INDUCTION AND REPRESSION OF ALKALINE PHOSPHATASE IN HUMAN CULTURED CELLS BY PREDNISOLONE, HYDROCORTISONE AND ORGANIC MONOPHOSPHATES

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We have recently described a simple technique which permits the detection and isolation of clones of human cells in tissue culture showing marked variations in specific alkaline phosphatase activities within the same cell strain (Maio and De Carli 1962). To date, three noteworthy features have emerged from our studies of the nature of this variation:

1) The differences in specific alkaline phosphatase activity may be as great as 1000-fold among individual sub-lines derived from the same parent strain, although it would appear that variants showing all intermediate enzyme activity levels may also be isolated.

2) Characteristic karyotype alterations accompany the variation in specific alkaline phosphatase activity (DeCarli, Maio and Nuzzo).

3) Organic monophosphate esters, prednisolone (Cox and MacLeod 1961; Cox and Pontecorvo 1961) and hydrocortisone not only induce increased specific alkaline phosphatase activity levels in cell lines derived from clones of strain EUE deficient

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in enzyme activity, but also repress enzyme formation in cell lines derived from the same strain showing high enzyme activity. This communication presents data illustrating this paradoxical effect.

MATERIALS AND METHODS

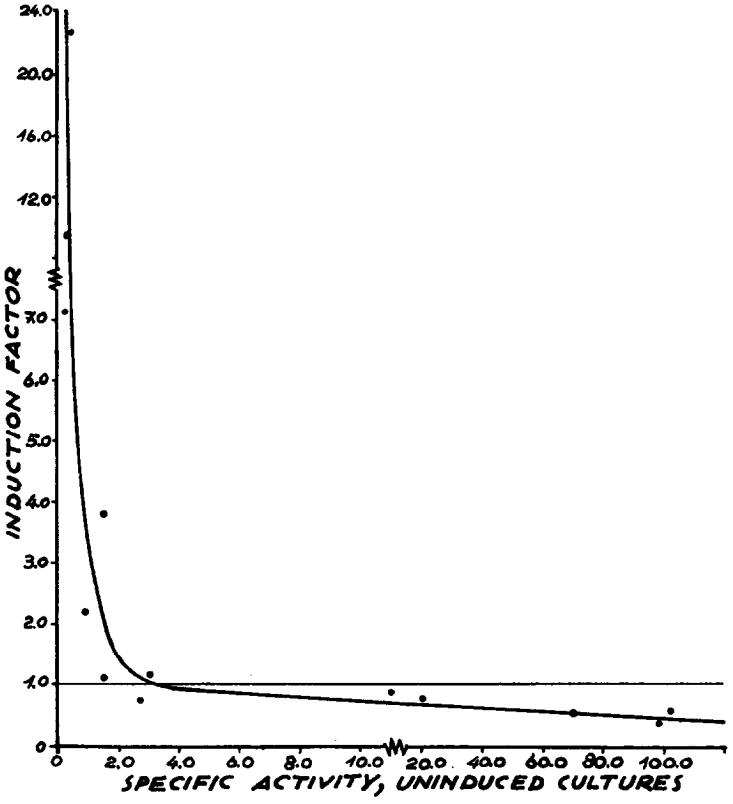
Human cell strain EUE, culture conditions, cloning procedures, isolation of variant clones and determination of specific alkaline phosphatase activities have been described in a previous publication (Maio and De Carli 1962). In the present work, however, 15% calf serum was routinely employed in the maintenance media and in the media used in the induction and repression experiments. Protein determinations were performed using the Folin-Ciocalteau reagent. Data presented here are derived from replicate experiments

RESULTS

Induction and repression of alkaline phosphatase formation. A series of 13 variant lines showing a wide range of specific alkaline phosphatase activities (0.13 to 125) were derived from isolated clones of strain EUE using the agar-pNPP (p-nitrophenyl phosphate) technique to distinguish "negative" clones having very low enzyme activity levels (sp. ac.*ca*. 0.1 to 1.0) from "positive" clones having intermediate to high enzyme activity levels (sp. ac. >1.0) (Maio and De Carli 1962). Immediately upon its re-establishment in continuous mass culture, each line, representing a clonal population of cells, was cultured for 6 days in medium 199 containing 10 γ/ml of the hydrocortisone analogue, prednisolone. Control or "uninduced" cultures were grown for the same period of time in the absence of the hormone.

