

Effect of Zinc on Serum Alkaline Phosphatase Activity in Patients With and Without Cancer.*

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Roche and his associates^{1,2} have reported that a concentration of 10^{-5} M Zn^{++} activated the serum alkaline phosphatase activity in 96 out of 101 individuals who were either normal or had non-malignant disease; the degree of activation ranged from 0 to 87%. In contrast, this concentration of Zn^{++} inhibited the serum alkaline phosphatase activity in 156 out of 185 patients with cancer; the effects in this group ranged from an activation of 24% to an inhibition of 73%.

Upon subjecting these results to statistical analysis, we found that the mean values were 25% for the degree of activation and 14% for the degree of inhibition. The difference between these two values was 39% or about 20-fold the standard error of the difference between the two means (2.1%) and hence highly significant. It was, therefore, considered of importance to determine the extent to which Roche's results were applicable to a population of patients with cancer in this hospital and, in addition, to investigate the effects of other concentrations of Zn^{++} .

The method for the determination of serum alkaline phosphatase was essentially that of A. Bodansky.³ The pH, determined electrometrically, of the serum-substrate-buffer mixture in the absence of any added Zn^{++} was 8.75 ± 0.05 . It was found, in preliminary experiments, that addition of $ZnSO_4$ to give

the desired concentrations of Zn^{++} caused a shift of the pH towards the acid side. Consequently, in all determinations where Zn^{++} was present, appropriate adjustment to give a final pH of 8.75 was made by the addition of a very small volume of 0.1 or 0.75 N NaOH. The pH was checked electrometrically in all determinations of phosphatase activity reported in the present work. The effect of Zn^{++} was evaluated as the ratio of the phosphatase activity in the presence of this ion to that in its absence.

Results. Table I shows that in a group of normal individuals and in patients without malignancy, the ratios at 10^{-5} , 10^{-4} , and 10^{-3} M Zn^{++} were, respectively, 1.01, 0.89, and 0.50. When these results were evaluated statistically, it was found that only the latter two values represented significant changes (Footnote, Table I). In other words, in this group, 10^{-5} M Zn^{++} had no effect on the serum alkaline phosphatase activity, whereas 10^{-4} M Zn^{++} inhibited the phosphatase activity about 10% and 10^{-3} M inhibited it about 50%.

The effect of Zn^{++} on the activity of serum alkaline phosphatase activity was studied in a group of patients with cancer in which the following were the types chiefly represented: leukemia, Hodgkin's disease, carcinoma of the colon, cervix, uterus, stomach, bladder, and breast. Examination of the ratios at each concentration of Zn^{++} showed that there was no apparent correlation of these ratios to age, sex, type of malignancy, level of phosphatase activity, or the pre-operative or post-operative state of the patient. Accordingly, the mean value for the effect of a particular concentration of Zn^{++} was calculated for the whole group of patients. The ratios at 10^{-5} , 10^{-4} , and 10^{-3} M Zn^{++} were, respectively, 1.00, 0.90, and 0.45. Again, only the latter two

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¹ Roche, J., van Thoai, N., Marcelet, J., and Desruisseaux, G., *C. R. Soc. de biol.*, 1946, **140**, 632.

² Roche, J., van Thoai, N., Marcelet, J., Desruisseaux, G., and Durand, S., *Bull. Acad. Med., Paris*, 1946, **130**, 294.

³ Bodansky, A., *J. Biol. Chem.*, 1933, **101**, 93.

TABLE I.
Effect of Zinc Ion on Serum Alkaline Phosphatase Activity in Patients With and Without Cancer.

Group	No. of cases	Ratio of phosphatase activity in presence of zinc to that in its absence			
		Range	Mean	Stand. dev.†	
Individuals without cancer*					
a. At 10 ⁻⁵ M Zn ⁺⁺	25	0.87-1.12	1.01	0.056	
b. At 10 ⁻⁴ M "	14	0.81-1.04	0.89	0.066	
c. At 10 ⁻³ M "	16	0.42-0.60	0.50	0.055	
Patients with cancer					
d. At 10 ⁻⁵ M Zn ⁺⁺	39	0.94-1.07	1.00	0.025	
e. At 10 ⁻⁴ M "	22	0.77-1.00	0.90	0.058	
f. At 10 ⁻³ M "	24	0.27-0.60	0.45	0.079	

* This group consisted of normal individuals and patients with miscellaneous diseases, chiefly heart disease. We are indebted to Dr. Harry Gold for the use of the latter patients from the Beth Israel Hospital Cardiac Clinic.

† The values for the standard deviation were used in calculating "P" values. The "P" values for the comparison of groups *a* with *d*, *b* with *e*, and *c* with *f* were, respectively, 0.4, 0.6, and 0.04. The "P" values for the comparison of groups *a* with *b*, *a* with *c*, *d* with *e*, and *d* with *f* were all less than 0.01.

values represented significant changes. Thus, in patients with cancer, as in those without, 10^{-5} M Zn^{++} had no effect on the serum alkaline phosphatase activity, whereas 10^{-4} and 10^{-3} M Zn^{++} produced significant decreases.

Statistical treatment showed that there was no significant difference between the mean values for the ratios of the individuals without cancer and the patients with cancer at any of the 3 concentrations of Zn^{++} . In view of the high "P" values for these differences, it was considered very unlikely that the extension of the study to larger numbers of patients would bring the "P" value down to 0.01 and

render the differences significant.

Summary. A concentration of 10^{-5} M Zn^{++} failed to affect the serum alkaline phosphatase activity in individuals either with or without cancer. These results are not in agreement with the findings of Roche *et al.*^{1,2} that this concentration of Zn^{++} activates the serum alkaline phosphatase activity of individuals without cancer and inhibits this activity in patients with cancer. Concentrations of 10^{-4} and 10^{-3} M decreased the serum alkaline phosphatase activity, but the extent of these decreases was the same in patients with and without malignancy.

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Multiplication of Influenza Virus in Dead Chick Embryos.

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Recently, the cultivation of *Rickettsia prowazekii* in dead chick embryos was described by Rabinowitz, *et al.*¹ These workers showed that the rickettsiae employed multiplied abundantly in embryos which, although dead, still

contained surviving cells. They confirmed the earlier work of Bucciante² and demonstrated that dead embryos may contain living cells for surprisingly long periods. After holding 3-day-old embryos at 4°C for 24

¹ Rabinowitz, E., Aschner, M., and Grossowicz, N., *Proc. Soc. Exp. Biol. and Med.*, 1948, **67**, 469.

² Bucciante, L., *Arch. exp. Zellforsch.*, 1931, **11**, 397.