Cortisone on *in vitro* Viral Infection. II. Effect on Distribution of Cytopathic Effect and Acid Phosphatase Arrangement in Human Cells Infected with Poliovirus.* (30258)

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(Introduced by H. Koprowski)
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Viral cytopathic effect has been attributed to the activation of lysosomal enzymes(1,2 3). Corticosteroids have protected some cells from the cytopathic effect of some viruses (4,5,6,7), and release of rat liver lysosomal enzymes induced by ultraviolet irradiation has been inhibited by hydrocortisone(8). The presence of acid phosphatase(9) allows histochemical staining of lysosomes(10,11). This paper reports the histochemical analysis of acid phosphatase in human cell cultures in relation to cortisone treatment and poliovirus infection. The results support the suggestion that cortisone inhibits viral cytopathic effect by its action on cells rather than virus(12).

Methods and results. Cells of human diploid strain WI-38(13) were used. They had been serially subcultured at least 6 times in medium containing 2.5 μg/ml cortisone acetate before these experiments.

Acid phosphatase staining. Based on Burstone's method(10), acid phosphatase staining was done on cells washed with warmed phosphate-buffered balanced salt solution and fixed one minute with cold 10% neutral formalin while chilled with dry ice. Fixed cultures were covered with 4% (v/v) Naphthol AS-MX phosphate (pH 5.2) with 0.5% (w/v) fast Blue RR in distilled water, incubated overnight at 37°C, washed with distilled water, and dried at room temperature. Some cultures were stained after substrate removal with 1% methylene blue for 30 minutes, then washed and dried. Dried cultures mounted in glycerol-gelatin were examined and photographed within 48 hours. Reagents were obtained in standardized form (Sigma Chemical Co., St. Louis, Mo.).

Effect of cortisone on acid phosphatase of cells infected with type 1 Mahoney poliovirus. Cortisone-treated cells were dispersed with trypsin (0.25 mg/ml) and grown to confluency in 50 mm plastic Petri dishes. There were 2 groups of 8 cultures, one group grown with cortisone-containing medium and the other group with cortisone-free medium. After being washed with the same medium without serum, 4 cultures of each set were overlaid with 0.2 ml of serum-free medium or Mahoney virus suspension (10^7 50% tissue-culture-infective doses/ml). Cultures were incubated 30 minutes at 37°C for virus adsorption, washed twice with serum-free medium, overlaid with cortisone-containing or free medium and incubated 18 hours at 37°C, and stained for acid phosphatase. Stained uninfected cells showed small, sharply defined cytoplasmic granules considered lysosomal (Fig. 1, 2). Diffuse rather than granular distribution of stain was relatively infrequent in these cultures. Incidence of diffusely stained cells in test cultures was graded from 0 to 4 by an observer unaware of culture treatment. Incidence of such cells (Fig. 3, 4) was elevated in infected compared with uninfected cultures, and the elevation was depressed with cortisone (Table I). Thus the incidence of diffusely stained cells in the infected cultures was increased over that observed in cells from uninfected cultures, and this increase was lessened by cortisone. The cells were stained 18 hours after virus infection, at which time the cells diffusely stained for acid phosphatase activity were defined more clearly. Staining was not performed after a longer period of infection because the progression of cytopathic effect would have obscured the histochemical changes in the cells.

