It is further indicated that progesterone may be obtained from the reaction in an alternate state Pr′. Preliminary studies indicate that Pr′ is water soluble, but further studies are in progress on the nature of this change.

One of the most meaningful aspects of this study may be the *in vitro* demonstration of the effect of a hormone on an enzymatic reaction at a subcellular level. It is furthermore indicated that the participation of the hormone in the enzymatic reaction results in its alteration (metabolism!). This specific example may, therefore, illustrate a concept of wide application and significance.

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A Simplified Method for Cultivation of Poliomyelitis Virus in Tissue Culture

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Roller tube tissue culture methods, as described by Enders and associates (*1, 2*) and extended by others (*3–6*), have opened new vistas in poliomyelitis research by providing techniques more economical than previous methods, requiring the use of monkeys, for poliomyelitis virus isolation, virus typing, and the assay of specific antibodies.

However, there still remain a number of practical difficulties and limitations, and foremost among them are the ingredients of the medium. Tissues such as human embryo and monkey testes either are not always readily available or are expensive. Horse serum is frequently inhibitory to virus, and chick embryo extracts are at times sources of contamination. While search is continuing for a suitable substitute for monkey testes, currently the most commonly employed tissue, we wish to report briefly on some changes in technique. These modifications permit the rotary drum to be dispensed with, and, when monkey testis tissue is used, the chick embryo extract and horse serum to be replaced by plasma hydrolyzate with rather satisfactory results.

Shortly after tissue culture study with poliomyelitis virus was instituted at this laboratory one year ago, it was observed that rotation of the tissue cultures is not essential for adequate cell or virus multiplication. If the culture tubes are kept stationary in slanted position so that the fluid just covers the tissue embedded at the lower end of the test tube, the cells as well as the virus grow very well. Other workers have had similar experience (*7–9*).

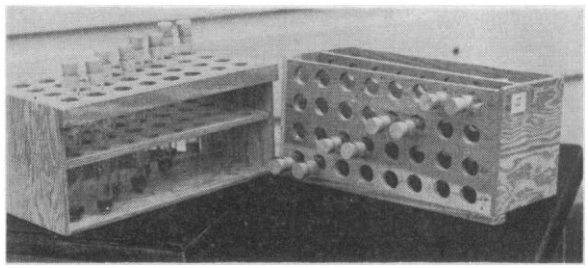


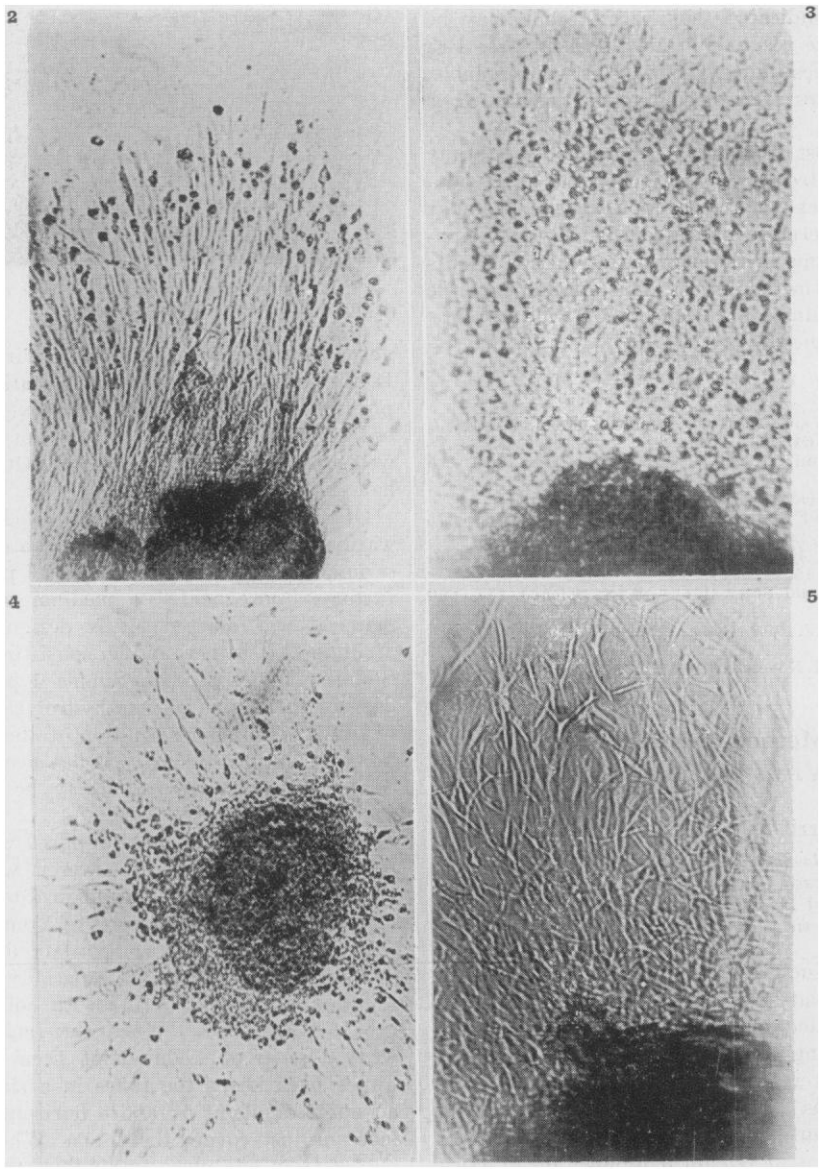
FIG. 1. Wooden racks holding tissue culture tubes in upright position or in slanted position.

