und bei hohen LET-Werten längerlebige und damit biologisch wirksamere Typen (z. B. H_2O_2) auftreten. Der Abfall der RBW bei höchsten LET-Werten läßt sich durch die zusätzliche Annahme eines Sättigungsvorganges erklären.

Durch ein derartiges Diffusionsmodell lassen sich auch die wesentlichen Züge der hier geschilderten Ergebnisse erklären.

Ob das auffallende Maximum der RBW des LET-Bereiches um 1360 MeV $cm^2\,g^{-1}$ allein von der Ionisierungsdichte abhängt, bleibt noch zu überprüfen. In bisher veröffentlichten Versuchen [13, 32] sowie in den hier geschilderten, wurden LET-Werte etwa dieser Größe durch Po-α-Strahlen erzeugt. Die übrigen LET-Werte >100 MeV $cm^2\,g^{-1}$ wurden dagegen meist, wie in den hier beschriebenen Experimenten, durch Beschleunigungsmaschinen geliefert, die eine pulsierende Strahlung abgeben. Da ^{210}Po-α-Quellen demgegenüber eine kontinuierliche Strahlung aussenden, kann ein möglicher Einfluß der Fraktionierung der Strahlung solange nicht ausgeschlossen werden, bis LET-Werte um 1360 MeV $cm^2\,g^{-1}$ auch durch Strahlung von Beschleunigungsmaschinen geliefert werden. Das gilt um so mehr, als bei UV-Bestrahlungen eine Reduktion der inaktivierenden Wirkung durch Ultrafraktionierung nachgewiesen werden konnte [33]. Daß jedoch ein realer Anstieg der RBW mit steigendem LET-Wert auch bei pulsierender Strahlung stattfindet, zeigen heute bereits die Werte für die im Linearbeschleuniger erzeugten He-Kerne.

Die Experimente mit den dicht ionisierenden Strahlen wurden im Donner Laboratorium der Universität von Kalifornien in Berkeley, U.S.A., durchgeführt. Den Herren Dr. TOBIAS, Dr. MORTIMER und Dr. BRUSTAD bin ich für Gastfreundschaft und Unterstützung der Arbeit zu Dank verpflichtet. Meiner Frau, Hildegard LASKOWSKI, verdanke ich wertvolle technische Assistenz bei der Durchführung der Versuche. Für die finanzielle Unterstützung der Versuche in Berkeley bin ich der Lalor Foundation zu Dank verpflichtet. Die übrigen Versuche wurden in der Abteilung für Biophysik des I. Physikalischen Institutes der Freien Universität Berlin durchgeführt. Herrn Dr. STEIN danke ich für viele anregende Dikussionen und vielfältige Unterstützung bei der Durchführung der Versuche. Der Deutschen Forschungsgemeinschaft sowie dem Bundesministerium für Atomkernenergie und Wasserwirtschaft sei für finanzielle Unterstützung gedankt.

[33] J. G. KEREIAKES, R. H. HODGSON u. A. T. KREBS, Science [Washington] **124**, 222 [1956].

Early Enzyme-Changes in Cultivated Cells as a Preparative Phase for the Biosynthesis of Poliovirus

By E. KOVÁCS, G. WAGNER and V. STÜRTZ

Aus der Abteilung für Serologie und Mikrobiologie, Deutsche Forschungsanstalt für Psychiatrie, Max-Planck-Institut, München

(Z. Naturforschg. **15 b**, 506—517 [1960]; eingegangen am 29. Januar 1960)

Verschiedene Hydrolasen (saure und alkalische Phosphatasen, Glucose-6-Phosphatase, 5-Nucleotidase, RN-asen, ATP-ase) wurden in kürzeren oder längeren Inkubationszeiten mit direkten Methoden an mit Poliovirus infizierten HeLa-Zellen-Kulturen und an normalen Kontrollen untersucht. Die Stimulation einiger mikrosomaler, mitochondrialer und nukleolarer Enzymsysteme ist das erste biochemische Zeichen der präparativen Phase der Poliovirussynthese. Solche Veränderungen treten kurz nach der Adsorption und Penetration der infektiösen Partikel auf und wurden in der vorliegenden Arbeit innerhalb 30 bis 60 Min. nach der ersten Wirt-Virus-Auseinandersetzung erfaßt. Während der gleichen Zeit waren andere Enzymaktivitäten unverändert oder sie wurden gehemmt. Diese unterschiedliche Beschleunigung oder Hemmung führte wahrscheinlich zur Gleichgewichtsstörung des Zellmetabolismus und schließlich zur Vernichtung des Wirtes. Primäre und sekundäre Erscheinungen konnten oft klar unterschieden werden, beide sind aber *irreversibel*, trotz der scheinbaren Ähnlichkeit mit den normalen Kontrollen, in der Zwischenzeit. Die erste Stimulation ist durch die Infektion und Virussynthese verursacht. Die zweite unkontrollierbare Aktivierungsperiode ist durch morphologische Zellalterationen bedingt; sie ist eine terminale Erscheinung, und zwar der Ausdruck der funktionellen und anatomischen Desintegration der Zelle. Verschiedene Aspekte und die Perspektiven solcher Untersuchungen wurden besprochen.

A direct method for the assay of various hydrolytic enzymes in living cell cultures was described previously [1]. The chemically defined simple substrate-media used for those experiments were found to support *survival* and *virus production* of the host for a reasonably long period of time [2] to allow comparative biochemical investigations. Precedent stu-

[1] E. KOVÁCS, V. STÜRTZ and G. WAGNER, Z. Naturforschg. **15 b**, 116 [1960].

[2] E. KOVÁCS and V. STÜRTZ, Z. Naturforschg. **15 b**, 238 [1960].

dies of one of us, carried out mainly during the end-stage of the poliomyelitis infection revealed drastic changes in the activity of some biocatalysts [3, 4, 5]. It was noted however, that the kinetics of various enzymsystems may go through a maximum at an earlier [6] or later phase of the pathological process [4–6]. The importance of biocatalytic activities for the building-up or maintenance of cell morphology and function, or the biosynthesis of the virus is obvious [7]. Thus it was a logical step to study with the help of improved techniques the earliest biochemical events in the synthesis of poliovirus [8, 9]. The fate of intracellular hydrolases will be described in the present report mainly during the first cycle of virus reproduction and later, eventually secondary alterations will be compared with early changes. Various aspects of the problem will be discussed on basis of experimental findings.

Materials and methods

In general the assays were carried out on HeLa cells [1, 2], although the behaviour of other tissue culture (TC) lines will be illustrated by examples. The techniques of cell cultivation and the preparation of substrate-media for the direct assay of various enzymes were the same, as those reported [1, 2]. The strict adherence to standard methods makes repetition unnecessary, therefore only the variations will be described here and for other pertinent details the reader is referred to precedent papers [1, 2]. The TCs were always of 48 hours subcultivation.

Inoculation: 60 minutes adsorption-time of 1 ml undiluted seed (Typ I poliovirus, Mahoney strain) was allowed for the "on step" growth [2]. The most frequently choosen period was the 20-minutes one to infect TCs for biochemical assays, but shorter and longer contact with the same amount of virus, or with smaller inocula were also assayed. For the homogeneous distribution of 0.1 to 0.2 ml virusinfected, centrifuged TC-fluid of a mean titre of 8.4 negative logarithms ID_{50}/ml, mechanical stirring of the TCs was made with help of a shaking device, moving with standardized low frequence [10]. Later, stirring was the routine procedure for all inoculations.

Washing of the cells to free them from unadsorbed or not firmly bound virus [10] was carried out 4 × with modified Hanks' solution [1, 2] or tris-buffered sucrose [1, 2], a last (5th) change being made with buffer-medium Iso II plus IP-free Hanks' solution (1 : 1), or complete medium for assays with the latter nutrient [1, 2].

