least become an important mechanism when dietary calcium is limited and therefore an adequate gradient for calcium absorption by diffusion may not exist.

Summary. Using an *in vitro* gut sac technic, active transport of calcium through the intestinal wall of the rat has been demonstrated. In the presence of physiologic amounts of Vit. D, active calcium transport is confined to the upper portion of the small intestine. In this area, large amounts of Vit. D increase, and cortisone decreases, calcium transport. In the jejunal and ileal areas, active transport occurs only when large amounts of Vit. D are present, and this transport is not reduced by cortisone.

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Effect of Hydrocortisone on Herpes Virus-Infected HeLa Cells.* (26437)

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Certain of the effects of steroids on cell metabolism and growth of both normal and virus-infected cells have been studied in tissue culture systems(1-7), and the magnitude of effect of steroid on cellular growth has been found to be related to the origin of the cells. Holden and Adams(1) and Grossfeld (2) found a 40-50% reduction of growth rate of fibroblastic cells in tissue culture in presence of hydrocortisone, whereas Grossfeld and Ragan(3) found that similar treatment of cells of epithelial origin had no effect. Franklin and Sinclair(4) found similar inhibition of growth of fibroblastic cells but reported growth stimulation of epithelioid cells treated with prednisolone. The mechanism of growth inhibition of fibroblastic cells would appear to be associated with cytotoxic effects of steroid since Kline *et al.*(5) found that cell lines of connective tissue origin were much more susceptible to the cytotoxic effects of steroid than were cell lines of epithelial origin.

The effect of steroid on cell metabolism has been studied by Grossfeld(6) who found that hydrocortisone increased rate of glycolysis and O_2 uptake of mouse fibroblast cells (strain L). Similarly Fisher and Fisher(7) found that HeLa cells treated with cortisone had a greater rate of glycolysis than did control cells, although control cells utilized slightly more glucose than did the cortisone-treated. Infection of these cells(7) with herpes virus resulted in an increase in rate of

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glycolysis of both steroid-treated and untreated cells as well as a significant rise in glucose used, and total acids produced by infected cells treated with cortisone over the values obtained with virus or steroid alone. That study indicated that virus and cortisone together produced qualitative and quantitative changes in cellular metabolism not found when either substance was added singly.

Experiments reported here were carried out to determine whether herpes virus infection influences uptake of hydrocortisone by HeLa cells and the effect of steroid and virus on glucose utilization by cells.

Methods. Virus. HF strain of herpes simplex virus grown in HeLa cell cultures and stored at −60°C was used as virus stock. Titer of this stock virus used in all experiments was about 10^6 pock forming units (PFU)/ml as determined by titrations on the chorioallantoic membrane of 11-day embryonated hens' eggs.

Tissue culture. HeLa cell cultures were grown as monolayers in tubes using a medium (N_{16}) described by Puck(8), supplemented with 30% fetal calf serum. At the start of experiments, glucose and serum concentrations were reduced to the levels indicated.

Hydrocortisone. Hydrocortisone (Solu-Cortef, Upjohn Co.) and C^{14}-4-hydrocortisone with a specific activity of 42.4 μc/mg (Tracerlab, Waltham, Mass.) were used. Labelled steroid was diluted with unlabelled hydrocortisone to give a final concentration of 200 μg of hydrocortisone/ml in the tissue culture medium, and concentrations of labelled hydrocortisone of 1% in the first experiment and 5% in the second. Isotope counts were made on a gas flow analyser.

Glucose. Determinations of glucose concentrations were made using the anthrone method described by Seifter *et al.*(9)

Results. Monolayer cultures of HeLa cells were washed and the medium replaced by one containing 5% fetal calf serum and 600 μg glucose/ml. Virus was added to the cultures in a concentration which resulted in a ratio of 1 PFU of virus for each 16-20 cells. The amount of glucose present in the virus inoculum was sufficiently low as not to add a detectable amount to that present in the medium. Labelled and unlabelled hydrocortisone were added to the cultures in the amounts previously indicated at the same time as virus inoculum. Cultures were incubated at 37°C and 4°C. After 40 hours' incubation at 37°C, virus-infected cells showed initial cytopathic changes on microscopic examination as evidenced by an increase in rounding and granularity of cells; by 96 hours cultures were almost totally destroyed. At the end of 40 hours' incubation, tissue culture fluids were removed from the majority of cultures for determinations of glucose. Hydrocortisone concentrations were measured by determining the amount of C^{14}-labelled steroid present. Similar determinations were made on the cells which were collected by trypsinization, washed, and counted in a hemocytometer. The amounts of steroid found in the tissue culture medium and with the cells accounted for the total amount of hydrocortisone added. The results of these experiments are shown in Table I, where each determination represents the mean of 5 separate samples, and each sample is a pool of 3 cultures. The amount of hydrocortisone associated with the cells was similar in both experiments, and virus infection did not influence the amount of steroid taken up. The amount of hydrocortisone found with the cells represents about 10% of total amount available based on calculations of uptake per 10^6 cells. This figure correlates well with similar determinations of hydrocortisone remaining in the medium after 40 hours' incubation. These results suggest that stress of virus infection does not influence uptake of steroid by cells in tissue culture. Similarly, results obtained with cultures incubated at 4°C were almost identical to those at 37°C indicating that uptake of hydrocortisone by cells is independent of temperature.

