kidneys contained most AC, ICD, and SD. These results are reported on dry basis. They appear to be just as significant when evaluated on wet basis.

Summary. 1. Normal and syphilitic rabbit testes, liver, heart and kidneys were assayed for α-ketoglutaric oxidase, pyruvic oxidase, fumarase, aconitase, lactic dehydrogenase, TPN isocitric dehydrogenase and succinic dehydrogenase. 2. In syphilitic testes there is a definite decrease in pyruvic oxidase, α-ketoglutaric oxidase, aconitase and TPN isocitric acid dehydrogenase. 3. Both normal and syphilitic heart tissue showed very considerable variations in aconitase activity. 4. In syphilitic kidneys there was a decrease in pyruvic oxidase activity and a moderate decrease in aconitase activity. 5. The decreased enzyme values found in the testes and kidneys of the syphilitic groups are probably a demonstration of a general reaction to the infection.

The author appreciates the assistance of John W. Clark, Jr., Carl S. Adams, and David L. Johnson.

1. Saurino, V. R., and DeLamater, E. D., *Am. J. Syph., Gonor. and Ven. Dis.,* 1952, v36, 353.

2. Anfinsen, C. B., *Methods in Enzymology,* 1955, v1, 695.

3. Sanadi, D. R., and Littlefield, J., *J. Biol. Chem.,* 1952, v197, 851.

4. Racker, E., *Biochim. et biophys. acta,* 1950, v4, 211.

5. Ochoa, S., *Methods in Enzymology,* 1955, v1, 699.

6. Neilands, J. B., *ibid.,* 1955, v1, 449.

7. Kun, E., and Aboad, L. G., *Science,* 1949, v109, 144.

Received April 3, 1956. P.S.E.B.M., 1956, v92.

Comparative Biochemical Studies on Normal and Poliomyelitis Infected Tissue Cultures. IV. Enzyme-changes in Host Cells. (22423)

ERNEST KOVACS.* (Introduced by R. D. Defries.)

Department of Hygiene and Preventive Medicine, School of Hygiene, University of Toronto.

The successful cultivation of the virus of poliomyelitis by Enders *et al.*(1) provides a unique opportunity to approach host-virus interaction in simple systems by excluding inflammatory reactions. Enzymes connected with nucleic acid metabolism were extensively studied in explants of normal Rhesus kidney with synthetic nutrients and in surviving tissues(2-4) to assure a baseline for the present work. Alterations occurring in similar biocatalysts during *in vitro* growth of poliomyelitis virus are here reported. Since enzymology in normal and in infected tissue culture (TC) is a relatively unexplored field(2,3) a brief synopsis of findings on nine different enzyme systems is presented. Details, together with various secondary problems investigated, will be published separately(5).

Methods. Assay material was obtained from the same source† as previously. In preliminary publications preparation of TC, media and homogenates was described, together with the assay methods for enzymes(2,3). Adherence to these technics makes repetition unnecessary and assures continuity with previous communications. The trypsinized Rhesus kidney epithelial cells were routine material from virus titrations, grown in roller tubes with synthetic medium 199(6) or its modified form, 597(2). All virus work was carried out in the poliomyelitis department. Titres were expressed in negative logarithms calculated by Kärber's method(7). Mahoney, MEFI and Saukett virus types were used. The strains were from TC passages and generally of high dilutions ($10^{-6.5}$ to $10^{-7.5}$). Sin-

* Aided by a Public Health Research Grant.

† Through the courtesy of Drs. A. E. Franklin, D. R. E. MacLeod, and H. G. Macmorine of poliomyelitis department of Connaught Medical Research Laboratories.

TABLE I. Turbidity Readings of Infected TC.

Explants,* nutrients, type of virus†			Virus titer	Turbidity, Klett units/ml
Roller tube cultures of trypsinized kidney cortex:				
Normal TC in medium 199, homogenates, pool of 50 tubes			—	900
Same, infected with Mahoney strain			$10^{-6.7}$	670
Idem			10^{-7}	610
Normal TC fluid in medium 597, pool of 20 tubes			—	30
Same, infected with Mahoney strain			$10^{-7.5}$	25
Normal TC extract in medium 597, pool of 20 tubes			—	45
Same, infected with Mahoney strain			$10^{-6.8}$	35
Total (homogenates)	10 exp.	Normal (mean)		575
		Infected "		515
Total (TC fluids)	4 "	Normal "		27
		Infected "		21