In many experiments the inoculum was only decanted, without washing; in some other assays the seed was not removed, but the first test made immediately, or just after 2 to 5 minutes adsorption, to detect the earliest changes under those experimental condition. *Titration* of virus concentration was carried out on tube-cultures [2].

Enzyme-assays were made with the help of substrate-media for the systems and buffer-media for the cell-controls (called buffer-control also) [1]; both were free of inorganic phosphates (IP), but the former contained a substrate for hydrolases and the IP set free enzymatically was determined by the method of Fiske and Subbarow [11, 1]. The controls served for the detection of spontaneous IP-changes in the TCs or substrate-medium. The *difference* between the IP values of the system and the TC-, respectively the substrate-controls was the measure of the relative activity of phosphatases [12, 1, 3], 5-nucleotidase [13, 1], glucose-6-phosphatase (G-6-P-ase) [14, 1], ATP-ase [15, 16, 1] and RN-ase [17, 1], King's total acid soluble phosphorus (TASP) determination [18, 1] serving for the latter. The phosphorus values were expressed as mcg/ml of the system and were plotted against time of incubation at 37 °C. The design of the experiments was similar to the preliminary assays [1, 2], but the volume of TC-fluid was diminished occasionally from 20 to 10, respectively 6 or 5 ml. The *indirect* assay for the detection of enzymes diffused into the supernatant medium was carried out as previously [1].

Protein was demonstrated by the biuret method [19, 1], in the nutrient fluid or in urea-desoxycholate lysate of the TC [20]. The UDC reagent was modified by rising the desoxycholate concentration to 10%. The cultures were frozen, for convenience, with or without the medium, generally the bottles laying on the cell-surface of the glass. This procedure allowed to bring the reagent directly on the TC and mix with the thawing fluid simply by turning the bottle or adding the previously decanted nutrient fluid. The original medium was used as diluent for total lysates or bidistilled water, when cells were

[3] E. Kovács, Proc. Soc. exp. Biol. Med. **92**, 183 [1956].
[4] E. Kovács, J. exp. Medicine **104**, 589 [1956].
[5] E. Kovács, Biochem. Z. **330**, 112 [1958].
[6] E. Kovács, Minerva Medica [Torino] **48**, 2157 [1957].
[7] S. S. Cohen, Biochemical Organization of the Cell and Virus, in: Burnet and Stanley, The Viruses, Academic Press, Inc., Pbls., New York 1959, vol. I, p. 15.
[8] E. Kovács and D. Wulf, Proc. IV. int. Conf. Biochemistry, Vienna 1958, Section 6, No. 6–13, p. 71.
[9] E. Kovács, Proc. 2nd int. Congr. Infect. Pathol., Milano, May 1959.
[10] L. C. McLaren, J. J. Holland and J. T. Syverton, J. exp. Medicine **109**, 475 [1959].
[11] C. H. Fiske and Y. J. Subbarow, J. biol. Chemistry **66**, 175 [1925].
[12] G. J. Shinowara, L. M. Jones and H. L. Reinhardt, J. biol. Chemistry **142**, 921 [1942].
[13] J. L. Reis, Enzymologia [Amsterdam] **5**, 251 [1938–1939].
[14] M. A. Swansson, J. biol. Chemistry **184**, 647 [1950].
[15] W. W. Kielley and R. K. Kielley, J. biol. Chemistry **200**, 213 [1953].
[16] S. N. Colowick and N. O. Kaplan, Methods in Enzymol., Academic Press Inc., Pbls., New York 1955, II. vol., p. 593.
[17] M. Kunitz, J. gen. Physiol. **24**, 15 [1940].
[18] J. E. King, Biochem. J. **26**, 292 [1932].
[19] T. E. Weichselbaum, Amer. J. clin. Pathol., Techn. Sect. **10**, 40 [1946] as recommended by Beisenherz et al., Z. Naturforschg. **8 b**, 576 [1953].
[20] E. Kovács, Arch. Biochem. Biophysics **76**, 546 [1958].

separately processed, added to 10% final urea concentration. The diluted lysates were stored at 4 °C for days, without deterioration [21].

Specific activity (s.a.) of the hydrolases was calculated as mcg of IP liberated per mcg of Protein of the TC, during 1 hour incubation at 37 °C. Protein determination was carried out at the end of the assays and the result was divided by the time, in hours with the assumption that no cell multiplication occured with substrate-medium [1, 2]. For instance s.a./24 means that the calculation was made on 24-Hrs basis.

Finally the most *important innovation* introduced with these assays was the identical treatment of the controls. The parallely assayed uninfected cells (always of the same batch as the infected ones) received a token-inoculum, which consisted of a centrifuged normal TC-fluid, prepared simultaneously with the virus-inoculum and administered similary to the uninfected group. Then they were washed and handled *identically* with the infected TCs, assuring comparability of the two groups.

Results

IP-changes in Cell Cultures Growing with Complete Medium

Fig. 1 illustrates the behaviour of the supernatant of HeLa cell cultures during infection with poliovirus. The typical "one step" growth-curve of the virus is illustrated also. The difference between the two parallel systems was the *absence* of inoculation and washings in the normal TCs. This fact explains the discrepancies observed between infected and control ones in the first 6 hours. However the IP values run parallel at 6 and 9 Hrs. post-infection (p.i.) when virus release attained logarithmic proportions [22, 23]. The IP concentration exhibits small fluctuation in the infected TCs, but *considerable variation* in the normals. There was only a slight deviation at 24 Hrs., but a considerable one at 48 Hrs. p.i., the normal being of significantly *lower* order. The virus of the extracellular fluid reached a peak at 12 and an almost steady level till 72 Hrs.

Fig. 2 illustrates a similar experiment, but the systems were *identically* handled through-out. As one would expect, there was no difference at Zero-time between the two parallel groups, but a significant deviation was found at 3 Hrs. p.i., the IP in *infected* TC-fluid remaining of lower order, till about the beginning of visible cytopathic effect (CPE). The IP values were significantly higher in the infected TCs at 48 and 72 Hrs. p.i. The latter findings are in agreement with those presented in Fig. 1, although the growth-curve of the virus exhibited some, possibly (in view of the titration techniques) unsignificant differences. The results emphasize the necessity of *uniform* and rapid handling for the registration of early changes. The fate of orthophosphate was followed in a *too complex* system. This set-up contained large amounts of IP,

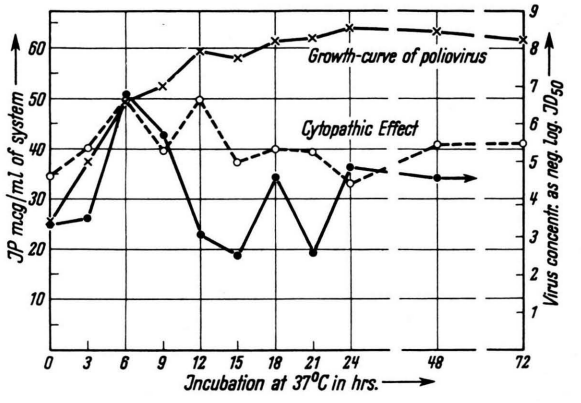

Fig. 1. Changes in IP concentration of the TC medium during virus multiplication. Effect of *not-uniform* handling. o— —o: IP mcg/ml in 48-hours subcultures of HeLa cells [1] infected with 1 ml undiluted inoculum (Type I poliovirus, Mahoney strain) about 8.4 neg. logarithms ID_{50}/ml; 60 minutes adsorption, followed by 3 washings with cold b. amniotic fluid (2 ×) and complete medium [1] (1 ×). Phosphorus determination in pooled portions of 4 parallel assays, by FISKE and SUBBAROW's techniques [11, 1]. X——X: "One step" growth-curve of poliovirus, as neg. log. ID_{50}/ml titrated on tube-cultures [1]. o——o: IP mcg/ml in normal TC-fluids of the same batch as above, without inoculation and washings, otherwise similarly processed as above.

