

## Research Reports: 1954

- 4) Urea clearance increased consistently, by as much as 8-fold.
  - 5) The duration of diuresis was 2 to 4 hours.
  - 6) A second dose of Adrenalin 2 to 4 hours later evoked a second diuretic response.
  - 7) Sodium and potassium clearances increased in one fish in which they were measured.
  - 8) No significant change occurred in plasma osmolarity.
- It is concluded that Adrenalin increases osmotic diuresis, but the osmotic components in the urine are not fully known.

### Comparative Histochemistry of Esterases and Mucopolysaccharides

A. G. Everson Pearse

University of Alabama, University of London

Tissues from the following species were used in this study.

|  |                           |
|--|---------------------------|
| <i>Strongylocentrotus droehbachensis</i> | <i>Halicystus</i>         |
| <i>Cuccummarai frondosa</i>              | <i>Buccinum undatum</i>   |
| <i>Patella vulgata</i>                   | <i>Mytilus edulis</i>     |
| <i>Metridium dianthus</i>                | <i>Mya arenaria</i>       |
| <i>Eutaenia sirtalis</i>                 | <i>Anodonta cygnea</i>    |
| <i>Pseudopleuronectes americanus</i>     | <i>Balanus balanus</i>    |
| <i>Lophius piscatorius</i>               | <i>Triton rubiculus</i>   |
| <i>Ameirus nebulosus</i>                 | <i>Pagurus bernhardus</i> |
| <i>Triturus viridescens</i>              | <i>Asterias vulgaris</i>  |

It was proposed to make a comparative study of the non-specific esterases and their association with mucopolysaccharides and mucoproteins. Phosphatase, 5-nucleotidase, and some other enzymes were studied incidentally. A single method of fixation and embedding was employed (cold neutral formalin fixation, cold acetone dehydration, paraffin embedding) which affords excellent histological preservation together with adequate preservation of alkaline phosphatase, lipase and non-specific esterases. Many of the tissues were also studied as frozen sections after cold formalin fixation. Some estimate was thus obtained of the losses of enzymes due to dehydration, clearing and embedding.

Coupling azo dye methods were used to demonstrate alkaline phosphatase and non-specific esterase and an indoxyl method for lipase and non-specific esterases. A standard Ca-CoS method was used for 5-nucleotidase. Polysaccharides and mucopolysaccharides were revealed by the PAS method (McManus - Hotchkiss); Alcian blue and toluidine blue studies of metachromasia were used to define the acid mucopolysaccharides. The whole of the material is now being subjected to a much wider spectrum of histochemical investigation.

## Research Reports: 1954

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 Ameiurus 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