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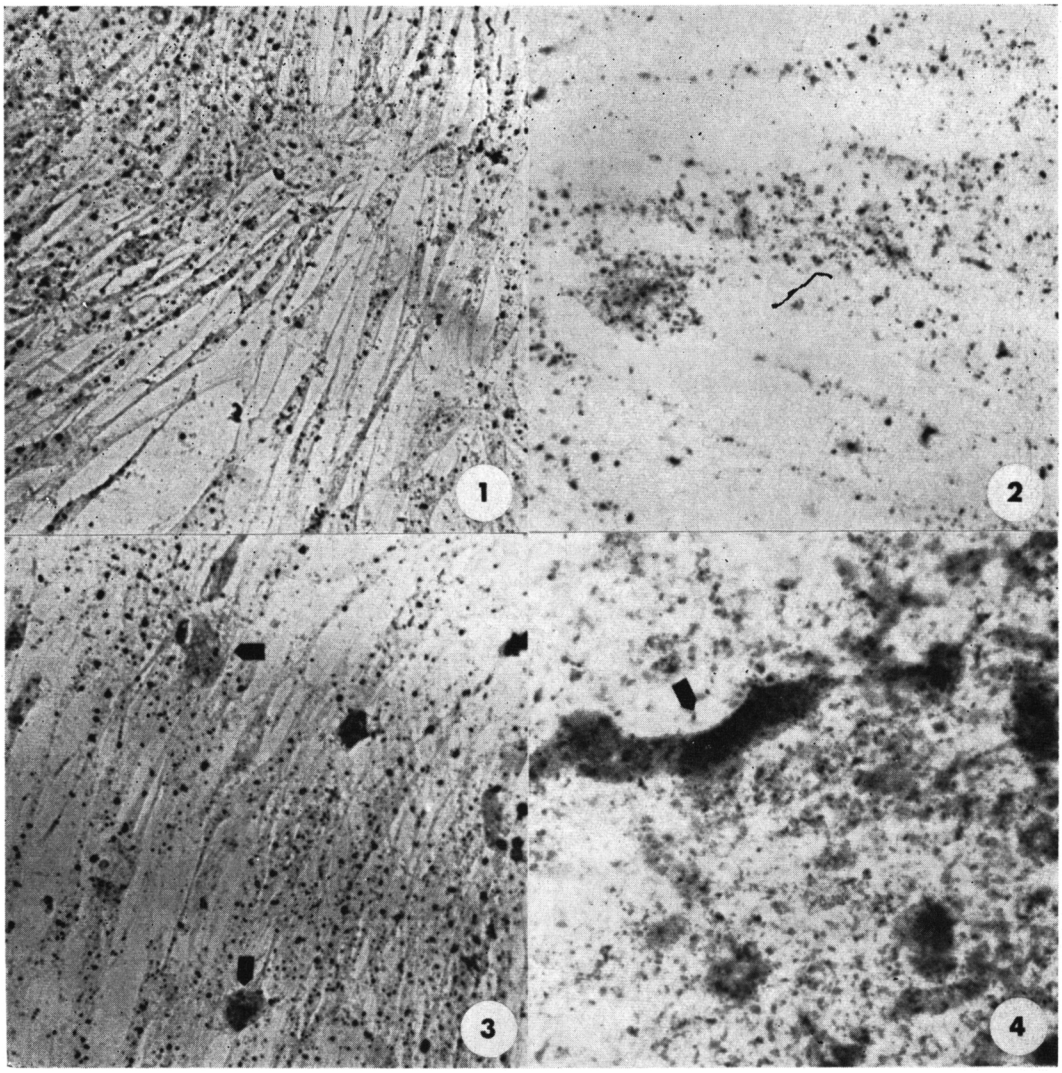

FIG. 1. Untreated WI-38 cells stained for acid phosphatase, × 250.
FIG. 2. Untreated WI-38 cells stained for acid phosphatase, × 625.
FIG. 3. Mahoney virus-infected WI-38 cells stained for acid phosphatase, × 225. Diffusely stained cells are indicated by arrows.
FIG. 4. Mahoney virus-infected WI-38 cells stained for acid phosphatase, × 625. Diffusely stained cell is indicated by arrow.

Effect of cortisone on acid phosphatase and plaque formation by cells infected with type 1 CHAT poliovirus. A similar protocol was followed for CHAT(14) as for Mahoney virus, except that dilutions of virus from 10^{-3} to 10^{-8} were employed. After virus adsorption, cultures were overlaid with agar with or without cortisone, and one set of infected cultures was stained with neutral red after 4 days while the other was stained for acid phosphatase after 48 hours. Infected cultures stained for acid phosphatase showed diffusely rather than discretely stained cells distributed in well-delineated plaques. Larger plaques showed a clear center produced by cell destruction with diffusely stained cells radiating toward peripheral uninfected cells (Fig. 5). With lower concentrations of infecting virus, or with higher concentrations in presence of cortisone, plaques were sur-

rounded by discretely stained cells (Fig. 6). As revealed by both acid phosphatase staining (Fig. 5, 6) and neutral red staining (Fig. 7, 8), plaques formed by CHAT virus with cortisone-treated cells were fewer and smaller. Distribution of cellular areas diffusely stained for acid phosphatase and areas of cell destruction shown by neutral red staining, whether separated as with low virus concentrations or confluent as with high concentrations, were similar in 4 repeated experiments with CHAT virus and 3 experiments with Mahoney virus.

Discussion. Evidence presented suggests an association between cortisone inhibition of poliovirus-induced cytopathic effect and plaque formation in human fibroblasts and the alteration of cellular acid phosphatase

