

Nothman's interesting discovery² of the presence of phosphatase in the pancreatic juice of dogs and its increase after ligation of the duct with subsequent rise of the level of serum phosphatase is, therefore, in full accord with the histochemical distribution of the enzyme in the pancreas of that species. Similar behaviour can be predicted for the rabbit. But caution is necessary in applying such results from animal experiments to clinical tests, and Nothman's suggestion that the level of serum phosphatase might be used as a diagnostic aid in pancreatic diseases (for example, duct occlusion) seems to be based on the erroneous assumption that a state of affairs exists in the human pancreas similar to that of the dog.

The cells of the acini themselves are histochemically negative with regard to phosphatase in all the species examined. It would thus appear that in the two 'positive' species (dog and rabbit) the epithelial cells of the duct system, particularly its finest ramifications, are responsible for the secretion of the enzyme. This interpretation would rest on the assumption that the site of the heaviest histochemical reaction for phosphatase is also the site of its formation. It is, however, conceivable that the enzyme, in these two species, is actually secreted by the cells of the acini and only becomes activated when present in the duct system (including the lumina between the centro-acinar cells), and that on applying the histochemical test, the adjacent epithelial cells and their nuclei supply suitable and perhaps necessary surfaces and interfaces for the reaction to take place, which then brings about the heavy salt precipitation in these cells.

Finally, it may be added that I found the islet cells strongly positive only in the dog; in the rat the peripheral cells of the islets sometimes show a weak (brownish) and doubtful reaction.

Further details of these and other comparative histochemical studies on the distribution of phosphatase will be published elsewhere.

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Demonstration of Phosphatase in Decalcified Bone

As the decalcifying fluids commonly used in histology destroy alkaline phosphatase, it has so far only been possible to demonstrate the enzyme in undecalcified bone. The disadvantages of current methods are threefold: (1) Only bones from embryos or very young animals can give sections sufficiently thin to enable localization of phosphatase to be established satisfactorily by the method of Gomori¹ or of Menten, Junge and Green.² (2) If the Gomori method for bone is employed, the preformed phosphate is stained black and the site of phosphatase purple. Controls only show the black bone salt. It is extremely difficult to decide whether there are areas in which both phosphatase and bone salt occur concurrently. (3) The Gomori method suffers from the obvious disadvantage of requiring treatment of the phosphatase-containing tissue with ammonium sulphide (which is an inhibitor of the enzyme³) before and during incubation of the sections.

The present method permits bones of adult rats and mice to be decalcified without adverse effects on the enzyme. It is based on the facts, established by Cloetens⁴,⁵, that alkaline phosphatase is reversibly inactivated by acid solutions having a pH greater than 4.5, and that it can be reactivated afterwards by alkaline solutions. Inactivation is retarded by the addition of Zn⁺⁺ to the acid medium, and reactivation is aided by glycine.

It was found that kidney sections kept in acetate or citrate buffer at pH 4.4-4.6 in the presence of Zn⁺⁺ (10⁻⁴M) can be completely reactivated even after 14 days by treatment with 0.075 per cent glycine in 1 per cent sodium barbitalone. (The glycine must be washed out before incubation as it interferes with the precipitation of calcium phosphate³.)

Small pieces of bone fixed in 80 per cent alcohol and brought to water were left in the buffers until decalcified, the liquids being changed daily and kept at 10° C. The time taken for decalcification varied from three to fourteen days according to the size and consistency of the bone. The tissues were then washed in water, 'reactivated' for two hours in glycine-barbitalone at 37° C., washed thoroughly in running water, dehydrated and embedded in paraffin (58° m.p.). Sections were cut at 8 μ and the phosphatase demonstrated by the original Gomori-Takamatsu method⁶.

The distribution thus found agrees essentially with that described by other workers^{1,4}. The ground substance of bony trabeculae or compact bone contains no phosphatase. Superficially placed osteocytes with their processes, and Sharpey's fibres, stand out black against the colourless matrix.

