TABLE III. Estimated Potency of Prednisolone and 6α-Methylprednisolone Relative to Hydrocortisone.

Steroid	Value of A	Potency relative to hydrocortisone	Stand. error of relative potency
Hydrocortisone	.0480	1	
Prednisolone	.0271	1.8	.5
6α-methylprednisolone	.0103	4.7	1.3

drocortisone. Hence, at the clinically useful 1% hydrocortisone level, fluorometholone was 1/.024 or 42 times as potent as hydrocortisone. Since these original observations were made, this high potency for fluorometholone has been demonstrated repeatedly in numerous additional assays where it has been compared both with hydrocortisone and with other steroids.

Summary and conclusions. 1. A reliable assay procedure has been presented for rapidly quantitating the anti-inflammatory potency of steroids on human skin. 2. Estimated steroid potency values can be used to predict reliably the proper steroid concentration to be studied in topical clinical trials. 3. Data have been adapted to the Stetten theory which concerns relationship of hormone dosage to physiological response. This permitted comparison of steroids with respect to capacity of target organ to bind hormone and affinity of hormone for binding sites. 4. The target organ has increased capacity to bind fluorometholone relative to hydrocortisone. This means that some optimum concentration of fluorometholone is capable of producing a greater anti-inflammatory response than that possible even with very high doses of hydrocortisone. 5. That fluorometholone is approximately 40 times as potent as hydrocortisone topically yet only about equal to hydrocortisone in systemic activity suggests that fluorometholone is a unique compound for topical anti-inflammatory therapy, since even if absorption from the skin occurred, the systemic effect would be negligible.

1. Robinson, R. C. V., Robinson, H. M., Jr., *J.A.M.A.*, 1956, v49, 260.

2. Goldberg, L. C., *Antibiot. Med. and Clin. Ther.*, 1958, v5, 372.

3. Stetten, D., Jr., *Science*, 1956, v124, 365.

4. West, K. M., *Metabolism*, 1958, v7, 441.

5. Kleeman, C. R., Koplowitz, J., Maxwell, M. H., *Clin. Res.*, 1958, v6, 54.

Received April 20, 1959. P.S.E.B.M., 1959, v101.

Evolution of Herpes Simplex Cellular Lesions Observed *in vitro* by Phase Contrast Microcinematography. (25042)

G. BARSKI AND R. ROBINEAUX (Introduced by A. B. Sabin)

Institut Gustave Roussy, Villejuif, (Seine) and Centre d'Immunopathologie, Hôpital St. Antoine, Paris

Since first attempts of *in vitro* cultivation of herpes simplex virus by Rivers *et al.*(1), Andrewes *et al.*(2), and others, many authors have described specific lesions produced by this virus in cells maintained *in vitro*. However, despite considerable amount of information accumulated so far, some points concerning phenomena occurring in herpes virus infected cells have remained controversial. Our purpose was to record by continuous observations with phase contrast microcinematography the herpes infectious process in living cells, and to compare these data with those obtained by routine histological techniques. We were thus able to follow step by step the interrelationships between different cell constituents and organelles during development of the herpetic cell lesion and, on an intercellular level, to study stage by stage the formation of giant cells characteristic of this infectious process.

Material and methods. Cultures of trypsinized rabbit kidney cells were prepared in

flattened tubes on 13 × 32 mm coverslips as previously described(3,4). The culture medium contained Morgan's 199 synthetic medium (57%), bovine amniotic fluid (30%), rabbit serum (10%) and bovine embryo extract (3%). The herpes simplex "5433" virus strain originating from a human case of acute stomatitis was adapted to, and has been carried in rabbit tissue cultures(3), since 1955. Since its first passages *in vitro* this strain has produced rapidly generalized lesions characterized by giant cell formation. For continuous phase contrast observation and microcinematography, the culture-bearing coverslips were mounted in special perfusion chambers(5) and maintained at body temperature on water-perfused heating stage(6). The Zeiss-Winkel phase contrast, long focal condensor, optical system was used. The microcinematography device has been described (7). The light source was a low voltage tungsten filament lamp with Wratten "60" green filter. Frequency of time lapse pictures varied from 1/sec. to 2/min., with 0.5 sec. exposure time. Cultures were light-protected between exposures by intermittent screening. Some microcinematographic records were analysed frame by frame and essential results computed by serial drawing. After observation in living state, cultures at different stages of infection were fixed in Bouin, Bensley or Pallade buffered osmic acid fixatives and stained with hematoxylin-eosin or ferrous hematoxylin. Feulgen and methyl green-pyronin stains were used after fixation with Carnoy solution. Virus titrations were performed by serial dilution method in cultures of rabbit kidney cells (3).