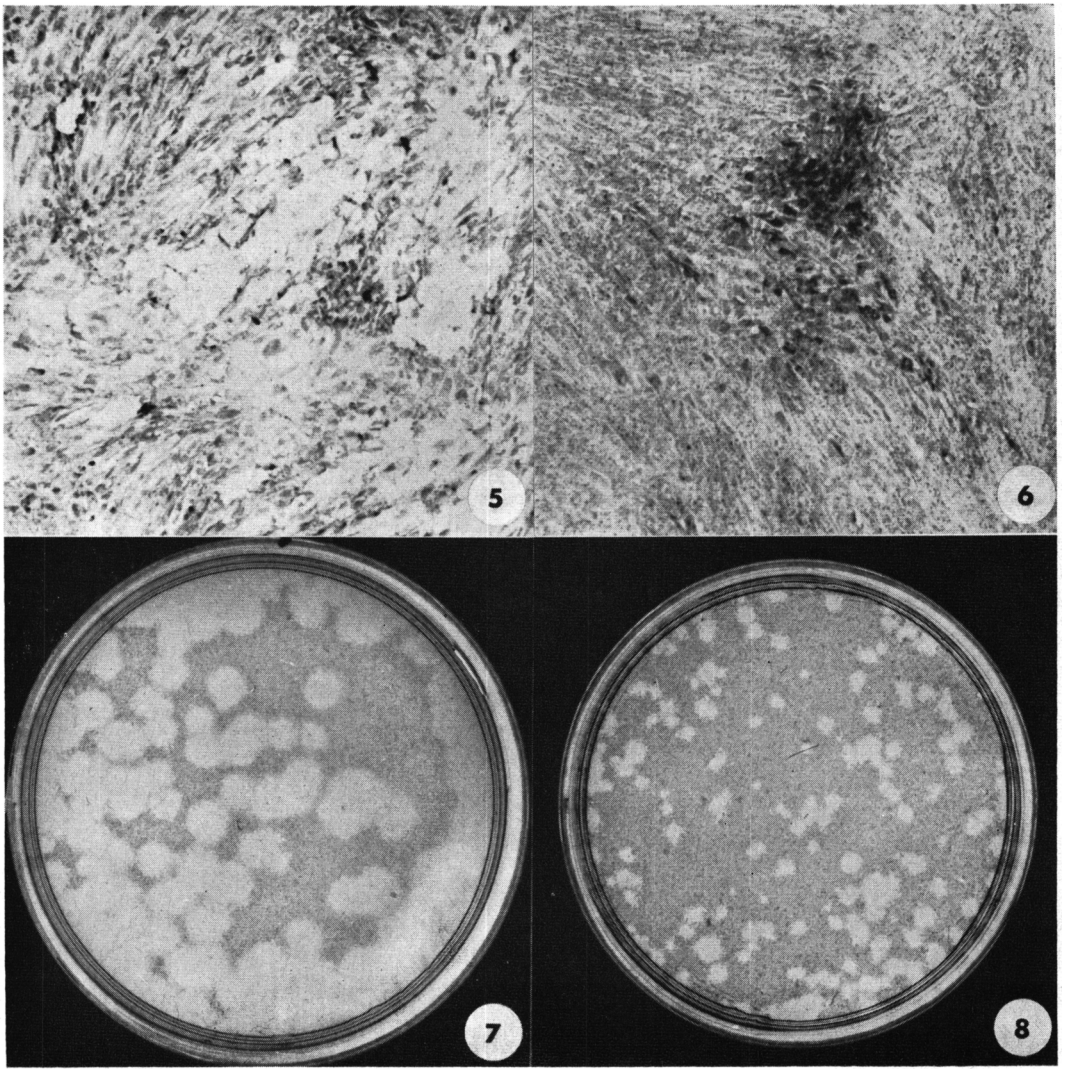

FIG. 5. Acid-phosphatase-stained WI-38 cells showing plaque from CHAT virus infection, × 70.

FIG. 6. Acid-phosphatase-stained cortisone-treated WI-38 cells showing plaque from CHAT virus infection under same conditions as in Figure 5, × 70.

FIG. 7. Neutral red-stained culture of WI-38 cells showing plaques from CHAT virus (10^{-4} dilution) infection, × 1.3.

FIG. 8. Neutral red-stained culture of cortisone-treated WI-38 cells showing plaques from CHAT virus (10^{-4} dilution) infection, × 1.2.

TABLE 1. Effect of Cortisone Treatment and Mahoney Virus Infection on Distribution of Acid Phosphatase Staining in WI-38 Human Embryonic Cell Cultures.

Virus inoculum	Culture maintenance medium	Relative incidence* of diffusely stained cells† in each culture			
		1	2	3	4
None	without cortisone	0	2	1	2
	with cortisone acetate 2.5 μg/ml	0	2	0	0
10^7TCID$_{50}$	without cortisone	4	4	4	4
	with cortisone acetate 2.5 μg/ml	1	1	2	2

* Grade on 0-4 scale (large number of diffusely stained cells) determined by independent observer.
† See Fig 1, 2 contrasted with Fig 3, 4.

distribution accompanying virus infection. While the present findings are insufficient to prove that all infected cells exhibited a diffuse distribution of acid phosphatase specifically linking the alteration in enzyme distribution with virus infection, as noted below such a link was suggested by the peripheral distribution of diffuse staining in cells surrounding enlarging virus-induced plaques. Thus altered acid-phosphatase distribution appeared to precede cellular destruction by virus infection.