Figure 1 shows the effect of prednisolone on the specific alkaline phosphatase activities of the series of variants with diverse constitutive enzyme activity levels. In this Figure, the "Induction Factor" is the ratio:

Figure 1



The explanation of this Figure is given in the text.

Specific alkaline phosphatase activity of induced culture
Specific alkaline phosphatase activity of uninduced culture

It may be seen that increased enzyme activity levels were induced in the negative lines by prednisolone, and that the induction factor was inversely proportional to the initial or constitutive enzyme activity levels of the variant lines. Those cell lines with constitutive enzyme activity levels of about 3 or higher either failed to be induced by the hormone or actually showed repression of enzyme formation to levels of about 10 to 40% of the activity of control cultures. Appropriate plating experiments showed that the altered enzyme activity levels were not due to the selection of negative variants in the presence of prednisolone.

Table 1 shows that hydrocortisone and β-glycerophosphate, compounds that induce enzyme formation in the negative lines (e. g., Sub 2) also repressed enzyme formation in the parent strain, EUE. Phenyl phosphate, an effective inducer of alkaline phosphatase activity in negative lines, could not be tested for possible repressive activity because of its rapid hydrolysis by cell lines having a specific alkaline phosphatase activity of about 4 or higher.

Table 1

Cell Strain	Specific Activity Uninduced	Phenyl Phosphate 2×10^{-2} M	β-Glycero-Phosphate 2×10^{-2} M	Predni-solone 10 γ/ml	Hydrocor-tisone 10 γ/ml
Sub 2	0.3 (11)	4.0 (5)	2.3 (10)	6.0 (19)	4.7 (16)
EUE	113.0 (39)	--	33.6 (18)	32.1 (19)	20.1 (27)
EUE	89.0 (26)	--	10.3 (19)	12.0 (23)	16.5 (26)

Induction and repression of alkaline phosphatase formation by hormones and organic monophosphate esters.
Cells were grown for 6 days in the presence of compounds as shown. Data are given as μM p-NPP hydrolyzed per min. per mgm. protein at 35°C. Numbers in parenthesis refer to the fold-increase in total protein of each culture during the 6-day growth period.

It is interesting to note that those lines with very low alkaline phosphatase activities grew at about 1/3 the rate of the positive lines under identical culture conditions. Furthermore, prednisolone could overcome this growth inhibition to some extent. Thus, a doubling in cell numbers and total protein of the cultures was frequently observed when the negative lines were grown for 6 days in the presence of 10 γ/ml prednisolone. Hydrocortisone was slightly less effective in stimulating growth. In some experiments, both hormones inhibited growth of the posi-

tive lines, but this effect was not consistent. These observations suggest that alkaline phosphatase activity may be a limiting factor in determining growth rates in the negative lines, but other hormonal effects cannot be excluded at present.