as an ingredient of the balanced salt solution [1] and organically bound phosphorus in nucleotides, lipids, phosphoproteins of the yeast extract, lactalbumin and blood serum present in the nutrient fluid. Although the virus growth illustrated in the curves is typical, the interpretation of the IP changes is difficult, in view of the complicated set-up; the IP level of the supernatant being dependent from the *original* concentration (about 16.6 mcg/ml before addition to the cells) and from the amount of IP liberated from organic compounds of the medium, respectively of the cells. The IP concentration could be influenced by the rate of phosphorylation and dephosphorylation [24, 1], which processes may differ

[21] E. KOVÁCS, Experientia [Basel] **15**, 425 [1959].
[22] J. L. MELNICK, Ann. New York Acad. Sci., **61**, 754 [1953].
[23] A. F. MAASSAB, P. C. LOH and W. W. ACKERMANN, J. exp. Medicine **106**, 641 [1957].
[24] F. LYNEN, in: Sympos. on Enzyme Chemistry, Tokyo 1957, Pergamon Press, London 1958, p. 25.

in infected or uninfected cells [25, 2]. Finally with the advent of cytopathic effect (CPE) the increase in IP over the values of normal controls may be the sign of functional damage or/and morphological desintegration.

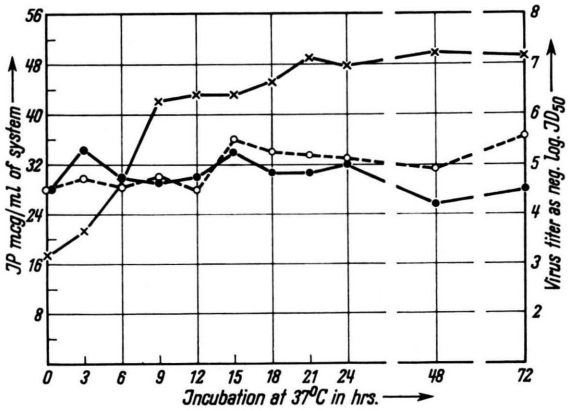

Fig. 2. IP-changes in TC-fluid and "one Step" growth-curve of Type I Poliovirus (Mahoney strain). Effect of uniform treatment. 48-hours subcultures of HeLa cells: Growth in complete Medium (CM) (2% Yeastolate, 0,5% lactalbumin, 10% Calfserum, 1% bovine embryo-extract in Hanks' solution) after 24 hrs. replaced by bovine amniotic fluid [1, 2]. o——o: IP changes in the supernatant of 4 infected flask cultures; portions pooled at 3-hr-intervals; IP determined as before[1, 10]. *Inoculation:* 1 ml undiluted virus-inoculum (5 recent passages on HeLa cells; titer $10^{-8.7}$ neg. log. ID_{50}/ml) layered directly over the cells and let adsorbed for 60′ at 37 °C; rest removed and washed with 2 × 10 ml amniotic fluid, (about 22 °C), 2 × with 10 ml Tris-buffered sucrose, 1 × assay-medium. *Cytopathic Effect:* At 21 hrs. 3 to 4+, controls intact; even after 72 hrs. control cells take *no* Trypan blue. The pooled portions stood accidentally 38 days at 4 °C before titration [2]. Virus concentration, as neg. log. ID_{50}/ml. *Reincubation:* w. 20 ml CM, pH 7.65, paralleled by 4 uninfected controls. X——X: "one step" growth-curve of poliovirus, titrated in pools of 4 portions at 3-Hr-intervals. ·——·: IP in TC-fluids of normal controls (of the same batch) treated with 1 ml *token-inoculum*, 5 washings and processed as above.

We might conclude, that in agreement with findings on normal TCs [1, 2] and other preliminary experiments [8, 9] the use of complicated systems for enzyme-assays in living cells has to be replaced by simple, chemically defined substrate-media, which do not contain IP and extrinsic enzymes, proteins, serum, or other source of phosphorus with exception of the *substrate* for the enzyme-system to be investigated.

Long-term assays of alk. phosphatase with substrate-media

Knowing the general behaviour of phosphate-ions, the accumulation of IP in simple system may be due to the hydrolysis of the substrate by extra- or intracellular enzymes of the TC [1], as will be substantiated by typical examples. Fig. 3 illustrated the relative

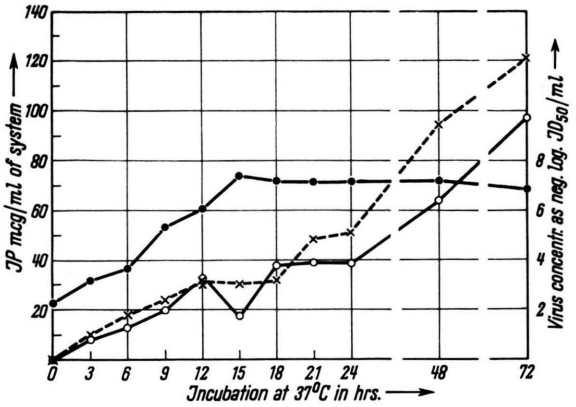

Fig. 3. Relative acitivity of alk. phosphatase and virus growth in chemically defined substrate-medium [1]. X——X: Relative activity in infected TCs, same batch as in Fig. 2, assayed with 20 ml Iso II and modified Hanks' solution (1 : 1) containing 5 g Na-b-glycerophosphate [1]; pH 7.65; IP determinations on pools of 4 parallels with substrate- and 2 parallels with buffermedium, processed as before. ·——·: "One step" growth-curve in pool of supernatants of above, inoculated and processed as in Fig. 2. o——o: Relative activity of alk. phosphatase of normal (uninfected) controls, as increase in IP mcg/ml of system, relative to buffer-controls, processed as above [1]. *CPE:* At 21 hrs. to 4 plus; controls relatively intact, even after 72 hrs. only a few trypan-positive cells [1, 2].

activity assayed with substrate-medium Iso II plus salts [1, 2], on HeLa cells of the same batch as those seen in Fig. 2. The growth-curve of the virus was typical [2] and the alk. phosphatase activity of the infected TCs, as revealed by IP determinations, was significantly *higher* during the intracellular accumulation of the virus, than the values of uninfected controls. The new virus was demonstrated in the medium between at 3 and 6 Hrs. p.i. and at 9 Hrs. there was already a logarithmical increase detected. At 12 Hrs., when the CPE was not yet manifest, the phosphatase activity of infected and normal TCs were about the same. The logarithmic increase of virus in the medium continued, reaching a peak at 15 Hrs. and remaining stationary till 72 Hrs. The IP values remained almost constant between 12 and 18 Hrs. in the infected, but fluctuated in the uninfected TCs, the difference of the over 100 mcg IP/system being highly significant. This is a *critical*

[25] J. Becker, N. Grossowiecz and H. Bernkopf, Proc. Soc. exp. Biol. Med. **97**, 77 [1958].

period in this experiment, when visible cytopathic desorganization of the virus-infected cultures starts and the *steady* levels of infectivity were paralleled by linearly increasing enzyme activities. The latter have been *higher* in the infected, than in the normal TCs, especially at 48 and 72 Hrs. The protein concentrations were exhibiting an increase during this phase in the supernatant fluid of infected cultures, substantiating the conclusion, that the explosive increase after 24 Hrs. p.i. may be due to progressive desintegration of the TCs and the primary events have to be looked for in the *moderate*, but significant *early* difference in activity, in favor of the infected system. Parallel assays with two, more, respectively less incomplete substrate-media (Tris-buffered isotonic Sucrose or Iso II without salts)[1, 2], which do not allow comparable cell survival and virus production[1, 2], somewhat different enzyme activities were observed, with a similar "terminal" rise in IP levels (not illustrated). These findings emphasize again that media of too low physiological value *cannot* be used for experiments lasting over 24 Hrs.