* 14 days' TC, except second pair, which were 9 days'.
† 6 days' infection, incubation at 37°C, except of second pair, which was of 1 day infection.

gle roller tube cultures assayed for enzymes individually gave a maximum variation of about ± 10%. Thus pools were prepared from as many tubes as possible, to reduce sampling error. When supernatants of TC fluids were employed they were mixed without shaking and centrifuged at 3000 r.p.m. for 10 minutes. The work with homogenates necessitated grinding of cells in the original tubes as reported(3). Tissue cultures were used fresh, usually after 6 days incubation with virus, occasionally stored at 4°C, or frozen at −25°C. Turbidity measurements of the pools were carried out by Loomey's technic as a check on tissue mass of homogenates and TC fluids. U.V. spectrophotometry was also used for this purpose, carried out on supernatants(2). The methods of Shinowara *et al.*(2,3,9) served for detection of acid and alkaline phosphatases (Na-glycerophosphate substrate), combined with Fiske and Subbarow's technic(10) for inorganic phosphorus (IP). Reis' method(11) for 5-nucleotidase with muscle adenylic acid and similar technics for simple nucleotidases with mixed ribonucleotides as substrate are adopted(2,12, 13). The total acid soluble phosphorus method for RNA-ses(2,3,14) and McCarty's(15) viscosimetric technics for DNA-se, as modified by Siebert *et al.*(16) were used, with highly polymerized nucleic acids as substrates (2-4). For pseudo-cholinesterase determination a spectrophotometric method was used (2,17), with benzoylcholine substrate. All experiments were on different batches and the assays were carried out at least in duplicate. Mean values of fully randomized samples are tabulated.

Results. A great number of infected TC were assayed, always controlled with identically treated, but uninoculated normals. Results are presented in six tables and two figures. Table I illustrates decrease in turbidity measurements of TC homogenates at the height of virus growth. The difference in mean values of random samples from infected and uninfected cultures is considerable. There seems to be linearity between virus titres and decrease in turbidity. Typical examples of supernatant fluid and centrifuged homogenates illustrate the small amount of material present and the difference between normal and infected samples with respect to turbidity. This is further evidenced by a characteristic ultraviolet absorption curve, which demonstrates qualitative and quantitative change in the medium due to the presence of virus(Fig. 1).

Table II presents examples and a summary of results for acid and alkaline phosphatase assays. The general pattern was a sharp decrease in both enzyme activities in infected cells. The difference between randomized normal and infected pools was pronounced in fresh tubes. However, if the cultures are stored for various times, after 6 days infection and incubation with virus, the pattern may

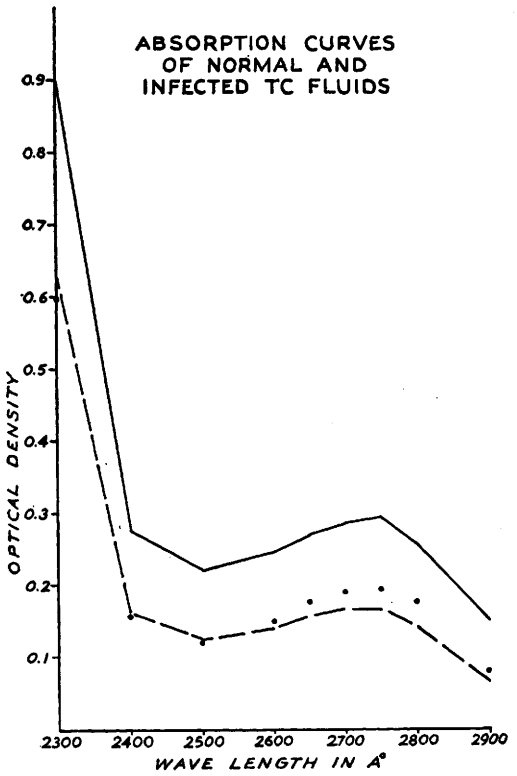

FIG. 1. ——— Normal, 1 ml, in phosphate buffer, pH 7.4. – – – – Same, after 3 days infection with Mahoney strain, titer 7.3, dilution 10^{-5}. ● Fresh medium 597, 1 ml, in same buffer. Reference: phosphate buffer.

be modified(5). By calculation of mean values in this synopsis, composition of nutrients, age of TC, virus strains, titers, and fractions of TC were disregarded.

