lar connective tissue of ankles and feet in rats has been regularly induced by oral administration of 6-sulfanilylindazole (SAI). The incidence of this inflammation probably has been caused by drug-induced provocation of latent PPLO infection. The mechanism of this effect of SAI is not known.

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Effect of Hydrocortisone on Alkaline Phosphatase in Cultured Cancer Cells.* (29688)

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Cox and MacLeod(1) have shown that prednisolone increased the level of alkaline phosphatase in HeLa cells. Melnykovych(2) studied the effects of a large number of steroids on HeLa cells and observed only glucocorticoids increased the alkaline phosphatase activity. Maio and DeCarli(3) reported that alkaline phosphatase activity is increased by prednisolone or hydrocortisone in isolated clones of cell strain EUE which normally exhibit a low enzyme activity. Clones which possessed high activity were not stimulated by prednisolone or hydrocortisone. The work reported here involved a study of some effects of prolonged hydrocortisone exposure on J111, H.Ep2 as well as HeLa alkaline phosphatase activity.

Materials and methods. Normal HeLa S_3B, J111 and H.Ep2 cells and those grown in the presence of 25 μg/ml hydrocortisone acetate for 6-8 weeks were grown in 50 ml Erlenmeyer flasks for six days at 37°C in 42.5% H-16 and 15% Saline F medium(4) supplemented with 15% calf serum. The cultures were started from an inoculum of 10^5 cells per ml per flask. After 3 days the old medium was replaced with fresh medium and incubated for another 3 days. The cells exposed to hydrocortisone were grown for an additional 3 days.

Determination of alkaline phosphatase activity was conducted according to the method of Melnykovych(2). At the end of the incubation period the medium was withdrawn by suction and the glass adherent cells were washed 3 times with 0.5 ml of balanced salt solution. To each flask was added 2.0 ml of 0.5 M glycine buffer pH 9.1, 0.5 ml of 0.05 M $MgCl_2$ and 0.4 ml distilled water. The flasks were shaken on an Aminco-Dubinoff shaker at 37°C for 30 minutes and 0.5 ml of substrate containing 1 mg of p-nitrophenylphosphate was added to each flask.

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TABLE I. Alkaline Phosphatase Determinations on 3 Cultured Tumor Cells.

Cells	No. of determinations	Exposure to hydrocortisone	μM/2 hr/mg enzyme activity (avg & range)	Fold change
HeLa	(6)	Normal	.20 .18–.23	
"	(5)	HCR	2.49 2.13–2.74	+12.5 x
J111	(5)	Normal	9.77 7.51–10.94	
"	(5)	HCR	1.39 .94–1.86	–7.0 x
H.Ep2	(5)	Normal	2.18 2.04–2.38	
"	(5)	HCR	1.45 1.29–1.55	–1.5

HCR = Hydrocortisone.

Shaking was continued for 30 to 60 minutes and the reaction was stopped by adding 1 ml of 20% sodium carbonate in 0.1 N NaOH to each flask. The concentration of liberated p-nitrophenol was determined colorimetrically at 410 mu with a Beckman D.U. Spectrophotometer. Protein was determined in duplicate cultures by the Bromsulfalein method (5). Alkaline phosphatase activity was expressed in terms of arbitrary units, each unit being equal to the number of M of p-nitrophenol liberated in 2 hours per mg of cell protein.

For histochemical determinations of alkaline phosphatase normal HeLa, J111 and H.Ep2 as well as hydrocortisone exposed cells were grown on glass slips under conditions identical with those above. The glass adherent cells were washed with distilled water and fixed with 10% formalin and stained for alkaline phosphatase by the method described by Sigma Chemical Co. Technical Bulletin No. 85(6).

Results. Table I shows that the low level of alkaline phosphatase activity in normal HeLa cells was markedly increased when the cells were grown in the presence of hydrocortisone. Conversely, the level of activity is very high in normal J111 cells and is greatly decreased by exposure to hydrocortisone. Likewise, the alkaline phosphatase activity which is relatively high in normal H.Ep2 cells is lowered significantly by hydrocortisone.

The histochemical determinations of alkaline phosphatase were conducted on all the above cells in order to localize the areas of altered enzyme activity. The results were not clear-cut due to a high level of enzymatic activity and concomitant dye concentration. However, the histochemical findings were in agreement, quantitatively, with the enzymatic determinations; high concentrations of the dye were observed in preparations showing high levels of the enzyme and vice versa.

Discussion. The results presented here are in agreement with those of Maio and DeCarli (3) who noted that the alkaline phosphatase induction factor is inversely proportional to the initial enzyme activity of variant lines of EUE cells. It appears that this relationship exists for cells of different strains as well as variants of the same strain.

Summary. The effects of prolonged exposure to hydrocortisone on the alkaline phosphatase activity of HeLa, J111, and H.Ep2 cells have been investigated by enzymatic and histochemical methods. Exposure of HeLa cells to hydrocortisone produced a 12.5-fold increase in activity, while exposure of J111 and H.Ep2 cells to the compound caused a 7- and 1.5-fold decrease respectively. The observations are in agreement with those obtained by other investigators using variants of cell strain EUE.

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