Enzyme-assays during the early phase of infection

Fig. 4 illustrates findings in short-term experiment. Alkaline phosphatase was assayed in cells infected by multiplicity. The inoculum was discarded without washing. Thus the time of reincubation for enzyme-test differs about 20 minutes only from the time of the *first contact* with large amount of virus. There was a definit increase in virus titres till about 24 Hrs. This fact and the 3 to 4 plus CPE speak for a successful infection. There was a great *stimulation* of the alk. phosphatase activity in the virus infected TCs, over the values exhibited by the uninfected controls during the first 6 Hrs., which period coincides with the intense intracellular accumulation of the virus[22, 23]. The phosphatase activity of normal TCs increased, that of the infected ones decreased at 8 Hrs, when the extracellular virus concentration rised. The increasing virus levels were paralleled by sharp increase in the protein content of the supernatant medium and a drastic drop of the enzyme activity, which features, with the exception of a small drop in the phosphatase values *were not* paralleled by the normal controls. Evidently morphological damage of the infected cells are responsible for the latter, possibly *secondary* changes, enhanced by accumulation of toxic products[26]. There was however a slight fluctuation in the activity of the controls at this time, caused maybe by metabolic and conditional factors, because the medium was not

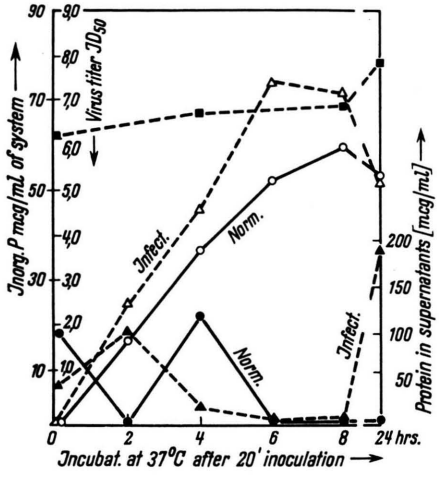

Fig. 4. Early changes in the activity of alk. phosphatase of 48-hours HeLa cell subcultures during infection with poliovirus. A— —A: HeLa subcultures, infected; 20 minutes adsorption with 1 ml inoculum (Mahoney strain, as before) no washing after decantation of the infected fluid. Assay-medium 10 ml Iso II plus Hanks' solution ($-PO_4$) containing glycerophosphate 5 g/*l*, as substrate[1]; 20 vol.-% assayed in 2 parallel systems and 2 buffer-controls at each time-intervals, substituted with fresh substrate-medium; pH was7.6; rsults as IP mcg/ml of system, after deduction of the IP values of the buffercontrols (TC plus buffer-medium)[1]. ■— —■ Virus concentration of pooled portions, as neg. log. ID_{50}/ml titrated in tube-cultures[2] of HeLa cells. o——o: Relative activity of alk. phosphatase in uninfected controls of above, treated with token-inoculum, and processed as above. Specific enzyme activity: Normal 0,0037 μg IP/μg Protein/hr. Infected: 0.042 μg IPμg Protein/hr. ▲— —▲: Protein concentration as mcg/ml in centrifuged, pooled portions of infected TC-fluids; biuret techniques[18, 1]. ●——●: Same in normal controls.

refreshed between the last 2 tests. The phosphatase activity *related* to the protein concentration of the TCs (s. a.) was almost 10 times higher in the infected, than in the controls, which fact underlines the significance of the results of these experiments. Thus we might conclude that an *early stimulation* can be observed with alkaline phosphatase, an enzyme mainly of cytoplasmic localization[27, 27a], during a very early stage, namely in the eclipse-, and intracellular growth-phase of poliovirus[22, 23].

[26] E. Kovács, Naturwissenschaften **45**, 91 [1958].

[27] G. M. Hageboom and C. M. Schneider, in: Chargaff and Davidson, The nucleic Acids, Acad. Press, Inc., Pbls., New York 1955, vol. II, p. 199.

[27a] M. Wachstein and E. J. Meisel, J. Histo-Cytochem. **2**, 137 [1954].

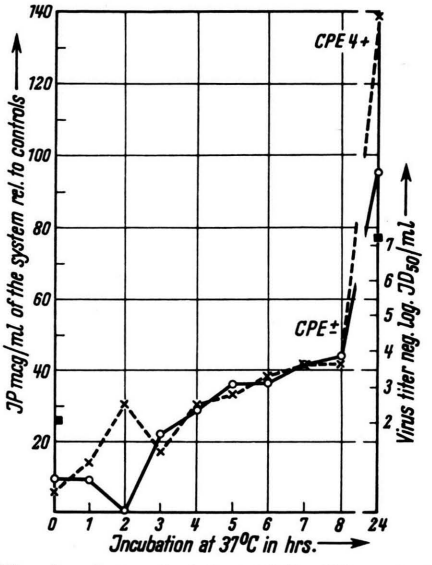

Fig. 5. Alk. phosphatase in infected TCs. Effect of short adsorption-time. X——X: Infected, 1 ml inoculum (Mahoney strain, as usual) 5 minutes adsorption, 5 washings; pooled portions of 2 systems and 2 buffer-controls assayed with 6 ml Iso II+modif. H a n k s' $-PO_4$; removed 20 vol.-%, pooled and replaced with fresh one [1, 2]; pH 7.6; processed as before [1]. ■: Virus titer in infected TC-fluids, as neg. log. ID_{50}/ml. o——o: uninfected controls, treated with shine-inoculation and washings, processed as above.

Other experiments were carried out with different designs to explore various aspects of the question. Fig. 5 illustrates typical findings with 4 times *shortened* adsorption-time of similar inoculum as before and the volume of the assay-medium was reduced also. Five washings assured a very *low* virus concentration till the appearance of the new product. The significantly lower IP value in the infected at Zero-time is noteworthy followed by significant *rise* at the *first hour*, reaching a peak at the second hour p.i. The fluctuation of the activity of normal TCs was observed during the same interval. Both systems exhibited almost equal levels in the next period, till 8 Hrs. p.i., but there were significantly higher IP values in the *infected* ones at the end of the assays. CPE was at the limit of significance at 8 Hrs., but 4 plus at 24 hours; thus the steep rise and the great difference from the controls during the endstage may be of secondary nature. The high *titres* indicate that the growth-cycle of the virus went to completion, thus the stimulation of alk. phosphatase observed during the eclipse-phase may be a *characteristic* feature of the infection. This event can be preceeded by an immediate transitory decrease in IP concentration due perhaps to the toxic effect of the inoculum [26]. The terminal hyperactivity may be a sign of *morphological* desorganization, in agreement with precedent findings.

Table I demonstrates with illustrative examples that the above behaviour is not exclusive characteristics of the HeLa cells. Furthermore the large variation in the specific activity of alk. phosphatase of various cell-lines is also noteworthy. Nevertheless out of 22 experiments, including about 100 individual phosphatase assays on various types of TCs[1, 2], only *two* were not conform to the general trend of early stimulation.