Previous work indicates that cytoplasmic granules appearing in normal cells with acid phosphatase staining are organelles described as lysosomes(11). These particles, sites of storage of hydrolytic enzymes, are involved in the breakdown of compounds for cellular metabolism or excretion(9) and in embryonic involution(15). The membrane enclosing the enzymes can be damaged by noxious agents which result in cell destruction(9,16). Cytopathic effects of *in vivo* infection with mouse hepatitis virus and *in vitro* infection of monkey kidney cells with vaccinia virus have been associated with lysosomal enzyme activation by Allison *et al*(2,3). In the present work, correlation of diffuse contrasted with sharply focal acid phosphatase staining of cells (the granular distribution being characteristic of uninfected cells) with distribution of cytopathic effect of poliovirus infection suggested activation of a lysosomal enzyme. The correlation was seen for both cell monolayers under liquid medium showing distributed cytopathic effect and cells under agar overlayer showing separated plaques. Peripheral distribution of diffusely stained cells about completely destroyed cells in the center of large plaques was consistent with precedence of lysosomal-enzyme activation before cell destruction. Previously reported capacity of cortisone to protect cells in part from viral cytopathic effects(4,5,6,7) is paralleled by present findings of partial prevention of acid phosphatase activation of virus-infected cells as histochemically revealed. The circumstantial evidence was provided by observed correlation between plaque size and number and distribution of cellular effects within plaques as revealed by neutral red and acid phosphatase staining. This suggestion supports the previous suggestion that cortisone protects cells by effect on cellular processes and not by selection of variant cells(13).

Summary. Diploid human cell cultures of embryonic lung origin were stained for acid phosphatase after infection with type 1 polioviruses in presence and absence of cortisone. Focal distribution of enzyme in untreated cells was changed to diffuse pattern with virus infection, and the incidence of such stained cells was depressed by cortisone treatment, as was viral cytopathic effect. Extent and distribution of cytopathic effects and arrangements of cells exhibiting normal and altered acid phosphatase staining was correlated under varied conditions of distribution of viral infection.

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Effects of Hypertonic Mannitol on Renal Vascular Resistance.* (30259)

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The renal hemodynamic effects of hypertonic mannitol infusions have recently become of interest. Infusions of this 6 carbon sugar alcohol appear to have the property of preventing postoperative oliguria(1) and acute tubular necrosis in cases of crossclamping of the abdominal aorta(2,3). Experimentally, these infusions have resulted in increased total renal blood flow in hypotensive(4,5) and normotensive(6) dogs as measured by clearance techniques, and in medullary blood flow in normotensive animals as determined by decreased dye transit time(7).

It has been suggested(7,8,9) that mannitol infusions act by decreasing renal vascular resistance. The present study was undertaken in an attempt to determine the mechanism whereby hypertonic mannitol exerts its effect on renal vascular resistance in the *in situ* perfused kidney preparation.

Methods. Ninety-three studies were carried out in 34 mongrel dogs (10.5-16.4 mg/kg) of both sexes. After intravenous pentobarbital anesthesia (25 mg/kg) and positive pressure endotracheal respiration with room air were established, the left kidney was exposed through a flank incision.

This kidney was perfused *in situ* (Fig. 1) by means of plastic tubing passing from the left carotid artery through a Sigmamotor pump into the abdominal aorta. The distal part of this system was a plastic cannula tied into the aorta 2 cm below the left renal artery. The aorta was occluded just above the left kidney by means of a Potts clamp, and all lumbar vessels in the area were ligated. The only tissue perfused by this system was the left kidney. Since this pump was capable of supplying a constant flow against variations in resistance pressure from 0 to 400 mm Hg, any changes in renal vascular resistance were directly reflected in changes in pressure in the circuit. Flows were adjusted to maintain renal perfusion pressure close to control arterial blood pressure. During measurements of changes in renal vascular resistance, flow rates were not changed from control values.

Pressures were measured in the brachial artery and in the perfusion tubing at a point between the Sigmamotor pump and the site of aortic cannulation. The latter pressure was taken as equivalent to renal artery pressure. Renal vascular resistance was calculated as the mean renal artery pressure (mm Hg) divided by pump flow (ml/min).

Isolation of the perfusion system was confirmed in each experiment by momentarily (1-3 seconds) turning the Sigmamotor pump off and observing the fall in renal artery pressure to approximately 5 mm Hg.

By means of an infusion pump, mannitol was infused directly into the renal artery as a 6.6% solution in distilled water at a rate of 1.9 ml/min. This was calculated to be

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