Early results have indicated that non-specific esterases are of widespread occurrence in the marine invertebrates and that they are consistently associated with cells producing acid mucopolysaccharides, as in Mammalia. A strong reaction was sometimes observed in the intestinal contents (e.g. Buccinum, Patella). Other sites containing strong esterases or lipases were digestive glands (Triton, Mya, Buccinum) and gonads. Some unexpected sites for esterase were revealed such as the nematocysts (including the spirals themselves) in the acontia of Metridium.

In the vertebrates Pseudopleuronectes and Lophius esterases were practically confined to the gut in paraffin sections, the liver and kidney enzymes being unable, apparently, to survive the dehydration and embedding process. In Ameirus the kidney enzymes survived but those of the hepato-pancreas did not.

It is evident that comparative histochemical studies of the distribution of esterases can only be complete if cold formalin fixed frozen sections are used. Studies of those esterases which are insensitive to dehydration can much more easily be made, however, on paraffin sections.

## Exploration of a New Method for the Dissolution of Calcium and Magnesium Kidney Stones

Martin Rubin
Georgetown University

The ideal agent to dissolve a calcium or magnesium kidney stone would be one that could be taken by mouth or administered by injection and which would, after passage through the kidney, act directly to dissolve a stone. Such an end result would require that the compound pass through the calcium and magnesium laden body fluids and yet appear in the secreted urine in an "unsaturated" form able to dissolve an alkaline earth concretion. Our prior studies of the application of chelating agents to problems of calcium and magnesium metabolism in animals and humans (Rubin, M. "Application of Chelating Agents to Calcium Metabolism" Transactions of the Fifth Macy Conference Macy Foundation, 1954) as well as studies of others (Hofstetter, R. Schweiz, Med. Wschs. **83**: 608, 1953; Spencer et al., J. Clin. Invest. **31**: 1023, 1952) can be interpreted as indicating that the type of result outlined above occurs to a minor degree after the parenteral administration of the chelating agent, Calcium Versenate. The present pilot study was designed to make a direct test of this interpretation.

Dogfish urine was collected in the usual fashion over a course of several 12 hour control experimental periods. The total calcium and magnesium concentration in each period was measured by Versenate titration at pH 10 in an ammonia buffer using Eriochrome Black T as the indicator. Urine samples which had an excess of divalent ion compared to EDTA were

titrated from wine red to the typical yellow green end point with 0.01 molar EDTA solution. On the other hand urines which had a predominance of the chelating agent were yellow green on addition of the indicator and were titrated to wine red with 0.01 molar standard magnesium ion solution. After several control collection periods the dogfish was given Calcium Versenate by intramuscular administration. Urine collection was continued as indicated in the Table for several 12 hour post injection periods. If the thesis developed in the brief presentation above has any validity it is to be expected that the amount of "free" calcium and magnesium in the urine would decrease following the administration of the chelating agent. If it should happen that more of the unsaturated chelating agent were excreted in the urine than calcium and magnesium, we would be required to add these alkaline earths to the urine in order to saturate the binding ability of the excreted chelating agent. The results obtained are given in the table.

*TABLE I*

Excretion of "Free" Calcium and Magnesium in Dogfish Urine Following Calcium Versenate, I.M.

| *Treatment Period* | *Excretion Divalent* m M/minute |
|---|---|
| Pretreatment control | 0.0011 |
| Pretreatment control | 0.0013 |
| Pretreatment control | 0.0015 |
| First post injection period | 0.0011 |
| Second post injection period | 0.0004 |
| Second injection, first period | -0.00034 |
| Second injection, second period | -0.00006 |

Data are presented for apparent "free" calcium and magnesium ion in the various experimental periods. It is evident that the normal divalent ion output/minute in the pretreatment period decreases after administration of the chelating agent, and finally goes through a negative phase before again reverting back to the more normal pattern.

This highly illuminating experiment opens a completely new avenue to the problem under consideration. Present knowledge of the relation of chemical structure to the physical-chemical and pharmacological action of chelating agents should permit rapid attainment of agents which will have maximum efficiency for the purpose in mind.