Assay of acid phosphatase by direct and indirect methods

This lysosomal [27, 28] resp. nucleolar [29] enzyme exhibited lower relative and specific activities in infected than in uninfected TCs assayed with the most physiological substrate-medium (Iso II + salts $-PO_4$) [1, 2] as shown in Fig. 6. The cells were not washed after inoculation, thus the evaluation of virus production and the findings is difficult [1, 2]. On the other hand the opposite results were obtained, when assay-media lacking salts and aminoacids were used [1, 2], as shown in Fig. 7. The protein concentration in the supernatants did not show great differences, notwithstanding the *indirect assays* revealed

cell-lines and specific activity *					
HeLa cells **		Human amnion cells (FL) ***		Rhesus kidney cells **** (subcultures)	
infected	uninfected	infected	uninfected	infected	uninfected
$4.6 \cdot 10^{-3}$	$1.7 \cdot 10^{-3}$	5.9!	14.2!	$5.4 \cdot 10^{-3}$	$1.3 \cdot 19^{-4}$

Table I. Alk. phosphatase in various cultivated cells. * Spa. a.=mcg inorg. phosphorus liberated/mcg of total TC protein/1 hour of incubation at 37 °C. ** in substrate-medium Iso II [1, 2]. *** in substrate-medium Iso II. **** R a p p a p o r t's modified synthetic maintenance medium [1].

[28] C. de'Duve, B. C. Pressman, R. Gianetto, R. Wattiaux and F. Appelmans, Biochem. J. **60**, 604 [1955].

[29] A. L. Dounce, in: Chargaff and Davidson, The Nucleic Acids, Acad. Press Inc., Pbls., New York 1955, vol. II, p. 93.

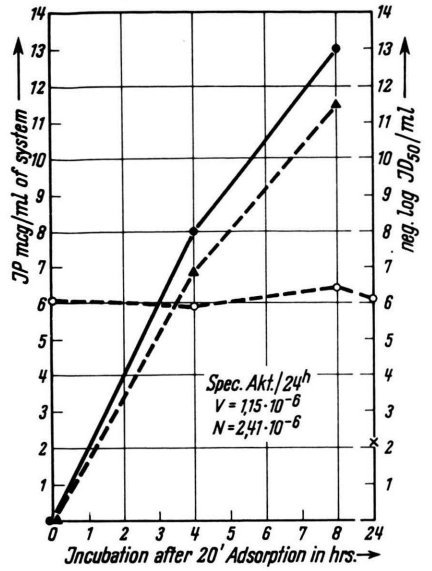

Fig. 6. Acid phosphatase during early phase of poliovirus infection. A— —A: Infected HeLa cells, 1 ml inoculum, (Mahoney, as before) 20 minutes adsorption, no washing; assay-medium Iso II plus H a n k s' solution, containing glycerophosphate 5 g/*l*; p_H 6.45; 10 ml TC-fluid, 20 vol.-% assayed at stated intervals and replaced with same volume of fresh medium. IP determined on pools of 4 parallels and the difference relative to buffer-controls and substrate-blanks is plotted, as mcg/ml of system. o— —o: Virus titer as neg. log. ID_{50}/ml as usual. A— —A: Uninfected controls, treated with token-inoculum, etc. as above. Specific activity as IPP mcg liberated/ Protein mcg, calculated for 1 hour incubation at 37 °C.

an abrupt rise in *diffused* enzymes in the supernatant of the infected cultures; a presumptive evidence of the infection. Glucose-6-phosphatase assayed in the same supernatant showed a slight rise in the infected and no change in the controls. Thus it seems that only the *lysosomal* enzyme is liberated at this time, due possibly to "unmasking";[28] by *early CPE*.

Although the condition of the experiments differ considerably, percluding direct comparability, in 5 out of 9 experiments on various cell-lines and assay-media, *lower* values were found in the infected groups, than in the uninoculated controls, although in agreement with older data [4, 5] the possible chance-occurrence of the above findings need a special study, with new techniques which allow good virus yields and enzyme-tests at acidic p_H, thus under *optimum* condition [30].

Glucose-6-phosphatase activity in infected tissue cultures

This enzyme is mainly of *microsomal* localization [27], thus its behaviour may shed some light on the function of those organelles. During preliminary work, no difference was observed in the virus yield and G-6-P-ase activity of HeLa cells, following 5 minutes adsorption of *large* (1 ml) and *smaller* (0.2 ml) volume of undiluted inoculum, during the first 8 hours of infection.

Fig. 8 presents activity curves of G-6-P-ase after *short* adsorption of a *small* inoculum; thus only about 40 minutes elapsed from the addition of the virus till the first test. After 1 hour p.i. there was some difference in favor of the infected one, which reached a peak at 3 hours, about 100% increase over the decreasing activity of normal controls. The sudden rise at 8 hours, preceeded by a drop, may be the final hyperactivity, in view of the *high* virus concentration of the supernatant at this time. CPE was 3 to 4-plus at

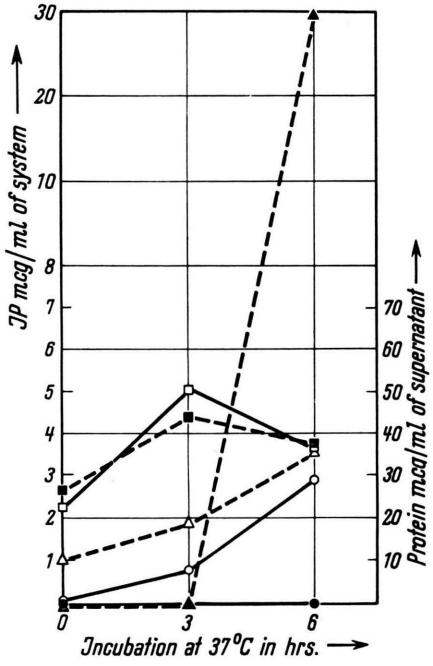

Fig. 7. Acid phosphatase in virus-infected tissue cultures assayed by direct and indirect methods. A— —A: Direct assay of infected TC; 20 minutes adsorption of 1 ml inoculum (Mahoney as usual) no washing; 20 ml substrate-medium Iso II [2], p_H 6.5; 20 vol.-% assayed and replaced; relative activity as increase of IP mcg/ml of system over the value of TC-(buffer) controls. o— —o: same in uninfected normal controls, treated with token-inoculum and as above. ▲— —▲: 0.1 ml of infected supernatant of buffer-control reincubated with 9 ml substrate-medium in testtubes, for 30 minutes at 37 °C. Reaction stopped by tricloroacetic acid, IP determination, as before [1]. ●— —●: Similar indirect assay in supernatant of normal buffer-control [1]. ■— —■: Protein concentration in infected TC-fluid, by biuret-method [18, 1], as mcg/ml. □— —□: Same in uninfected controls.

[30] Unpublished, or to be published.

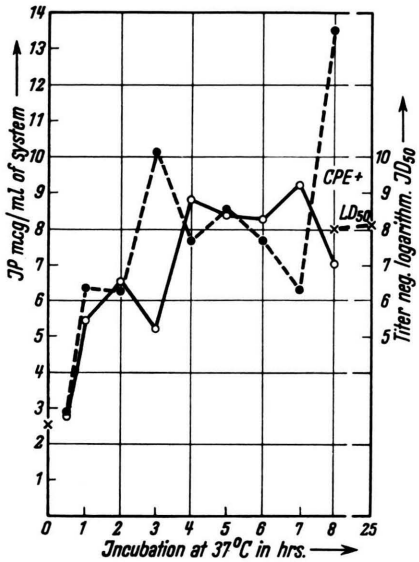

Fig. 8. Glucose-6-phosphatase during early phase of poliomyelitis infection *in vitro*. ·——·: Infected, 3 parallels with substrate-, 3 with buffer-medium, one ml inoculum (Mahoney strain) as usual; *10 minutes* adsorption, 5 washings; 5 ml Iso II plus H a n k s' solution (no PO_4) containing 0.0025 M glucose-6-phosphate [13, 1]; 10 vol.-% assayed on pools and replaced at stated intervals; rel. activity as Pmcg/ml relative the controls. o——o: Same in uninfected HeLa cells with token-inoculation, treated as above. X——X: Virus concentration in infected TC-fluid as neg. log. ID_{50}/ml. Pools assayed on tube-cultures.