Tables III and IV illustrate the behaviour of 5-nucleotidase and simple nucleotidases. The former (Table III) is less sensitive to the effect of virus than the latter, or than the phosphatases. They may enter the supernatant mostly during cell disintegration(2,3), persist there much longer and suffer less decrease, or even show an increase in some old cultures with low virus titers(5). Simple or nonspecific nucleotidases (Table IV) are very sensitive to virus and their decrease is connected in part with the decrease of phospha-

TABLE III. 5-Nucleotidases in TC Infected with Poliomyelitis Viruses.

Explants,* nutrients, type of virus†	Virus titer	5-Nucleotidase, as increase in IP (μg/ml), pH 8.5, incubation 2 hr, 37°C
Roller tube culture of trypsinized kidney cortex:		
Normal TC in medium 199, homogenates, pool	—	1.53
Same, infected with Mahoney strain	10^{-7}	.64
Total: 20 exp. (TC fluids and homogenates)	Normal (mean)	.88
	Infected "	.58

* 14 days' TC.
† 6 days' infection and incubation at 37°C.

TABLE IV. Simple-Nucleotidases in TC Infected with Poliomyelitis Viruses.

Explants,* nutrients, type of virus†	Virus titer	Simple-nucleotidase as increase in IP (μg/ml), pH 8.5, incubation 2 hr, 37°C
Roller tube cultures of trypsinized kidney cortex:		
Normal TC in medium 199, supernatant, pool	—	1.92
Same, infected with Mahoney strain	$10^{-6.5}$	.73
Total: 10 exp. (TC fluids and homogenates)	Normal (mean)	.81
	Infected "	.49

* 14 days' TC.
† 6 days' infection and incubation at 37°C.

TABLE II. Phosphatase in TC Infected with Poliomyelitis Viruses.

Explants,* nutrients, type of virus†	Virus titer	Acid and alkaline phosphatase increase in IP (μg/ml), incubation 4 hr at 37°C	
		pH 5	pH 10.9
Roller tube tissue cultures of trypsinized kidney cortex:			
Normal TC in medium 597, supernatant, pool	—	.64	1.73
Same, infected with Mahoney strain	$10^{-6.5}$	.16	.29
Total: 20 exp. (TC fluids and homogenates) Normal (mean)		.46	.87
Infected "		.28	.37

* 14 days' TC. † Example is of 5 days' infection.

TABLE V. Effect of Poliomyelitis Viruses of Ribonucleases of TC.

Explants,* nutrients, type of virus†	Virus titer	RNA-se, as increase in total acid soluble phosphorus (μg/ml) pH 5	pH 7.6
Roller tube culture of trypsinized kidney cortex:		Incubation 4 hr, 37°C	
Normal TC in medium 597, homogenate, pool	—	8.80	0
Same, infected with Mahoney strain	$10^{-7.1}$	4.00	0
		Incubation 2 hr, 37°C	
Normal TC in medium 597, supernatant, pool	—	1.86	2.98
Same, infected with Saukett strain	$10^{-6.9}$	2.26	2.40
Normal, whole TC in medium 597, original tubes	—	2.80	2.06
Same, infected with MEF strain	$10^{-6.8}$	4.00	5.26
Total: 15 exp. (TC fluids and homogenates) Normal (mean)		15.40	5.80
Infected "		6.96	9.80

* 14 days' TC. † 6 days' infection and incubation at 37°C.

tases(2,3).

Two types of ribonucleases, an acid and an alkaline, were examined in the infected TC (Table V). The examples presented show a drop of RNA-se activity in the infected TC at the time of "harvest" although exceptions are also illustrated individually and in mean values as an increase of RNA-se activity. The large individual variation of nucleases in normal(2,3) and infected samples makes evaluation of the findings somewhat complicated. However, much significance should be attached to the increase of RNA-ses in the early phase of the infection, which seems to be connected with the synthesis of the virus(5). Table VI presents findings on two types of DNA-se. Both main groups exhibit a marked decrease in nuclease activity at the height of poliomyelitis infection. Similar behaviour of the pseudo-cholinesterase(2) was observed in preliminary experiments. It showed no activity in infected TC fluid (Fig. 2) as measured by decrease of optical density at 2400 Å. Spectra of TC and substrate alone, in buffer did not show any change during the same period of incubation.

TABLE VI. DNA-ses in TC Infected with Poliomyelitis Viruses.