Results. As previously observed(3) trypsinized rabbit kidney tissue produced *in vitro* a mixed cell growth in which fusiform and polygonal cells were present along with typical epithelial sheets. The herpes virus "5433" strain when introduced at 10^5-10^6 ID50 concentrations in cultures containing 1 to 2 × 10^5 cells produced a generalized cytopathic effect with intense giant cell formation in less than 24 hours. No preferential attack of the virus upon epithelial or fibroblastic cells was observed.

The first detectable virus released from cultures, thoroughly washed after infection, was found in the medium at $10^{0.5}$ ID50/ml concentrations around 10th hour after infection. The virus titer increased to $10^{0.8}$-$10^{1.8}$ levels between 23rd and 28th hour. However massive accumulation of virus in the medium, attaining 10^6-10^7 ID50/ml levels occurred, starting from 48th-50th hour. A massive release of virus from cells followed with some delay, generalization of the nuclear lesions observed in cultures as early as 24 hours after infection.

The first discrete alterations in living infected cells could be observed in the nucleus. From 9-10th hour after infection nucleoli in many cells lost their optical homogeneity, becoming partially less opaque, lobulated and granulated. In this way the nucleoli disappeared gradually both by dissolution and disintegration (Fig. 4-6). Only isolated nucleolar debris stuck to the nuclear membrane and could be detected after fixation and staining as rarely dispersed pyronin-positive granules. Simultaneously and independently very dark, spherical granulations appeared throughout the nucleus. These formations corresponded in size and number to Feulgen and methyl green positive granulations seen in stained control preparations. The Brownian movement in the nucleoplasm became progressively more intense and granulations tended to accumulate close to the nuclear membrane, the outlines of which became thicker and less regular. All these changes were asynchronous in cells of the same culture, even when infected with highly concentrated virus suspensions.

Later a zone of optical densification could be observed in the central area of many nuclei, but in no case did this take on an aspect of a sharply delineated inclusion body (Fig. 1-3). Such inclusion bodies located in the nuclear center and well separated from the nuclear membrane by a clear halo could be identified however in many cells of the same cultures after fixation with Bouin or Bensley mixtures and hematoxylin-eosin staining. Yet no definite pictures of intranuclear inclusion bodies were seen when Pallade's buffered osmic acid mixture was substituted for the classical fixatives.

At initial stage of infection, during the 10-20th hour period, the cytoplasm of many cells

