HORMONAL REGULATION OF ALKALINE PHOSPHATASE IN DISPERSED CELL CULTURES[1]

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Received April 9, 1963

Increase in activity of alkaline phosphatase of human leucocytes in vivo has been observed following the administration of ACTH or adrenal glucocorticoids (Valentine et.al., 1954). The latter also accelerate the appearance of the enzyme during development of chick and mouse intestinal epithelium in the intact embryo and in organ fragments in vitro (Moog, 1959). Similarly, increase in phosphatase activity of dispersed cell cultures has been described following growth with prednisolone (Cox and MacLeod, 1961). Since we have reported previously differences in activity and thermal stability of this enzyme in various human cell cultures (Nitowsky and Herz, 1961; Herz and Nitowsky, 1962), it was of interest to determine whether qualitative as well as quantitative changes resulted from action of this steroid.

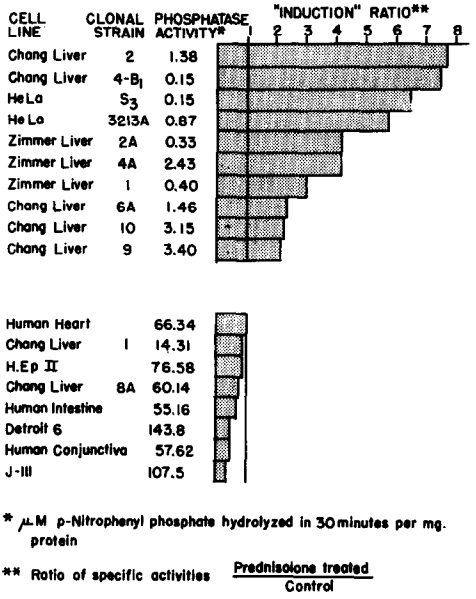
Methods: Stationary cell cultures were grown for intervals up to 8 days in Eagle's or Puck's medium containing 10% or 20% undialyzed human serum respectively. Prednisolone 21-phosphate, 0.5 ug/ml (10^{-6}M), was added 24 hours after cell transfer. At various times thereafter cells were harvested with 0.05% trypsin, and then washed with, and suspended in, 0.25 M sucrose. Alkaline phosphatase activity of sonicates was measured with p-nitrophenyl phosphate in 2-amino-2-methyl-1-propanol (AMP) buffer, pH 10.6, and specific activity was expressed as umoles p-nitrophenol liberated in 30 minutes per mg protein. The ratio of specific activities of steroid-treated to control cultures is referred to as the induction ratio. Thermal stability of alkaline phosphatase was investigated by incubating cell-free sonicates with AMP buffer at 56°C in a ther-

1. Supported by a U.S. Public Health Service grant, A-494.

mostatically controlled water bath and subsequently measuring activity by the standard assay procedure at 38°C.

Results: The effect of prednisolone on the phosphatase activity of sonicates of established human cell lines after a 7-day growth cycle is shown in Figure 1. The change in enzyme activity, or induction ratio, was characteristic of, and reproducible for, each of the parental cell lines or clonal strains derived following single cell platings. In general, two types of response were noted. With certain cell lines growth with prednisolone resulted in increased activity, 2 to 8 times that of control cultures. With other cell lines, either no increase or a decrease in activity was observed. While this difference in response could not be related to cellular morphology or the nutrient medium employed, there was a correlation with the phosphatase activity of the particular cell line. Enzyme induction was observed with low phosphatase cell lines, i.e., those having a specific activity of approximately 5, or less.

EFFECT OF PREDNISOLONE ON ALKALINE PHOSPHATASE ACTIVITY OF ESTABLISHED HUMAN CELL CULTURES



* μM p-Nitrophenyl phosphate hydrolyzed in 30 minutes per mg. protein

** Ratio of specific activities $\frac{\text{Prednisolone treated}}{\text{Control}}$

Figure 1

Induction of enzyme activity following addition of the steroid was noted within 24 hours, and after 48 hours the induction ratio remained relatively constant. After transfer to steroid-free medium, the specific activity fell to the level of control cultrues within 2 to 3 days, suggesting dilution of enzyme activity by cell multiplication. Incubation of mixtures of cell-free extracts appeared to exclude the possibility that the differences were due to activators in the steroid-treated cultures or to inhibitors in the controls. No changes in activity were observed following incubation of cell-free extracts with prednisolone in vitro. Similarly, no changes were noted with intact cells attached to glass after 48 hours in a balanced salth solution containing the steroid but incapable of supporting cell growth or with cells growing in a complete medium with added steroid and puromycin (Table 1). The latter observations support the conclusion that the changes in phosphatase activity reflected induced synthesis of enzyme. The activities of other enzymes, including glutamic-oxalacetic and glutamic-pyruvic transaminases, acid phosphatase, lactic dehydrogenase, glucose-6-phosphate dehydrogenase, and rhodanese, showed no significant changes in cells grown with prednisolone.

