CC/NUMBER 17
APRIL 23, 1984

This Week's Citation Classic™

Posen S, Neale F C & Clubb J S. Heat inactivation in the study of human alkaline phosphatases. *Ann. Intern. Med.* **62**:1234-43, 1965.
[Dept. Medicine, Univ. Sydney, and Dept. Biochemistry, Sydney Hosp., New South Wales, Australia]

This paper describes differences in the heat inactivation rates of alkaline phosphatase in sera from different groups of patients. Alkaline phosphatase in serum from patients with skeletal disorders was inactivated at a more rapid rate than serum alkaline phosphatase from patients with hepatobiliary disorders. [The *SCI*® indicates that this paper has been cited explicitly in over 180 publications since 1965.]

Solomon Posen
Royal North Shore Hospital
St. Leonards, New South Wales 2065
Australia

January 17, 1984

"During 1963, a number of patients at Sydney Hospital were found to have unexpected, transient, and spectacular elevations of their serum alkaline phosphatase values. John Clubb, who was then a resident, found that all these patients had received infusions of albumin prepared from human placentas,[1] and we discussed ways and means to distinguish this contaminant alkaline phosphatase (presumably of placental origin), from the endogenously occurring material.

"At Frank Neale's suggestion we tried heat denaturation. We chose 56°C in the first instance because the thermostat of a water bath in the microbiology department (used for complement work) was set at that temperature. It soon became clear that human placental alkaline phosphatase, whether obtained from fresh placentas, from maternal pregnancy serum, from placental albumin preparations, or from the serum of recipients, was uniquely heat stable and differed from other human alkaline phosphatases in a variety of other parameters.[2]

"Differences between serum alkaline phosphatase from patients with skeletal disorders and patients with hepatobiliary disorders were less spectacular but, nevertheless, highly significant.

"Skeletal material (whether derived from bone or from blood) was more readily denatured by physical and chemical agents than biliary alkaline phosphatase ('bone breaks'). We concluded, 'Serum alkaline phosphatase in subjects with skeletal disorders is of bony origin while in hepatic disorders the enzyme is derived from the contents of the biliary tree.'

"When this paper was submitted to the *American Journal of Medicine*, the then editor (Alexander B. Gutman—a great believer in the 'unitary' nature of alkaline phosphatase) returned it with a curt note saying that it needed more than such a crude method to convince him that there were multiple tissue origins of this enzyme.

"After its publication in the *Annals of Internal Medicine*, this method generated a good deal of interest for a variety of reasons. Serum alkaline phosphatase assays constitute one of the most commonly performed tests in clinical medicine.[3] The heat denaturation technique is universally available so that our results were rapidly confirmed in other laboratories.

"In spite of its inherent inaccuracies (no worse than those of electrophoretic methods), the test is still widely employed in the study of tissue sources of circulating alkaline phosphatases. Most importantly, this paper changed clinical thinking about the mechanism of serum alkaline phosphatase elevation in hepatic disease.

"More work needs to be done to clarify the nature of the differences between skeletal and biliary alkaline phosphatases which are probably due to posttranslational factors."[4]

1. **Neale F C, Clubb J S & Posen S.** Artificial elevation of the serum alkaline phosphatase concentration. (Letter to the editor.) *Med. J. Australia* **2**:684, 1963.
2. **Posen S, Cornish C J, Horne M & Saini P K.** Placental alkaline phosphatase and pregnancy. *Ann. NY Acad. Sci.* **166**:733-44, 1969.
3. **McComb R B, Bowers G N & Posen S.** *Alkaline phosphatase*. New York: Plenum Press, 1979. 1004 p.
4. **Goldstein D J, Rogers C & Harris H.** Evolution of alkaline phosphatase in primates. *Proc. Nat. Acad. Sci. US* **79**:879-83, 1982.