Of a number of substances tested with a view toward replacement of chick embryo extract and horse serum, bovine plasma hydrolyzate[1] proved most satisfactory. Consequently, a medium incorporating this material was devised; it is designated Medium E. Medium E is rather simple, consisting of 75% Hank-Simms (Hank's 3 parts, Simms' 1 part) solution and 25% of the bovine plasma hydrolyzate. It is adjusted to pH 7.0–7.2 by the addition of sterile sodium bicarbonate solution. Recently it has been found that the Simms serum ultrafiltrate may be omitted entirely with equally good results.

The tissue cultures are prepared with fragments of monkey testicular tissue covered with 2 cc of the medium in ordinary bacteriological test tubes, 15 × 150 mm, similar to the techniques of Youngner, Ward, and Salk (*5*) and Ledinko, Riordan, and Melnick (*6*). The tubes are inserted into especially designed wooden racks, 11½ × 5½ × 5½ in., with holes for 4 rows of tubes, 8 tubes/row. When the rack is placed sidewise, a strip of wood about 1 cm thick across the upper back keeps the tubes in a slanted position at an angle of about 5° above horizontal level, and the medium just covers the tissue. When the tubes are moved from one place to another, the rack is carried in an upright position to prevent the fluid from touching the rubber stoppers (Fig. 1).

The cultures are incubated at 35° C for 5–7 days. No change of the medium is required during this period. The medium is then removed, and the virus, or other material desired to be cultured (in an amount of about 0.1 cc), is placed in contact with the cells for about 10 min, after which 2 cc of fresh medium is added. Incubation is continued and the cells are observed. With this procedure, both the fibroblasts and the virus grow well in Medium E, and, although the cells do not grow so richly and densely as those cultured in Medium A of Youngner, Ward, and Salk

[1] Obtained commercially as Travamin, a sterile 5% solution of bovine plasma hydrolyzate, w/v in water, from Baxter Laboratories, Inc., Morton Grove, Ill. Recently Melnick and Riordan (*8*) reported that lactalbumin enzymatic hydrolyzate is also satisfactory.



FIGS. 2–5. The cytopathogenic effect of poliomyelitis viruses on monkey testicular tissue culture Medium E without rotation, showing various degrees of cellular degeneration. 75 ×. Fig. 2. First degree of degeneration. Beginning degeneration of the periphery of the outgrowth zone. Twelve-day viral growth. (Mouse-adapted Type III Leon virus.) Fig. 3. Second degree of degeneration. Massive degeneration of the entire outgrowth. Four-day viral growth. (Tissue-culture-adapted Type I Mahoney virus.) Fig. 4. Third degree of degeneration. The outgrowth is almost entirely gone. The periphery of the mother tissue itself shows degeneration. Seven-day viral growth. (Tissue-culture-adapted Type I Mahoney virus.) Fig. 5. Control. Ten-day cellular culture without inoculation of virus.

(*5*), they are not granular as are those observed in Medium A. The pH of this medium does not drop so rapidly as does the medium containing chick embryo extract and horse serum. Consequently the number of necessary changes of medium is reduced.