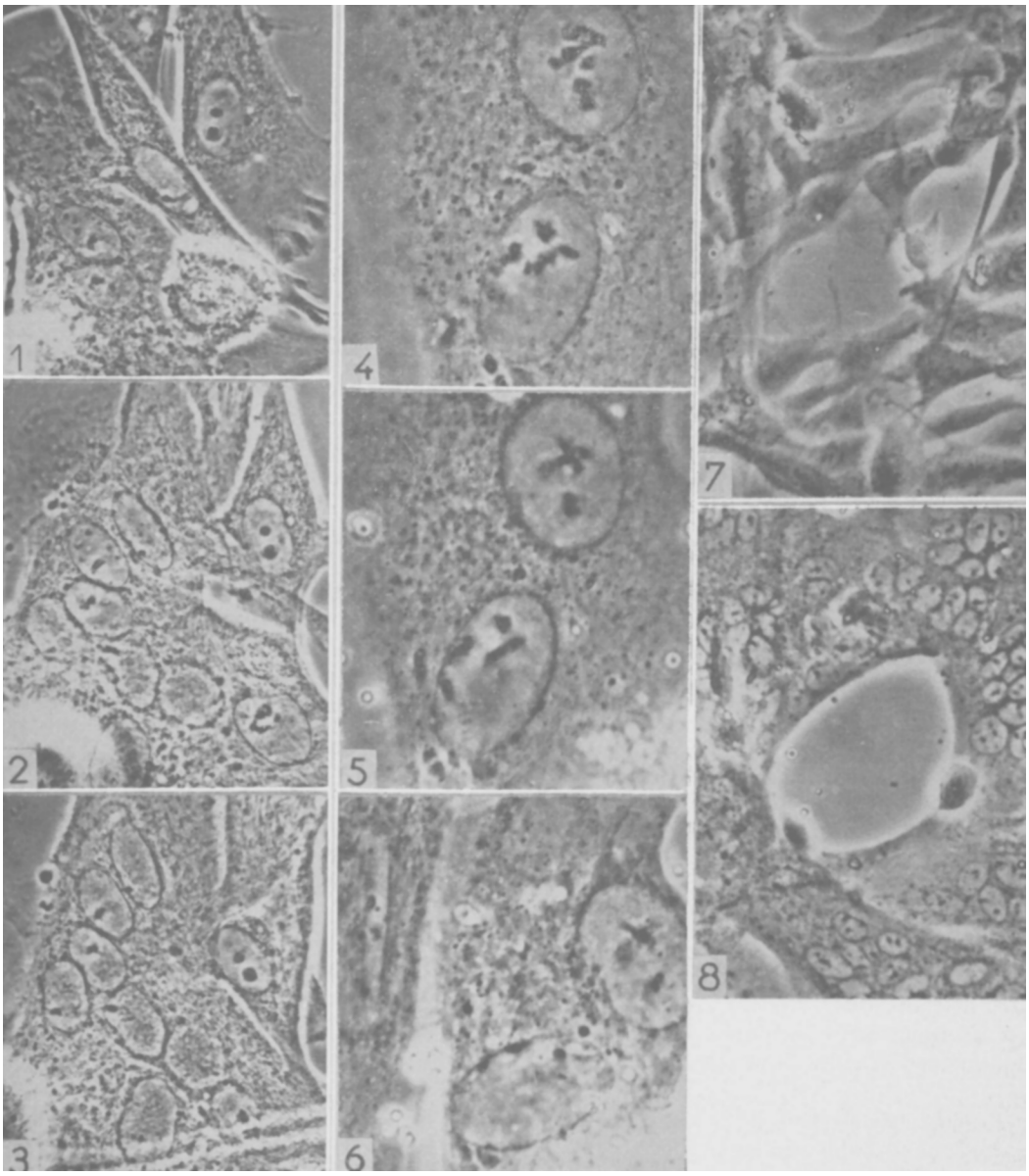

FIG. 1-3. Three successive stages of herpes virus lesions in a group of rabbit kidney cells, 8 hr 55 min., 10 hr and 10½ hr after infection; dissolution of intercellular limits, disappearance of nucleoli, optical densification of central nuclear area visible in last 2 pictures. (Phase contrast, magnif. 30 × 6.)

FIG. 4-6. Nuclei in herpes infected cells 10½ hr, 11 hr 50 min. and 15 hr 10 min. after infection; progressive dissolution and dispersion of nucleoli, increasing heterogeneity of nucleoplasm, thickening of nuclear membrane. (Phase contrast, magnif. 68 × 4.)