Effects of hormones and organic monophosphates in combination. Table 2 shows that in negative strains Sub 2 and Sub 24, the hormones prednisolone and hydrocortisone present in combination

Table 2

Culture Conditions	Cell Strain and Specific Activity			
	EUE (Expt. 1)	EUE (Expt. 2)	Sub 2	Sub 24
No inducer	86.0	168.0	0.3	0.2
Phenyl phosphate	--	--	2.2	1.4
β-Glycerophosphate	35.5	24.6	1.3	0.7
Prednisolone	30.8	76.0	1.8	1.1
Hydrocortisone	42.2	18.5	1.7	1.3
β-Glycerophosphate + Prednisolone	18.0	24.8	3.7	1.9
Phenyl phosphate + Hydrocortisone	--	--	4.0	2.3
β-Glycerophosphate + Hydrocortisone	--	18.5	2.6	1.8
Phenyl phosphate + β-Glycerophosphate	--	--	2.0	1.2
Prednisolone + Hydrocortisone	36.8	62.4	1.8	1.3

Effects of hormones and organic monophosphates in combination. Phenyl phosphate and β-glycerophosphate were each employed at 10^{-2} M concentration during the 6-day induction period. Prednisolone and hydrocortisone were each employed at 7 γ/ml. It was not possible to test the combination phenyl phosphate + prednisolone because of the rapid degeneration of the cultures under these conditions. Presumably, this degeneration was due to the hydrolysis of phenyl phosphate which occurs even in cultures of negative lines upon induction.

in the culture medium did not induce levels of alkaline phosphatase activity higher than those levels attained in the presence of either compound alone. This was also true in the case of β-glycerophosphate or phenyl phosphate added singly or in combination. However, either hormone in combination with β-glycerophosphat or phenyl phosphate usually induced higher enzyme enzyme activity levels than could be attained by culturing the cells in the presence of any single compound. In fact, the induction effects were often additive in this case (Table 2). These facts might suggest that at least two active sites may be involved in the induction mechanism and that one of these sites responds to the hormones and the other to the organic monophosphates. Alternatively these compounds may function synergistically at common sites.

It may also be seen from Table 2 that prednisolone and hydrocortisone in combination did not repress alkaline phosphatase formation to a greater extent than either compound added singly. In some experiments, the combination of β-glycerophosphate with either prednisolone or hydrocortisone did exert an additive effect. However, this result was not always consistent (Table 2).

Michaelis-Menten Kinetics. Determinations of the constants K_m and K_i did not reveal any marked differences in the alkaline phosphatase formed by strain EUE under normal growth conditions, under repression, or in the alkaline phosphatase formed by Sub 2 under enzyme induction (Table 3).

The inhibition of p-NPP hydrolysis by β-glycerophosphate and phenyl phosphate was competitive. Prednisolone and hydrocortisone had no effect on enzyme activity when present in the reaction mixture at a concentration of 100 γ /ml. Because of the extremely low activity levels, it was not possible to obtain accurate K_i values for alkaline phosphatase from uninduced negative strains.

Table 3

Cell Strain	Specific activity	K_m, p-NPP	K_i, β-glycerophosphate	K_i, phenyl phosphate
EUE, no repression	120.0	7.0×10^{-5} M	2.9×10^{-3} M	4.5×10^{-4} M
EUE, repression by β-glycerophosphate	26.3	6.4×10^{-5} M	2.1×10^{-3} M	3.8×10^{-4} M
EUE, repression by prednisolone	30.0	9.1×10^{-5} M	2.9×10^{-3} M	2.7×10^{-4} M
Sub 2, induction by prednisolone	2.8	1.1×10^{-4} M	2.6×10^{-3} M	7.1×10^{-4} M

K_m and K_i values of alkaline phosphatase of strain EUE and Sub 2. Cell extracts were incubated with 6 different concentrations of p-NPP ranging from 10^{-4} M to 5×10^{-3} M at pH 9.4. The inhibitors β-glycerophosphate and phenyl phosphate were added to a final concentration of 10^{-2} M.

Summary

A series of human cell variants derived from the same strain and having greatly diverse constitutive alkaline phosphatase activity levels show a paradoxical response to prednisolone, hydrocortisone, and organic monophosphates. In those variants with very low constitutive levels of enzyme activity, alkaline phosphatase formation is induced by both classes of compounds. The same compounds repress enzyme formation in those variants with high constitutive levels.

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