The cellular degeneration caused by poliomyelitis viruses in Medium E is not only clearly defined but also, because nonspecific degeneration does not readily occur in uninfected tissue, the specific effects may be more easily recognized. There appears to be no difference in virus yield when this technique is compared to techniques employing the other media with and without the use of the rotary drums. For instance, a culture of Type I virus (Mahoney strain[2]) in Medium E without rotation yielded a pooled fluid with a tissue culture titer of 10^{-5}. This is the highest titer we have obtained as yet with any medium with or without rotation.

The cytopathogenic effects of the viruses on tissue culture cells in Medium E without rotation are shown in Figs. 2–5.

[2] Kindly supplied by J. E. Salk, Pittsburgh, Pa.

In a number of tests, cultures in Medium E without rotation have proved satisfactory for primary isolation of virus and for the performance of neutralization tests with the three types of poliomyelitis virus. Poliomyelitis viruses have been isolated from infected monkey cord, mouse cord, and human stools. One of the stool specimens was negative by monkey inoculation, but positive results were obtained by this tissue culture method. The fluid phase of this tissue culture, produced paralysis in two monkeys inoculated with it, and poliomyelitis was confirmed by histopathologic findings.

Neutralization tests, with the Mahoney strain as the test virus, have been performed by this method on a group of 80 human serum specimens with quite satisfactory results. In non-neutralized mixtures first evidence of cellular degeneration was usually observed on the second day after inoculation with the virus. This became fairly definite by the third day, and the final readings were possible on the fourth day.

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On a New Derivative of Thiamine with Cysteine

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The mechanism of thiamine in the metabolism in the living body is not yet clear. Zima and Williams (*1*) previously proposed a redox system between the thiol form of thiamine and thiamine disulfide.

The present authors have recently hypothesized a mechanism of thiamine in a medium that contains cystine, cysteine, glutation, or proteins having thiol groups, and have studied as a model experiment the reactions between thiamine and cystine, and between thiamine disulfide and cysteine by paper partition chromatography.

(1) Thiamine and equivalent cystine were dissolved in a phosphate buffer solution (pH 7.5), left standing at 37° C for 20 min, and then subjected to paper partition chromatography using the solvent prepared by shaking a mixture of alcohol, acetic acid, butanol, and water (1:1:4:10) and then drawing off the lower layer (Table 1).

The five paper strips on which the specimen was developed under the same conditions were dried in air and sprayed separately with the reagents indicated in Table 1.

The zones of Rf 0.04–0.05, 0.28–0.29, 0.29–0.31, and 0.36–0.38 correspond to cystine, cysteine, thiamine, and thiamine disulfide (VI), respectively. Besides these a new zone of Rf 0.14–0.15 was clearly detected by the reagents D, N, and I between the zones of cystine and thiamine.

When the paper strip sprayed with the reagent B was heated at 70–80° C for 5–10 min and then sprayed with the reagent A, the zone corresponding to thiamine showed strong fluorescence of thiochrome under the quartz mercury lamp, while the zones of Rf 0.14–0.15 and thiamine disulfide (VI) did not. However, when the paper strip was sprayed with the reagent C and heated at 70–80° C for 15–30 min in order to reduce the substances before spraying with the reagents B and A, all the three zones showed strong fluorescence.

From the result it seems evident that the new zone of Rf 0.14–0.15 means a new compound having the structure (IV) which was produced from the thiol form (II) of thiamine and cystine (III).

TABLE 1

Reagents	Cystine	Thiamine cysteine (IV)	Cysteine	Thiamine	Thiamine disulfide (VI)
D	—	0.14–0.15	—	0.29–0.31	0.36–0.38
N	0.04–0.05	0.14–0.15	(0.28–0.29)	—	—
I	0.04–0.05	0.15	—	0.30	0.37
B + A	—	(0.15)	—	0.30	—
C + B + A	—	0.14–0.15	—	0.29–0.31	0.36–0.38

D = the Kraut-Dragendorff reagent (*2*).

N = 0.2% solution of ninhydrin in the water saturated with butanol.

I = 0.05 *N* aqueous solution of iodine containing 1.5% of sodium azide. The reagent used for the detection of sulfur-containing amino acids (*3*) (thiamine and some related compounds can be detected by this reagent).

B = this reagent is prepared by adding 10% solution of sodium cyanide to the water saturated with bromine until the color of the bromine has disappeared (this is used instead of potassium ferricyanide to oxidize thiamine to thiochrome).

C = 0.4% solution of cysteine hydrochloride in water.

A = 5% solution of sodium hydroxide in the water saturated with butanol.

The mark (—) implies weak detection.