Explants,* nutrients, type of virus†	Virus titer	DNA-se activity, as % drop of relative viscosity, incubation 2 hr, 37°C pH 5	pH 7
Roller tube culture of trypsinized kidney cortex:			
Normal TC in medium 597	—	7.0	5.4
Same infected with Mahoney strain	$10^{-6.5}$	3.0	0
Total: 15 exp. (TC fluids and homogenates) Normal (mean)		9.0	2.0
Infected "		3.0	1.0

* 14 days' TC.
† 6 days' infection and incubation at 37°C.

Discussion. It was thought that explanta-

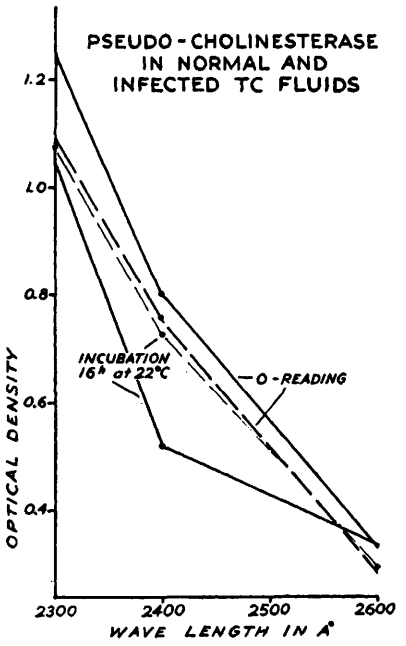

FIG. 2. ——— Normal, 0.25 ml + benzoylcholine (5×10^{-5} mol.) substrate, in phosphate buffer 7.4. — — — Same, infected with Mahoney strain, titer 6.5, dilution 10^{-7}. Reference: phosphate buffer.

tion and cultivation procedures render non-neural tissue susceptible to poliomyelitis infection(2,3). The present findings shed light on the general effect of the virus upon the host cell. At the peak of the growth curve there is a considerable drop in the enzyme activities of fresh TC with complete synthetic nutrients. This change precedes the cytopathogenic effect by at least 24 to 48 hours, depending on virus titers, and can be measured quantitatively. Some enzymes go through a maximum before decrease. Day-to-day experiments on portions of large batches revealed the various phases of these changes(5) due to the presence and later to the multiplication of virus. It remains to be decided if the enzymes are destroyed or only temporarily inhibited. The decrease of their activity may be caused by the virus directly, or by some toxic substances elicited by its presence, because this deleterious effect can be transferred with infected TC fluids in the presence of crude or purified enzyme systems *in vitro*(8). This indicates that a strong inhibitory mechanism must be present, which, like the virus, is carried through the serial dilutions. Various enzymes were sensitive to varying degrees to the action of virus. However, this fact does not protect the cell from the disorganization of its functional balance, especially if essential biocatalysts have been attacked. This results in severe alterations of the metabolism, with progressive accumulation of metabolites and virus, leading to (necrosis) death and disintegration of the cells. The acid RNA-se and 5-nucleotidase seem to be the most resistant, while acid phosphatase, simple-nucleotidases, pseudocholinesterase and alkaline phosphatase are the most sensitive to virus effect. Because of the very large amount of material these biocatalysts will be described individually in detail(5). In this way many important problems connected with the subject can be dealt with. Finally, decrease in turbidity and in optical density of infected TC may be due to disturbed nucleoprotein metabolism and suggests that basic cell constituents may have been destroyed and not replaced during one or another phase of the invasion. It is striking how clearly the absorption curve reflects the modified metabolism and interchange of substances between medium and cells. It remains to be settled whether the point of attack of the virus is primarily at the site of nucleolytic enzymes, or whether damage of oxidative biocatalysts(9,10) represents the first stage in this profound alteration of cell physiology. Further studies are being made to correlate the nuclease pattern observed in the CSF of poliomyelitis patients(13) with the findings presented.

Summary. 1. Marked changes in the activities of 9 enzyme systems were observed at the height of poliomyelitis infection *in vitro*. 2. The decrease of alkaline phosphatase, pentanucleotidase, simple nucleotidase, "acid" RNA-se and DNA-se is so marked, that this may serve as a quantitative measure of cytopathogenic effect of the poliomyelitis virus. 3. The bearing of these findings on cellular physiology and pathology is discussed, as a working hypothesis.

The technical help of Mr. Louis Szendy and Mr. Joseph Kovacs is gratefully acknowledged.

1. Enders, J. F., Weller, T. H., and Robbins, F. G., *Science,* 1949, v109, 85.

2. Kovacs, E., *Can. J. Biochem. Physiol.,* 1956, v34, 273.

3. ———, in press, 1956, v34, May issue.