The amount of glucose taken up by cells over a 40-hour incubation period was calculated by subtracting the amount present at 40 hours from that present in fresh medium. When glucose uptake is calculated in terms of a unit cell population rather than in total culture, the increase in glucose uptake in presence of hydrocortisone is changed to a

TABLE I. Hydrocortisone and Sugar Uptake by HeLa Cells in Presence and Absence of Herpes Virus.

Exp.	Virus	Hydrocortisone	Hydrocortisone uptake/10^6 cells at 37°C	Calculated glucose uptake		Cell conc. of glucose/10^6 cells
				Total culture	Per 10^6 cells	
				μg		
1	—	—		261	155	7.0
	—	+	12	297	145	2.8
	+	—		208	107	6.1
	+	+	15	258	120	3.7
2	—	—		382	187	6.2
	—	+	17	378	170	2.6
	+	—		308	134	5.7
	+	+	15	358	150	3.9

decrease because of greater numbers of cells present in these cultures (Table I). It is not known whether this increase in cells in steroid-treated cultures is the result of increased growth or a better survival of cells under conditions of reduced nutrients in the medium (5% serum and reduced glucose). The possibility of growth stimulation in presence of hydrocortisone is supported by the work of Franklin and Sinclair(4). Addition of virus significantly reduced the uptake of glucose; however, in the presence of hydrocortisone, the effect of virus in reducing glucose uptake was not as great. The glucose content of cells is shown in the last column of Table I. Cellular levels of glucose are decreased by addition of hydrocortisone and left virtually unchanged by infection with virus, suggesting that cellular metabolism of glucose is changed in the presence of steroid while uptake is only slightly affected. The differences in cellular levels of glucose between steroid-treated and control cells and between steroid-treated cells infected and not infected with virus are significant to 1% and 10% levels respectively.

Discussion. The finding that hydrocortisone was associated in equal amounts with infected and noninfected cells at 37°C and 4°C indicates that the union of steroid and cell is nonspecific in these epithelial cells. However, it may be that epithelial and fibroblastic cells react differently in this regard as they do in others(1-5).

The results of studies on glucose uptake by herpes-infected HeLa cells are in partial disagreement with those of Fisher and Fisher (7) in that steroid and virus did not increase uptake of glucose above that of control cultures. This difference in results may be due to the larger virus inoculum (about 50 times greater in terms of PFU/cell) used in the present study resulting in a greater physiological impairment of cells by 40 hours, which was the same time after infection that Fisher and Fisher(7) made their observations. The decrease in amounts of glucose in cells treated with hydrocortisone tends to support the observation of Fisher and Fisher (10) that alternate pathways of glucose metabolism are operative in steroid-treated cells which in the present case has resulted in either a greater utilization of endogenous reserves or a shift away from processes leading to glucose storage. Effects of hydrocortisone on herpes-infected HeLa cells seem to result in a partial return of cells to a normal state in terms of glucose uptake and storage.

Summary. Association of C^{14}-labelled hydrocortisone with herpes virus-infected HeLa cells appeared nonspecific in that equal amounts of steroid were taken up at both 37°C and 4°C by noninfected as well as infected cells. The action of steroid on the cells resulted in a slight decrease in glucose uptake and a proportionally greater decrease in glucose stored. When steroid-treated cells were infected with virus, there was an increased uptake of glucose over that of virus-infected controls, and more glucose stored than in control cells treated with hydrocortisone suggesting that the action of steroid results in a partial return of stressed cells to more normal physiologic function.

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Effect of Antiserum on Adsorption of Vaccinia Virus to Earle's L Cells.* (26438)

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Neutralized virus is, by definition, not measurable by conventional titration methods. For this reason it has been difficult to assess the part played by altered host cell-virus particle adsorption in the overall neutralization of virus by antiserum. Recent work(1) with Newcastle disease virus, carrying radioactive label, has shown a blocking of adsorption of neutralized virus to host cells. A more direct demonstration of this vital step in the infectious sequence is desirable.

We have described(2) the methods for counting virus particles released by sonic energy from host cells shortly after adsorption. This paper contains preliminary results of application of these methods which show that a substantial part of the neutralization of the infectivity of the virus for L cells is manifest at the point of adsorption.

Materials and methods. Vaccinia virus, WR (mouse neurotropic) strain was obtained from the American Type Culture Collection, passed 18 times in L cells and stored at −70°C. L cells were obtained from Dr. W. Earle's laboratory. Medium and other details of the cell cultures have been described(3). Antiserum was produced by vaccination of young New Zealand white rabbits with active virus. Further applications of live virus were made 5 and 12 weeks later with the last application resulting in a typical immune response. Blood was drawn at the 13th week and serum was stored at −20°C. Normal serum was obtained by cardiac puncture from rabbits before vaccination. All sera were heated 30 minutes at 56°C before use. Counting of virus particles was done by the agar sedimentation process (4) employing the electron microscope.

Experimental. Mixtures of active virus and serum were made in growth medium such that they contained 10^9 virus particles per ml and a serum dilution of 1/5. These were incubated 4 hours at 37°C with occasional agitation. L cells were counted, sedimented in pointed centrifuge tubes and resuspended in the various serum-virus mixtures in such quantity that final concentration was 5×10^5 per ml. There were thus 200 virus particles per cell. Adsorption proceeded at 37°C with frequent agitation for 1½ hours, after which the cells were washed twice and the remaining adsorbed virus released by 2 minutes treatment with 9 KC sonic energy. This cell-

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