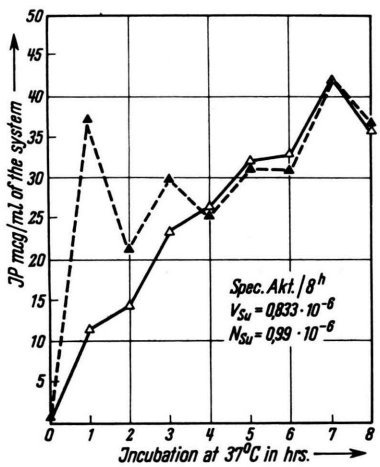

Fig. 9. Glucose-6-phosphatase activity in TCs infected with different type of poliovirus. Effect of substrate concentration. A——A: Infected with 0.2 ml inoculum (Type III, Leon strain) 10 minutes adsorption, 5 washings; assay-medium 5 ml, Iso II containing 0.005 M glucose-6-phosphate; p_H was 6.5; pool of 3 parallel systems and 2 parallel buffer-controls were assayed, 10 vol.-% was removed and replaced at stated intervals. A——A: uninfected normal controls, treated with token-inoculation and processed as above. Specific activity, as in precedent Figures.

24 hours, which fact confirms the *typical* course of the infection. Essentially similar results were obtained in 3 additional assays under the same experimental conditions, exhibiting intense virus-production. However specific activities calculated at the end displayed some variation.

Assays with differend virus type

The assay with small inocula of Type III poliovirus (Leon strain) revealed a strong stimulation at the *first hour p.i.* lasting 4 hours, when the values of uninfected controls attained the same level and run parallel till the end of the experiment (Fig. 9). The activity increased linearly with substrate concentration, the specific activities however did not differ significantly at 8 hours, the total proteins showing about 10% difference in favor of the infected one. Virus production was moderate.

Repetition of the assays revealed somewhat higher activity in the infected throughout, although the relative values were sometimes of dubious significance (not illustrated). About 10% higher protein content of the controls and the higher specific activity of the *infected* ($1.31 \cdot 10^{-7}$ mcg) than the uninfected TCs ($1.13 \cdot 10^{-7}$) underlines the rightness of the observations and means that the eventual discrepancies may depend on biological factors, such as the relatively lower titres of the seed (Leon strain).

We might summarize that out of 7 G-6-P-ase experiments (with two different virus Types and media) only *one* did not show early stimulation of this hydrolase during the eclipse-phase of poliovirus infection *in vitro*.

Assays on 5-nucleotidase

This specific nucleotidase, supposedly a nuclear enzyme [29, 27a] did not show essential differences from normal, during early stage of the infection; however in salt-free medium the activity of the enzyme *decreased* significantly at the hight of the primary pathological process (Fig. 10). Moderate virus increase was observed in the medium and the specific activities of the normal were over those of the infected TCs. In presence of salts [1, 2] the virus production attained exceptional levels, *without* significant differences in relative and specific activities of 5-nucleotidase, both in the infected and control groups. Total proteins were the same and there was no drop in enzyme activites at 24 hours, which fact may emphasize the better physiological value of the

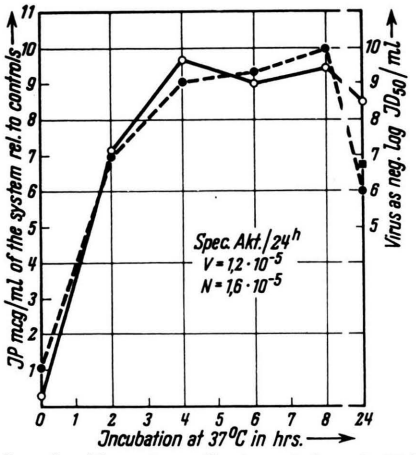

Fig. 10. 5-nucleotidase in poliovirus infected HeLa cells. Effect of incomplete medium. ·——·: Infected with 1 ml inoculum (Mahoney strain as before) 20 minutes adsorption, no washings; 10 ml Iso II medium (no salts) containing 0.01% Muscle Adenylic Acid; 20 vol.-% replacements; pool of 2 parallels assayed [12, 1], p_H 7.75. ■: Virus titer, as neg. log. ID_{50}/ml. o——o: Normal control with token-inoculum, procedures as above [12, 1]. Specific activity calculated as before.

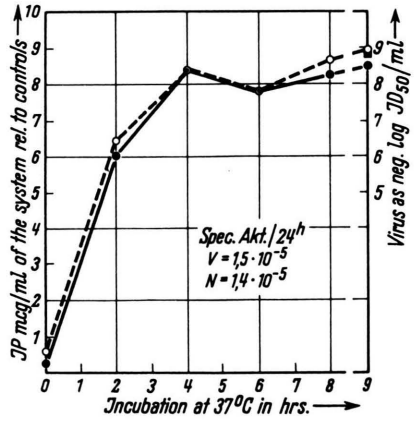

Fig. 11. 5-nucleotidase in infected HeLa cells. Effect of salts. o——o: Same batch as Fig. 10 and infected as before, substrate-medium however contained Hanks' solution (no PO_4) procedure as above [12, 1]. ·——·: Same, uninfected; with token-inoculum, etc. ■ Virus titer as neg. log. ID_{50}/ml. S. a., before.

assay-medium used (Fig. 12). Other experiments, although limited in number, were in agreement with the findings presented above and may suggest that the *normal state* of the organelle or of the cytological site, where this biocatalyst is located, *persists* intactly for a relatively long time during poliovirus infection of the cell.

Acid and alkaline ribonuclease assays

Fig. 12 illustrates RN-ase assays in two different p_H ranges, namely in acid and in alkaline "milieu".

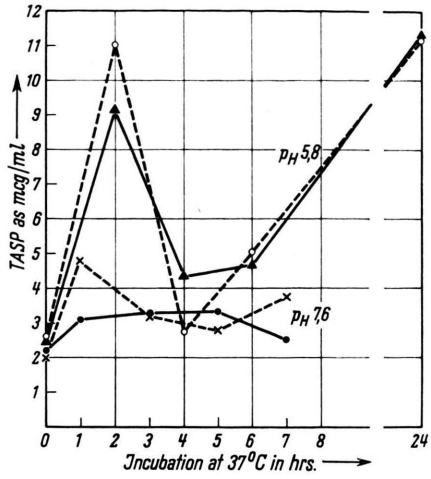

Fig. 12. RN-ases in TCs infected with poliovirus. Effect of p_H. o——o: Acid ribonuclease in HeLa cells infected with 1 ml inoculum (Mahoney, as before) 20 minutes adsorption, no washing; assay-medium Iso I containing 0.02% purified yeast nucleic acid [16, 1]. Twenty ml substrate, respectively buffer-medium added to 2 TCs and same volume of buffer-medium to cell-controls; 20 vol.-% removed (and replaced) at stated intervals, for determination of TASP [16, 17, 1]; p_H was 5.8; mean results of 2 parallel assays are plotted, as TASP mcg/ml of system, after deduction of the value of buffer-, respectively RNA-controls. A——A: Uninoculated normal TCs treated with shine-inucolum and processed as above. X——X: Alkaline ribonuclease in similarly treated infected TCs of the same batch, assayed at p_H 7.6; procedure as above [1]. ●——●: Normal control as to alk. RN-ase assays, processed as above.