4. ———, *idem.*

5. ———, to be published.

6. Morgan, J. F., Morton, H. J., and Parker, R. C., PROC. SOC. EXP. BIOL. AND MED., 1950, v73, 1.

7. World Health Organization Technical Report Series, 1954, No. 81.

8. Loomey, J. M., and Walsh, A. I., *J. Biol. Chem.,* 1939, v130, 635.

9. Shinowara, G. Y., Jones, L. M., and Reinhardt, L., *ibid.,* 1942, v142, 921.

10. Fiske, C. H., and Subbarow, Y. J., *ibid.,* 1925, v66, 375.

11. Reis, J., *Enzymologia,* 1938-39, v5, 251.

12. Stern, H., Allfrey, W., Mirsky, A. E., and Saetrens, H., *J. Gen. Physiol.,* 1952, v25, 539.

13. Kovacs, E., *J. Pediatrics,* 1955, v46, 691.

14. Kunitz, M., *J. Gen. Physiol.,* 1940-41, v24, 15.

15. McCarty, M., *ibid.,* 1946, v29, 123.

16. Siebert, G., Lang, K., Lucius-Lang, S., Herkert, L., Stark, G., Rossmüller, G., and Jöckel, H., *Hoppe-Seyler's Z. Physiol. Chem.,* 1953, v295, 229.

17. Kalow, W., *J. Pharmacol. Exp. Therap.*, 1952, v34, 122.
18. Kovacs, E., *Proc. Can. Physiol. Soc. 19th Annual Meeting*, London, Ont., 1955, Oct. 22-23.
19. Howe, H. A., and Flexner, J. B., *J. Biol. Chem.*, 1947, v167, 663.
20. Ackermann, W. W., and Johnson, R. R., *J. Exp. Med.*, 1953, v97, 315.

Received April 9, 1956. P.S.E.B.M., 1956, v92.

Transcapillary Loss, Equilibrium Time, Half-Return Time of Thiocyanate and Heavy Water in the Forearm.* (22424)

EDWARD D. FREIS AND HAROLD W. SCHNAPER.
Cardiovascular Research Laboratory, Georgetown University School of Medicine and Veterans Administration Hospital, Washington, D.C.

We previously described a method for estimating the transcapillary exchange of permeable substances in the human forearm(1). The method later was extended to the pulmonary capillaries of man(2). No attempt was made in these experiments to estimate the length of time the permeable substances remain in the tissues or their rate of return to the circulation. More recently we have described a method for determining the late washout slopes of injected tracer materials in the human forearm(3). The method takes advantage of the fact that the forearm circulation is small in comparison to the general circulation. Thus, brachial arterial injection of tracer materials in dosages sufficient to produce significant concentrations in the effluent ipsilateral veins produces negligible concentrations when diluted in the general circulation. In this way contamination and consequent distortion of the late washout slopes due to recirculation of significant amounts of labelled material can be avoided.

The present report is concerned with the transcapillary loss and later return of the extracellular electrolyte, thiocyanate, and of heavy water. We believe that these studies provide a more complete picture of the circulation of extravascular substances under physiological circumstances than has been available heretofore.

* Supported in part by research grants from the N.H.I., National Institutes of Health, U. S. Public Health Service and the Squibb Institute for Medical Research, New Brunswick, N. J.

Method. Preparation of labelled materials and manner of injection and sampling have been described in other communications(4,1, 3). The method of analysis of thiocyanate and deuterium oxide also has been described previously(1). The subjects were young or early middle-aged males who were on the wards of the Veterans Administration and Georgetown University Hospitals. They all were ambulatory and afebrile at the time of testing and were not suffering from circulatory disease which might interfere with normal transcapillary exchanges in the forearm. Twenty-two tests were carried out using T-1824 and thiocyanate. In 8 subjects D_2O was determined. In 13 tests forearm and hand capillary beds were included. In one of these subjects (M.W., Table I) simultaneous sampling was carried out from cephalic and median veins. In 8 subjects tracer materials were limited to the forearm by inflating a cuff about the wrist to pressures of 100 mm Hg above systolic pressure. In the remaining 2 cases, permeability characteristics of the hand alone were studied, injection being made into a radial artery with subsequent sampling from a vein near the wrist. The site of transcapillary exchange (forearm or hand or both) did not appear to make any significant difference in the results obtained.

Definition of Terms. Methods for determining rate of net return have not been described previously and, therefore, requires some explanation. The method of determining transcapillary percentage loss has been