruses. However, recent results from this laboratory (1) have shown that 2 immunologically related poliomyelitis strains, Lansing and Yale SK, differ in their ability to elicit antibodies after feeding to cynomolgus monkeys. The work of Enders, Weller, and Robbins(2,3) on the growth of poliomyelitis viruses in tissue culture has opened new avenues for exploration in this field. These workers have recently reported the capacity of Lansing and Brunhilde strains to cause cell injury and death in human embryonic tissues(4). We had found that the poliomyelitis viruses will grow in monkey testes *in vitro* (but not *in vivo*),

* Aided by a grant from the National Foundation for Infantile Paralysis.

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as have Syverton(5) and Evans(6), using the suspended cell cultures as recommended by Enders(2).

The present report is concerned with the growth of poliomyelitis virus in roller tube cultures of monkey testes, and particularly with the description of a technic whereby in 4 days, it is possible to titrate the virus using degeneration of fibroblasts of monkey testes as the criterion of virus growth. We have used this technic to compare the relative cytopathogenic effects of the antigenically related Lansing and Yale SK (Y-SK) strains of poliomyelitis virus.

Materials and methods. Tissue cultures. Roller tube cultures were employed in these experiments. The technic, used with some modification, has been described in detail(7). Minced testes from immature rhesus *(Macaca mulatta)* monkeys, and, in a separate series of experiments, minced human embryonic skin-muscle, were used. The medium consisted of 9 parts of the "HS" mixture as described by Weller and Enders(8), which consists of 3 parts of Hanks salt solution and 1 part of Simm's ox serum ultrafiltrate, and 1 part of chick embryo extract prepared from 10-16-day-old embryos extracted with acetone according to the procedure of Margoliash(9). This extraction of chick embryo tissue was found to be necessary in order that fibroblasts with no granular lipid deposits are obtained during growth. In this way, any degeneration due specifically to the virus may be clearly seen. Fifty units of penicillin and 50 μg of streptomycin per ml of medium were added. 0.9 ml of this mixture was added to each culture tube containing 6 to 10 pieces of tissue. The nutrient medium was changed whenever a drop in pH made it necessary. The tissue was allowed to grow 5 to 7 days before addition of virus. At this time, fibroblastic proliferation is moderate. *Viruses.* Lansing and Y-SK strains of poliomyelitis virus were used as 10% mouse cord suspensions. The LD_{50} of Lansing was 3.5, and that of Y-SK 3.0. The Lansing strain had undergone 267 passages in mice, while the Y-SK only 11 passages. 0.1 ml of each virus was added to each of 4 culture tubes. Four culture tubes inoculated with 10% normal mouse cord served as controls. *Titrations.* Virus titrations were performed by adding serial tenfold dilutions of the suspensions to be titrated to growing tissues. Three to 4 roller tubes were used per virus dilution, and 0.1 ml was used as the inoculum. Mouse titrations of the same suspensions were done by intracerebral inoculation (0.03 ml) of adult albino Swiss mice. Eight mice were used per virus dilution and they were observed daily for 5 weeks. The Reed-Muench method was used to calculate the LD_{50}. *pH changes.* pH changes were followed by taking the average of the readings of the pH of the medium in the 4 roller tubes before the addition of fresh medium. The pH was determined by colorimetric comparison with standard buffer solutions, which had the same buffer and the same concentration of phenol red as the medium. The pH of the fresh medium varied from 7.5 to 7.7.

Experimental. In order to compare the cytopathogenic effect of Y-SK and Lansing viruses, the 2 strains were inoculated at the same time into different tubes of the same growing testicular tissue. Four days after virus inoculation, the roller tubes inoculated with Y-SK, when examined microscopically, showed a complete destruction of the testicular fibroblasts. Granulation and loss of structure were evident. In marked contrast, the tubes inoculated with Lansing showed only a slight tissue destruction. These observations suggested that a titration of Y-SK, but not of Lansing, might be possible in roller tubes. This proved to be so. Consequently, several dilutions of the fluid phase of Y-SK inoculated tubes, at various intervals following virus inoculation, were titrated both in tissue culture and in mice. At this time the medium was also renewed. The culture fluids of the Lansing inoculated tubes could be titrated only in mice. The results, typical of a number of experiments, are given in Table I.