TABLE I

EFFECT OF PUROMYCIN ON STEROID INDUCTION OF ALKALINE PHOSPHATASE IN HeLa S_3

Addition	Alkaline Phosphatase (umoles/30min/mg protein)	Cell Protein (mg/flask-6 day growth)
Control	0.22	1.34
Prednisolone (0.5ug/ml)	0.84	1.36
Prednisolone (0.5ug/ml) +puromycin (0.3ug/ml)	0.59	1.10
Prednisolone (0.5ug/ml) +puromycin (0.5ug/ml)	0.31	0.70

Previous studies of the physical-chemical properties of alkaline phosphatase of cell cultures showed that preparations with low activity were relatively stable during incubation at 56°C in AMP buffer. In contrast, cell lines with high en-

zyme activity, with certain exceptions, showed rapid inactivation under these conditions. The results of studies of thermal stability with sonicates of steroid-treated and control cells are shown in Figure 2. Zi liver clone 4A, a cell strain with low and heat-stable phosphatase activity, showed an induction ratio of 2.5 with prednisolone (not shown here) but no change in the first-order reaction kinetics of heat inactivation of the enzyme. Sonicates of Chang liver clone 8A, which had a 20 to 30 times higher phosphatase activity than Zi liver clone 4A, showed a diphasic heat inactivation course. Although after growth with pred-

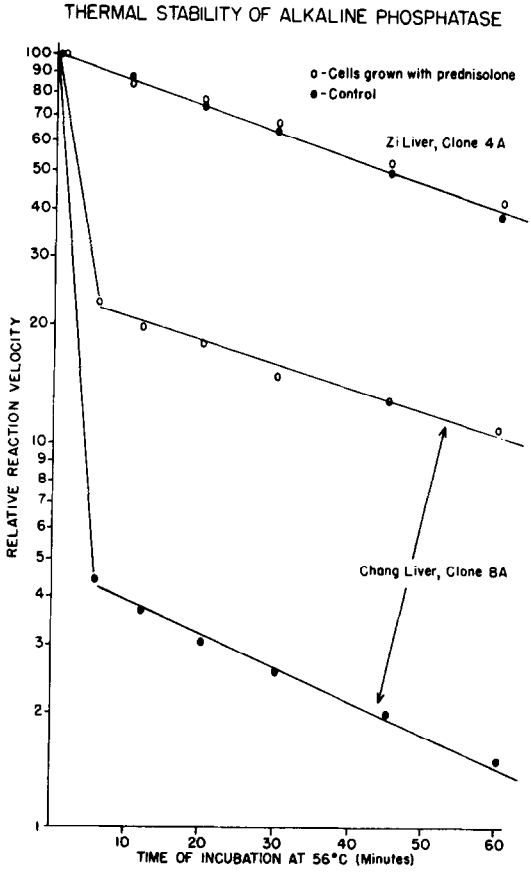


Figure 2

nisolone for 7 days, phosphatase activity decreased to 75% that of control cultures, there was a significantly greater heat-stable fraction. The proportion of heat-stable phosphatase in relation to total activity can be calculated by

extrapolation of the slower heat-inactivation rate to zero time. The changes in total and heat-stable alkaline phosphatase during the course of a growth cycle are shown in Figure 3. While total phosphatase activity in steroid-treated cul-

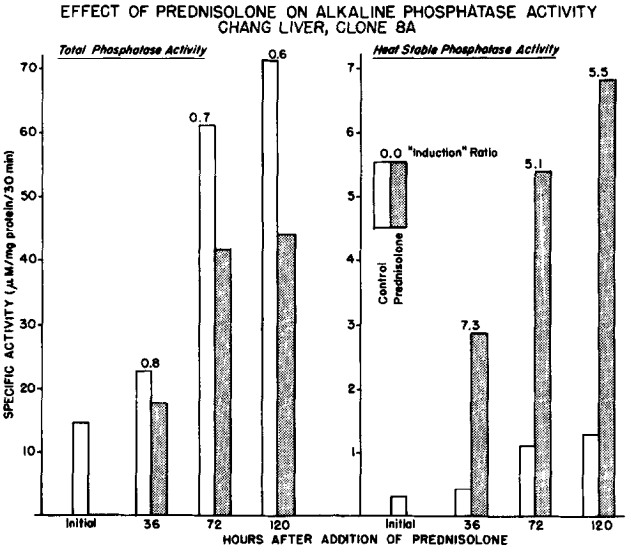


Figure 3

tures of Chang liver clone 8A fell to 80% that of controls 36 hours after addition of prednisolone, reflecting a smaller heat-labile fraction, there was a striking increase in heat-stable activity. As reported previously, there was a progressive increase in specific activity of alkaline phosphatase during a 7-day growth cycle and this also was characteristic of the heat-stable fraction. The steroid effect was superimposed on these changes so that the induction ratios of total and heat-stable phosphatase showed little alteration. Thermal inactivation experiments with mixtures of preparations excluded the likelihood of extrinsic heat-inactivating or heat-stabilizing factors to account for the differences observed. The changes in total and heat-stable alkaline phosphatase with several high activity cell lines after growth with prednisolone are summarized in Table 2.

TABLE 2

CHANGES IN TOTAL AND HEAT-STABLE ALKALINE PHOSPHATASE AFTER GROWTH WITH PREDNISOLONE

Cell Line	Alkaline Phosphatase (umoles/30min/mg protein)		Residual Activity After 10 min. at 56°C in AMP Buffer	
	Control	Prednisolone	Control	Prednisolone
Detroit 6	79.7	36.2	4%	58%
Heart	77.1	73.0	5%	23%
Intestine	33.1	22.1	2%	20%

Our previous findings of heat-stable and heat-labile alkaline phosphatase activities with similar catalytic properties suggested the presence of multiple molecular forms of the enzyme. In all established human cell lines studied to date, prednisolone induced activity of the heat-stable fraction, and, in high activity cell lines, resulted in a reduction in the heat-labile activity. The divergent responses of these two fractions following the addition of the steroid supports the interpretation of molecular heterogeneity of the enzyme in established human cell cultures.

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