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Vitamin Storage and Utilization in the Organism

DR. T. K. WITH's suggestion¹ that carotenoids, such as cryptoxanthin and β -carotene, are vitamins in their own right commands considerable sympathy, but we do not think that the data advanced in that communication settle the point. The efficiency of utilization and the storage of vitamin A in the liver of the rat and the chick are affected by many factors which control the growth response or quantity found. These include the amount and kind of vitamin A fed², the amount of sparing agents, more particularly the tocopherols fed at the same time³, and the idiosyncrasies of the animal⁴, which is another way of saying that we do not yet know the complete physiology of vitamin utilization and liver storage.

We believe that the high value of utilization of cryptoxanthin by the chick may be explained completely by the large quantities of γ -tocopherol and other covitamins and sparing agents which are present in yellow corn. At least Dr. With's contentions would be considerably strengthened if the exact contribution made by the sparing agents could be measured.

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Effect of Röntgen Irradiation on the Serum Content of Hæmagglutinins in Human Blood

THE behaviour of the natural antibodies present in the blood of normal individuals, under irradiation with Röntgen rays, has not been much investigated. The object of our present study was the behaviour of natural anti-sheep agglutinins in men who had been subjected to röntgenotherapy.

We have chosen as the indicator of the effect of irradiation the anti-sheep agglutinins, since in recent years this normal antibody has been the subject of extensive studies. Their standard titre in normal individuals is generally equal to the dilutions 1:4-1:8 of the serum. In our own investigations, on some hundreds of normal individuals, we have found in normal men only exceptionally a titre as high as 1:16. It is well known from clinical observations that the titre of anti-sheep agglutinins may rise considerably in some definite conditions, particularly in infectious mononucleosis and after injections of horse serum (normal or immune). In the first condition we have seen the titre in one case as high as 1:2,800.

The interpretation of this rise is relatively easy in serum sickness, as horse serum belongs to Forssman's antigens. In the case of infectious mononucleosis, the rise of the titre may be due to an unknown etiological agent, which is acting as Forssman's antigen, or to some unspecific stimulation of the reticulo-endothelial system. The clinical and experimental work of this Institute has shown that X-rays in small doses act as a powerful stimulant of this system. In our observations we therefore studied the possible influence of radiological stimulation of the reticulo-endothelial system on the titre of anti-sheep agglutinins.

In all we have had under observation thirty-two persons, who received röntgenotherapy for different causes: cancer, leucæmia, inflammatory states, and so on. Dosage varied from 50 r. to 6,000 r. In no case did the titre of sheep agglutinins after irradiation rise above the dilution of 1:10; it therefore remained normal.

These observations show that X-rays do not influence the behaviour of normal anti-sheep agglutinins; hence the rise of the titre in some conditions probably does not depend on the stimulation of the reticulo-endothelial system, but on active immunization.

The first appearance of hæmagglutinins in infants at the age of four to six months (earlier on artificial than on breast feeding) is probably due to changes of the intestinal flora at this period, some intestinal micro-organisms acting as Forssman's antigen.

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Nutritional Studies on Blood-sucking Arthropods

A GREAT deal of study has been devoted in recent years to the nutritional requirements of insects, particularly those of economic importance. Blood-sucking insects, however, have suffered neglect, although they present problems of unusual interest. We have undertaken investigations on the rate of development of nymphs of the bed-bug, *Cimex lectularius* L., and of the fertility of the resulting adults, by feeding them directly on a number of different hosts or *in vitro* through a membrane.

It was found possible to rear first instar bugs to the adult stage by feeding them on defibrinated hæmolysed blood through a mouse skin membrane. Attempts to vary the nature of the diets fed through the membrane were hampered by the refusal of the bugs to consume many of the diets offered them. Moreover, slight changes in the composition of the blood, such as slight dilution with isotonic saline solution, resulted in the death of all the nymphs by the time the third instar was reached. In spite of these difficulties, it was possible