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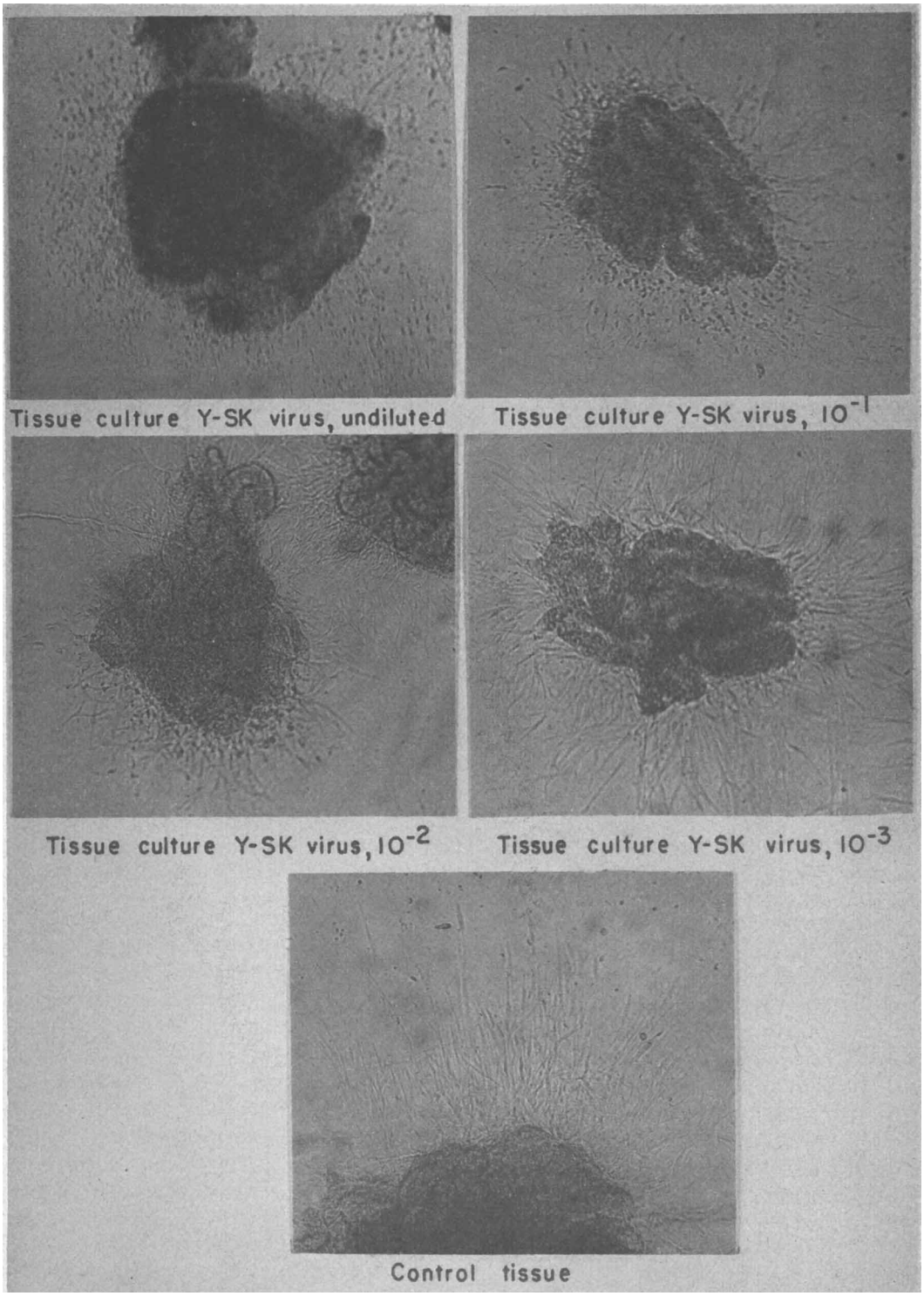

FIG. 1. Titration of Y-SK strain of poliomyelitis virus in roller tube cultures of monkey testicular tissue. Magnification 67 ×.

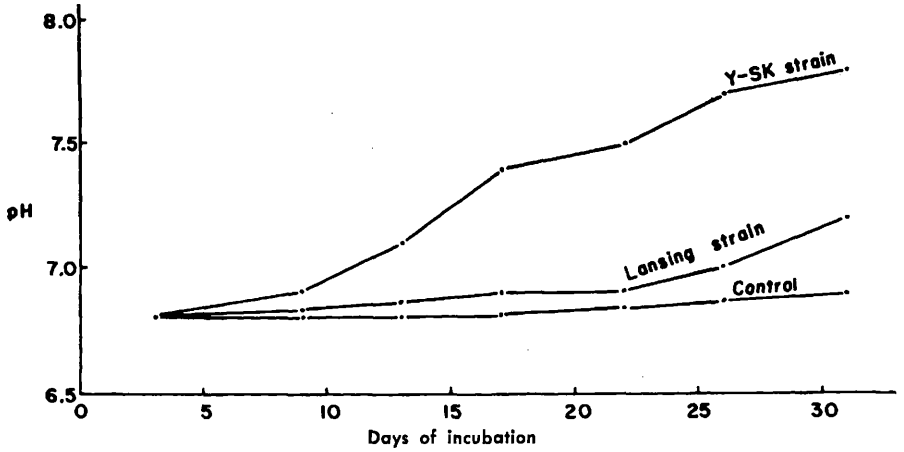

FIG. 2. pH determinations of fluid phase of roller tubes of monkey testicular tissue inoculated with Y-SK and Lansing poliomyelitis viruses.