The stimulation of both types of diesterase was observed, one, respectively two hours p. infectionem. In an other experiment the same trend, namely *higher* activity was found in infected TCs, than in normal controls *30 minutes* p. infectionem. In further instances however, there was no difference observed between the two groups (not illustrated). Because incomplete nutrients (Iso I without salts) and various cell-lines were used, furthermore no "one step" growth-curve of poliovirus with the substrate-media containing ribonucleic acid is yet available [30]. The findings, although very suggestive, must be considered of preliminary character. The majority of the experiments followed however the course illustrated above and further work is carried out to assure the reproducibility of the results [30].

Similary the draw-backs of the generally used ATP-ase techniques was discussed before [1]. Because they cast some doubt on the results obtained with the standard methods [15, 16] reinvestigation of the behaviour of these enzyme-systems in poliovirus infected TCs will be carried out with refined direct techniques [30].

Discussion

The general physiological and biological backgrounds of the assays with chemically defined substrate-media were amply discussed in the precedent papers [1, 2]. The experimental evidence presented now indicates that the adsorption and penetration of poliovirus starts important biochemical events in cultivated cells. The *early stimulation* of the activity of certain biocatalysts has been demonstrated during initial stage of poliovirus infection of cultivated cells. This feature is considered as a *preparative phase* of virus synthesis by enhencement of enzyme activities, which may have a part in the biosynthesis of the infective particle.

For the finality of the reactions it maybe of secondary importance that this stimulation occurs upon the penetration of an *intact* virus particle engulfed by the cell [31], – or by the invading nucleic acid moiety [32, 33] in macromolecular (or depolymerized) form. It seems that the methods allow exploration of the *function* of some organelles, as reflected in the activity of enzymes having predilectional cytological localization [1, 27–29]. If this assumption is true, the higher activity of G-6-P-ase is the sign of increased microsomal [27] activity, within one hour following host-virus contact. The activity of alkaline phosphatase may be of a similar nature, although this enzyme-system might have multiple intracellulat loci [27 27a], thus an encounter with the infectious principle may be possible at various depths, beginning from the cell-membrane till the nucleus [29]. There is some suggestion that *nuclear* sites where the 5-nucleotidase resides [27 a, 29] may not be involved before detectible morphological changes occur. The same could hold for ATP-ase a mitochondrial [27, 30] and nuclear enzyme [29]. The "inhibition" of acid phosphatase, needs confirmation, because an other "lysosomal" enzyme, acid RNA-ase [28] exhibits early increase in activity, although the conditions of assays of biocatalysts having acidic p_H requirements, were not favorable, neither for virus production [34], nor for p_H optimum of the enzyme [12, 28, 29]. The interpretation of the results with alkaline RN-ase, a mitochondrial [27] and nuclear [29] diesterase is also difficult. Further work with more specifically localized enzyme-systems may furnish important data on the *state* of various organelles during virus infection *. Additional studies on normal cells may contribute to the control of enzyme localizations, carried out till now mainly by gradient centrifugation of homogenates [27–29] or histochemically.

The other approach measuring by indirect method the enzymes diffused into the supernatant medium furnished data, which substantiate the assumption, that in intact cells *mainly* intracellular enzymes were assayed by the direct method, *in situ* [1, 2]. The sudden rise of the enzyme activity of infected fluids (alkaline phosphatase) [30] at the end of growth-cycles of the virus, is unparalleled in the uninfected controls and may be the consequence of fine morphological changes. In other instances an abrupt rise (2 Hrs. p. i.) of alkaline phosphatase was observed [20], followed by rapid disappearance of any activity. Interpretation of these findings have to be postponed, because of the lack of enough experimental data. The dilution of the (used) TC-fluid increases the enzyme activity as a rule, especially of the infected one, which observation emphasizes the presence of natural and/or specific *inhibitors* [26].

The study of glucose consumption and glycolytic enzymes by other [35, 36, 25] revealed increased activity in shorter [35, 25] and relatively longer periods of incubation of various cell-lines infected with poliovirus. These are enzymes of the soluble cell-fraction [27], thus their exploration with our direct and indirect techniques would be desirable.

Similary enhenced incorporation of radiophosphorus [37, 38, 25] by poliovirus-infected cells, starts about 60 [23, 27] resp. 30 minutes [38] following reincubation for test, although only few [35, 38] assays are comparable with our short-term experiments, because of the too long adsorption-time followed by ice-cold washings and the uneven treatment of the controls. The same holds for the alkaline phosphatase assays [39], revealing increased activity of in-

[31] P. W. Brandt, Exp. Cell Res. **15**, 300 [1958].
[32] J. S. Colter, H. H. Bird, A. W. Moyer and R. A. Brown, Virology **4**, 522 [1957].
[33] H. E. Alexander, G. Koch, I. M. Mountain, K. Sprunt and O. Van Damme, Virology **5**, 172 [1958].
[34] G. E. Gifford, H. E. Robertson and J. T. Syverton, Proc. Soc. exp. Biol. Med. **93**, 321 [1956].
[36] D. Matzelt, J. Homann and H. Lennartz, Biochem. Z. **330**, 260 [1958].

* See E. Kovács, Cellular metabolism and virus, Symp. Ital. Soc. Stud. Infect. Parasit. Dis. St. Margherita, June 10–11, 1960.

[37] W. W. Ackermann, Proc. VII, Int. Congr. Microbiol., Stockholm, Aug. 1958, p. 229.
[38] G. Miroff, W. E. Cornatzer and R. G. Fischer, J. biol. Chemistry **228**, 255 [1957].
[39] W. Albrecht, H. K. Mittelstrass and R. Sauthoff, Z. Naturforschg. **13 b**, 71 [1958].

fected tube-cultures as compared to unifected controls about 4 hours p. i.

In agreement with SYVERTONS group [10] long adsorption time employed at inoculation deprives the observer from precious opportunity to record immediate or early effects of the virus, without rewarding him with differently higher synchrony in infection. Direct contact for 2 to 20 minutes, under gentle agitation, or no extra adsorption-time at all, allows reproducible results and registration of the *earliest* biochemical changes. Quick washings with isothermic fluids help to remove residual inoculum and free the cultures form desquamated cells, although the adverse effects of these procedures regarding diffusion of cell material [40, 41] have to be taken into consideration.

Furthermore it seems that the inoculum *inhibits* some enzyme-systems [26] because the zero-values were consequently higher in uninfected TCs, although the difference was not always significant. This observation would be in line with earlier findings on enzyme-toxic principles in virus-infected TC-fluids [26], or the cytotoxic affect of the inoculum [42]. This factor needs further clarification for its possible *primary role* elicitating changes in the physiological state of the host, and/or starting specific biochemical events. It is the most important therefore to have identically handled controls (see Fig. 1 and 2) with token-inoculum and washings, in parallel with the infected group. The costumary ice-cold rinsing causes *kinetical-changes* in the intracellular enzymes [30], thus the necessity of rapid, uniform and isothermic treatment is the sine qua non of *comparability*, which rule was often forgotten previously.

The stimulation of various enzyme-systems within 30 to 60 minutes may be in agreement with the results of LEBRUN [43], who demonstrated the fluorescence-labelled antigen of poliovirus one hour p. i. (+30 minutes adsorption) in the *cytoplasm* of the host. Although the findings with different techniques are not directly comparable, the coincidence in time with our results ist, at least, very *suggestive*. The absence of fluorescent material in the nucleus for over 5 hours p. i. substantiate those said in connection with 5-nucleotidase.