FIG. 7-8. Transformation of area of typical connective tissue cells into multinucleated plasmodes. Fig. 7—8½ hr following infection with herpes virus. 2 hr 45 min. elapsed between pictures 7 and 8. No nuclear division but only nuclear aggregation was seen in this area. (Phase contrast, magnif. 8 × 6.)

showing heavy intranuclear alterations kept its full activity as evidenced by persistence of normal filamentous mobile mitochondria, by presence of non-retracted cytoplasmic pseudopodia and by energetic activity of undulating membranes (Fig. 9-12). All this activity

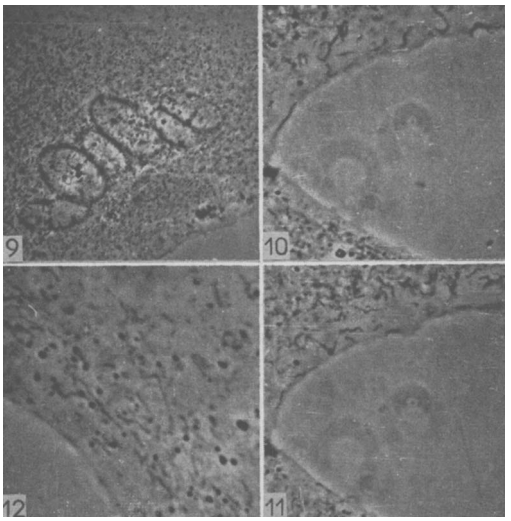

FIG. 9. A formed giant cell with typical herpetic nuclear lesions. (Magnif. 20 × 4.)

FIG. 10 and 11. Same cell as Fig. 9; ectoplasmic membranes in full activity, 10 min. elapsed between 10 and 11. (Magnif. 45 × 4.)

FIG. 12. Same cell as Fig. 9; cytoplasmic area with active mitochondria. (Magnif. 45 × 6.)

later ceased rather abruptly with a general cell retraction and arrest of movement in both cytoplasm and nucleus.

During the early 10-24 hour period, concurrently with the described intranuclear changes, very rapid and generalized giant cell formation, equally involving epithelial sheets and fibroblastic cell agglomerations was observed. The essential picture was that of progressive disappearance of cell boundaries and massive accumulation of nuclei in certain areas which were transformed into big plasmodes. This process occurred rather rapidly: thus formation of typical plasmodes containing 20 to 50 nuclei from apparently normal, isolated cells often occurred in no more than 2 to 3 hours (Fig. 7, 8).

We observed no nuclear duplication in areas of giant cell formation but were able to follow easily migration of nuclei through the cytoplasm. In certain cases this migration was so rapid that it could be checked by direct observation. Occasionally, following a retraction of cell sheets one notices a free flow of nuclei through the plasmode as if cytoplasm offered no more resistance than an astructural liquid of very low viscosity. Another indication of cytoplasm liquefaction during giant cell formation was the increasing intensity of random movement of intracytoplasmic granulations, especially in immediate vicinity of nuclei.

Some nuclei participating in giant cell formation had nucleoli in a state of dispersion whereas some nuclei appeared normal in this respect. Size of giant cells was variable, many containing 50 or more nuclei. No ordered accumulation of nuclei around hollow cytoplasmic centers, as occurs with certain other viruses,* was observed.

Occurrence of giant cells was not influenced either in frequency or in rapidity of formation by substitution for the usual culture medium of a medium supplemented with 30 mg% glutamine, or by other media such as Enders' amniotic fluid medium.

Discussion. Presence of inclusion bodies in a cell has long been considered an essential sign of virus infection, also for herpes virus. Consequently efforts were directed to discover in specific inclusion bodies centers of virus formation. Melnick and Reissig(8) demonstrated, however, that no identity nor straight correlation exists between intranuclear herpes inclusion body and accumulation of virus particles. These authors claimed further that appearance of herpes inclusion is dependent on fixative employed. We were able to substantiate this claim only partially. According to our observations an optical densification regular in shape or slightly lobulated appeared in the central nuclear area of living infected cells (Fig. 1-3) but this densification never took on the aspect of routinely recognized herpes inclusion body, sharply delineated and separated from the cell wall by a clear halo. This kind of formation, seen only after fixation and staining, is possibly due to a modification of the physico-chemical state of the nucleoplasm. Both the initial heightened intensity and later nearly complete arrest of Brownian movement in the nucleoplasm, as well-known changes in stain affinities(9) are all signs of profound reorganization of the nucleoplasm as a whole during the infectious process.