These results indicate approximately equal susceptibility of monkey testicular tissue in roller tubes and the intact mouse to the Y-SK strain of poliomyelitis virus. This was substantiated by the fact that the tissue culture titration of the original inoculum gave an endpoint of 10^{-3}, the same as that found when the virus was titrated in mice. The fact that the Lansing strain grew equally as well as the Y-SK strain and still did not show as much degeneration points to a fundamental difference in the cellular pathogenicity of these two strains.

A typical tissue culture titration is illustrated in Fig. 1. The undiluted virus causes complete destruction of the fibroblasts, involving granulation, shrinkage, and loss of the normal structure shown by the control tissue. As the virus is serially diluted, correspondingly less fibroblastic degeneration is seen. The endpoint here is 10^{-2}, where definite degeneration of the tissue is still evident. In the tubes showing degeneration, the amount of tissue proliferation is less than that of the controls. The tissue inoculated with the 10^{-3} dilution of virus shows as much fibroblastic proliferation as the control tissue. Specific immune serum prevents tissue degeneration by the virus. Undiluted Lansing virus gives about the same amount of degeneration as the 10^{-2} dilution of Y-SK in Fig. 1.

Because of the marked tissue degeneration caused by the Y-SK strain of poliomyelitis virus in contrast to that caused by the Lansing strain, it might be expected that these two strains would also differ in their capacity to alter the metabolic activity of their host tissue. One manifestation of such an alteration would be a change in the amount of acid production corresponding to the amount of damage caused by the virus. Therefore pH changes induced by the two viruses were also followed throughout the experiment (Fig. 2). For the first 9 days of incubation, no significant pH change occurred in any of the roller tubes. Then from the 13th day until the termination of the experiment on the 31st day, the Y-SK inoculated roller tubes began to show a progressive decrease in acid production, with the pH rising from an initial value of 6.8 to 7.8. No such marked change occurred in the Lansing tubes, although the initial pH of 6.8 slowly rose to 7.2 by the 31st day of incubation.

To determine what the cytopathogenic effect of the Y-SK and Lansing strains used in the above experiments would be on another tissue, human embryonic skin-muscle roller tube cultures were tested. These experiments indicate that these two strains also differ in their cytopathogenic effect on this tissue. The Y-SK strain again caused a greater amount of fibroblastic degeneration in 4-6 days than the Lansing strain. The testing of various culture fluids, in a series of experiments similar to that described in Table I, revealed that the two viruses grew to an approximately equal

TABLE I. Tissue Culture and Mouse Titrations of Y-SK and Lansing Poliomyelitis Viruses in Roller Tube Cultures of Monkey Testicular Tissue.

Days after inoculation	Negative logarithm of tissue culture fluid — Y-SK: Tissue culture titration	Y-SK: Mouse titration, LD_{50}	Lansing: Mouse titration, LD_{50}
3	1*	.8	1.2
6	2	1.8	1.2
9	2	1.5	1.6
13	2	1.2	1
17	2	1.1	.9
22	1	.4	.4
26	1	.2	.3
31	1	.6	.5

* The tissue culture endpoint is the final dilution of virus which produces tissue degeneration in 4 days, as shown in Fig. 1. In the series shown there, the end point was found in the 10^{-2} concentration of the culture fluid being titrated.

TABLE II. Mouse Titrations of Y-SK and Lansing Poliomyelitis Viruses in Roller Tube Cultures of Human Embryonic Skin-Muscle.

Days after inoculation	Viruses — Mouse titration (negative logarithm) Y-SK LD_{50}	Lansing LD_{50}
3	*	*
6	1.4	1.7
9	1.2	1.6
12	1.1	1.6
15	1.1	1.5
19	1	1.1
23	*	*

* Not done.

extent in the human tissue. A representative experiment is given in Table II. Moreover, the pH changes in this experiment were similar to those obtained for the monkey testicular tissue (Fig. 3), in that the Lansing strain induced less of a pH change than Y-SK. After 23 days of incubation, the initial pH of 6.8 rose to 7.6 for the Y-SK strain, and to 7.2 for the Lansing strain.