No change, or the *inhibition* observed with some nuclear or lysosomal enzymes is in line with data on a differential activation or inhibition, leading to a vitious circle of the cell metabolism [40, 3, 5]. Besides the cytological localization of the primary infectious principle its mechanism of action has to be considered. The macromolecules may act physiochemically or may interact chemically, combining with the active sites of the enzyme system [44, 45]. At this time one may speculate only, how the early changes described in the present paper may be generated.

A great effort was made to standardize the assays. The success was partial only; regarding the assay techniques a great refinement [1, 2] was achieved over methods generally used. The intactness of the normal controls was controlled by vital staining [1, 2] and by successful subcultivation after 2 – 3 days maintenance in substrate-media. Virus production and the kinetics of virus release is not different in *substrate-media* + modified Hanks' solution (1 : 1) from those obtained with *complete growth-media* [22, 23, 30]. Virus titres and the distribution between cells and medium [22, 23] compare favorably even in substrate-medium Iso II (no salt), at 7 Hrs. p. i. slightly more virus being intracellularly, than in the TC-fluid [30]. The difference in cell count of individual TCs from the same batch (biological variation) made necessary the use of references for the calculation of specific activities. The activity referred to protein-content is generally used [36, 39, 1]. Although one has to bear in mind in connection with unpurified enzymes, tissue "brei" and cell lysates [1, 20], that *not all* the proteins are enzymes, or one part of the proteins is certainly denaturated in poliovirus infected cells. Thus the specific activity is an approximation only and additional standards have to be considered, such as number and viability of cells [45a], proportion of living to non-functioning cells [45a], or nucleic acids (NA), especially DNA content, as discussed previously [1]. Other factors, for instance the IP and enzyme content of the inoculum, the splitting of substrate-media by purified enzymes [1] were checked also and found that they do not represent additional source of errors, under the conditions of assays [1, 2].

There is some controversy in the literature regard-

[40] E. KOVÁCS, Canad. J. Biochem. Physiol. **34**, 600 [1956].
[41] W. E. MAGEE, M. R. SHEEK, B. P. SAGIK, Proc. Soc. exp. Biol. Med. **99**, 390 [1958].
[42] W. W. ACKERMANN, Arch. ges. Virusforsch. **8**, 1 [1958].
[43] J. LEBRUN, Ann. Inst. Pasteur **93**, 225 [1957].
[44] C. G. BELING and E. DICZFALUSSY, Biochem. J. **71**, 229 [1959].
[45] L. M. KOZLLOFF, Cold Spring Harbor Sympos. quantitat. Biol. **18**, 209 [1953].
[45a] J. T. SYVERTON, personal communication.
[46] E. KOVÁCS, Canad. J. Biochem. Physiol. **36**, 619 [1956].

ing protein and NA synthesis in poliovirus infected cells. Protein determinations and ultraviolet spectrophotometry was a routine in our assays determined mainly on total cell lysates [1, 20]. In agreement with others [47, 48a] decrease of protein and nucleic acid content (in average about 10%) was observed at the end stage. However there have been some findings of an increase in 4 to 8 hours p. i. [30], which data would be in agreement with ACKERMANNS results [37, 49] not confirmed by others [47, 48, 50, 35]. A yet unexplained periodical fluctuation in enzyme activities was observed during earlier [4–6, 36], or the present work and demonstrated recently by others for protein and nucleic acids in *uninfected* HeLa cells [51]. Thus the reinvestigation of similar questions has to be carried out with *reproducible* and strictly controlled experiments [30]. Further, eventually radio-biological work is needed to prove the penetration of the substrates through the cell membrane [1]; the fate of phosphorus in phosphate-free milieu, glycolysis, respiration and phosphorylation in simple substrate-media will be studied also [30]. Various cell-lines displayed greatly varying enzyme activities in normal and infected TCs. These observations suggest the use of only one cell-type for one definit study; further considerations on the cause of similar findings were outside of the scope of the present work.

The well-preserved state of the normal controls [1, 2] the low enzyme levels in the supernatant (especially when 1/2 concentration of balanced salt solution was added) the high virus yield [2] prove that the changes described, explored in over 60 experiments (about 220 individual assays) are *characteristic* and due to the pathochemical process of virus *infection*. The role of RN-ases in the biosynthesis of poliovirus was described in precedent papers [52, 3, 5]. Application of improved technical methods to test the activity of other enzymes or special problems connected with viral infections will be reported [30].

The kind interest of Priv. Doz. Dr. G. POETSCHKE, the help of Miss G. MÜLLER-NEUDORF and Mr. E. SOIDERER is gratefully acknowledged.

[47] N. P. SALZMAN, R. Z. LOCKART, Jr. and E. D. SEBRING, Virology **9**, 244 [1959].
[48] E. L. ROTHSTEIN and L. A. MANSON, Virology **9**, 141 [1959].
[49] W. W. ACKERMANN, P. C. LOH and F. E. PAYNE, Virology **7**, 170 [1959].
[50] H. GOLDFINE, KOPPELMAN and E. A. EVANS, Jr., J. biol. Chemistry **232**, 577 [1958].
[51] N. P. SALZMAN, Biochim. biophysica Acta [Amsterdam] **31**, 158 [1959].
[52] E. KOVÁCS, G. MALATT, Infett. Parasit. **10**, 190 [1958].

Vergleichende Untersuchungen über die antigene Wirksamkeit verschiedener gereinigter Serumprotein-Fraktionen*

I. Mitt.: Humanalbumin sowie γ-Globulin vom Rind als Antigene. Zum Antikörpernachweis OUCHTERLONY-Technik und Immunoelektrophorese nach GRABAR und WILLIAMS

Von F. SCHEIFFARTH, H. GÖTZ und A. DUTSCHKE

(Z. Naturforschg. **15 b**, 517—525 [1960]; eingegangen am 6. Februar 1960)

In vorliegender Arbeit wurde in tierexperimentellen Studien der antikörper-produzierende Effekt von Albumin und γ-Globulin geprüft. Bei gleicher Versuchsanordnung und gleichem Sensibilisierungs-Modus ergab sich ein zeitlicher und quantitativer Unterschied der Antikörper-Produktion. Die Untersuchungen wurden unter Verwendung von Geltechniken (Ouchterlony-Technik und Immuno-Elektrophorese nach Grabar und Williams) und der Papierelektrophorese ausgeführt.

Zur Frage der Spezifität von Präzipitinen** sind bereits in den dreißiger Jahren unseres Jahrhunderts umfangreiche, vorwiegend tierexperimentelle Untersuchungen angestellt worden [1–7]. Unabhängig von der Art der verwendeten Antigene, des Sensibilisierungs-Modus sowie der für die Immunisierung

* Unter Verwendung verschiedener Antikörper-Nachweismethoden, insbesondere neuerer Geltechniken sowie der Präzipitat-N-Bestimmung nach Heidelberger-Kendall.

** Präzipitierenden Antikörpern.

[1] A. K. BOOR u. L. HEKTOEN, J. infect. Diseases **46**, 1 [1930]; R. DOERR, Die Immunitätsforschung, Bd. 1, Antikörper, 1. Teil, S. 141, Wien 1947.
[2] M. HEIDELBERGER u. F. E. KENDALL, J. exp. Medicine **62**, 697 [1935].
[3] L. HEKTOEN u. A. K. BOOR, J. infect. Diseases **49**, 29 [1931].
[4] S. B. HOOKER u. W. C. BODY, J. Immunology **26**, 469 [1934].
[5] E. NICOLAS, C. R. Séances Soc. Biol. Filiales **109**, 1249 [1932].
[6] T. SATOH, Z. Immunitätsforsch. exp. Therap. **79**, 117 [1933].
[7] H. R. WOLFE, a) Proc. exp. Biol. Med. **27**, 146 [1929]; b) Physiol. Zool. **6**, 55 [1933]; c) J. Immunology **29**, 1 [1935].