Consequently, the typical herpes inclusion body visible in fixed preparations has to be looked upon as the result of these changes

* Unpublished data.

rather than as a restricted site of virus formation or accumulation. Similarly, the cytoplasmic "central body" we described as a specific lesion in polio virus infected cells(10) proved not to contain any special virus agglomeration.*

Activity of mitochondria and of undulating membranes in cells in advanced stages of nuclear lesions is a striking phenomenon. This continuous functioning of the basic cytoplasmic machinery, during early stages of viral development in the cell, seems to be a common feature of cells infected with many viruses as has been observed with Rous(11), polio(10) and adeno(12) viruses.

Another phenomenon, formation of giant cells in virus-infected tissue cultures, also appears to be a quite general one having been observed in measles, varicella, ectromelia* and monkey foamy viruses among others. However, since it occurs for herpes virus with extreme rapidity, the process could be in this case readily observed and its confluence mechanism definitely established. We could not confirm the observations of Gray *et al.* (13) on amitotic divisions in giant cells. Yet the extremely rapid intrusion of nuclei from outside into a crowded area could easily imitate pictures of amitotic division. Here our observations agree with those of Ross and Orlans(14) McNair Scott(15) and Stoker and Newton(16).

The essential factor influencing formation of giant cells seems to reside in structural changes of the cytoplasm, evidenced by its increased fluidity during early stages of infection. This kind of alterations, as well as previously described changes in the nucleus, visualizes a rather profound and general molecular reorganization of both cyto- and nucleoplasm triggered by virus infection.

Summary. Continuous phase contrast observation and microcinematographic recording of living rabbit kidney cell cultures infected with herpes simplex virus, established the following phenomena: 1. Progressive dissolution and dispersion of nucleoli, loss of nucleoplasmic homogeneity, and appearance of intranuclear densification zone but not of a well delineated inclusion body. 2. Persistent activity of cytoplasmic organelles in cells with severe nuclear lesions. 3. Rapid (2-3 hours) formation of giant cells by cell confluence but not by cell division mechanism, accompanied by signs of cytoplasmic liquefaction. The significance of these data is discussed.

1. Rivers, J. M., Haagen, E., Muckenfuss, R. S., *J. Exp. Med.,* 1929, v50, 665.

2. Andrewes, C. H., *J. Path. Bact.,* 1930, v33, 301.

3. Barski, G., Lamy, M., Lépine, P., *Ann. Inst. Pasteur,* 1955, v89, 415.

4. Barski, G., *ibid.,* 1956, v90, 512.

5. Barski, G., Robineaux, R., *ibid.,* 1956, v90, 514.

6. Robineaux, R., Barski, G., *ibid.,* 1956, v91, 106.

7. Robineaux, R., *Mikroskopie,* 1956, v11, 31.

8. Reissig, M., Melnick, J. L., *J. Exp. Med.,* 1955, v101, 341.

9. Wolman, M., Behar, A., *J. Infect. Dis.,* 1952, v91, 63.

10. Barski, G., Robineaux, R., Endo, M., PROC. SOC. EXP. BIOL. AND MED., 1955, v88, 57.

11. Doljanski, L., Tenenbaum, E., *Cancer Res.,* 1942, v2, 776.

12. Barski, G., Cornefert, F., *Ann. Inst. Pasteur,* 1958, v94, 724.

13. Gray, A., Tokumaru, T., McNair Scott, T. F., *Arch. f. ges. Virusfsch.,* 1958, v8, 58.

14. Ross, R. W., Orlans, E., *J. Path. Bact.,* 1958, v76, 393.

15. McNair Scott, T. F., McLeod, D. L., *Ann. N. Y. Ac. Sci.,* 1959, in press.

16. Stoker, M. G. P., Newton, A., *ibid.,* 1959.

Received April 21, 1959. P.S.E.B.M., 1959, v101.