Discussion. Viral variation is well established. Variation within an immunologically related virus group may involve such diverse properties, as pathogenicity, toxicity, and stability, as has been extensively studied in the case of the influenza group of viruses. It is reported here that the 2 immunologically related strains, Lansing and Y-SK, differ in their cytopathogenic effect on monkey testicular tissue, and human embryonic skin-muscle, as evidenced by a difference in the amount of tissue destruction, and in the alteration of the host metabolic activity. The fact that Y-SK produces a more marked tissue damage, raises

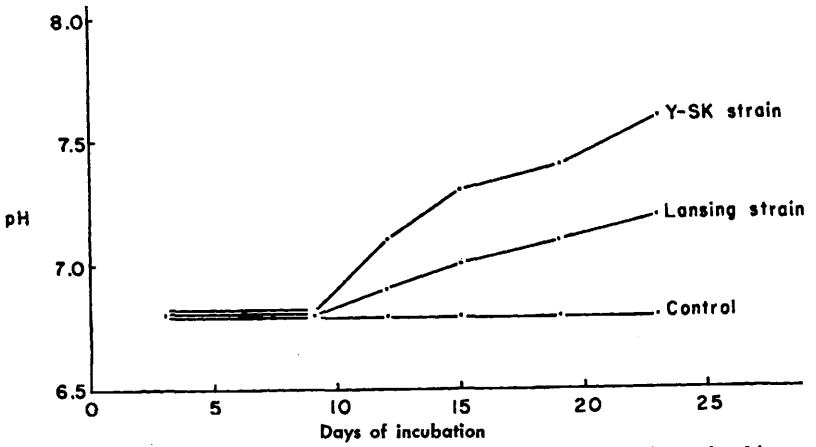

FIG. 3. pH determinations of fluid phase of roller tubes of human embryonic skin-muscle inoculated with Y-SK and Lansing poliomyelitis viruses.

the question whether this difference from the Lansing strain is due to the fewer number of mouse passages which the Y-SK has had, or to an inherent difference between the two strains. It is known that many laboratory virus variants have appeared as a result of continuous passage in a new animal host. Therefore the possibility exists that both viruses initially had the same cytopathogenic ability, and that for the Lansing strain, this ability was lost by continuous mouse passage. In this connection, Theiler(10) has found that continuous mouse passage of the Lansing strain resulted in a marked drop in its capacity to infect monkeys.

Summary. The Y-SK strain of poliomyelitis virus may be titrated in roller tube cultures by observation of the degeneration of monkey testicular fibroblasts within 4 days following the addition of virus to the tissue culture. The extent of fibroblastic degeneration is inversely related to the metabolic activity of the tissue, as measured by the amount of acid production. The 2 immunologically related Y-SK and Lansing strains of poliomyelitis virus differ in the degree to which they cause fibroblastic degeneration, and alter the amount of acid production by the tissue. In contrast to the Lansing strain, the Y-SK strain produces more extensive degeneration in fibroblasts of monkey testicles and also in those of human embryonic skin-muscle.

Received July 17, 1951. P.S.E.B.M., 1951, v78.

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Growth Promotion in Tetrahymena by Folic Acid Analogs.* (18983)

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The most widely used of the substituted folic acids in inhibition studies is 4-aminopteroylglutamic acid (Aminopterin). Early studies have shown it to be inhibitory to all organisms tested,† with the single exception of the rabbit, which had not been previously rendered folic acid deficient(1). It has been shown(2,3) that inhibition by low concentrations of inhibitor can be reversed by large doses of folic acid but reversal fails when the inhibitor concentration is high. More recently(4,5) reversal of inhibition has been obtained over wide ranges with citrovorum factor.

It is of considerable interest, therefore, to find that the animal microorganism, *Tetrahymena,* is not only uninhibited by Aminopterin but that this compound will replace folic acid in its diet. The same is true of N^{10}-methylfolic acid (Methopterin), although in this case the growth promoting power is lower and mild inhibition results at very high concentrations.

This report deals with Aminopterin, Methopterin, pteroylaspartic acid, 4-aminopteroylaspartic acid and citrovorum factor.

Experimental. The organism used was *Tetrahymena geleii* W, grown in bacteria-free culture. The medium used was Medium

* Aided by a grant recommended by the Committee on Growth acting for the Am. Cancer Society and a grant from the Research Corporation.

† A note by G. E. Foley reports that the partial utilization (probably by deamination) of 4-aminopteroylglutamic acid by *Streptococcus faecalis* 8043 in appropriate pteroylglutamic acid-free substrate has been demonstrated (personal communication).

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