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Hemoglobin Excretion in the Hornpout, *Ameiurus nebulosus*

O. C. Jaffe

University of Arkansas

The passage of hemoglobin through the glomeruli and the site of absorption of this protein was examined in a series of experiments on the hornpout kidney. This species was used as an example of a teleost having a differentiated renal tubule. Commercial hemoglobin (DIFCO) dissolved in saline was injected intraperitoneally into the test animals. The kidneys were examined in paraffin sections and as dissected tubules. With the latter method the tubules were partially dehydrated (50% alcohol) and stained with benzidine and peroxide. The benzidine stain was also used with paraffin sections, in addition to the Dunn hemoglobin stain, the PAS and others. Both methods of examination indicated that hemoglobin passed readily through the glomeruli and was reabsorbed in the proximal tubules. Some evidence of peroxidase activity in the distal tubule was indicated but this aspect requires further study. The filter-ability of this protein and its site of absorption corresponds to that described in the mammal. Further studies, in which the tubules were dissected in saline and stained with Janus green, indicated that protein absorption in the proximal tubule is accompanied by transformations in the form of the mitochondria as described in the mammal by Oliver.

These experiments were undertaken as part of a comparative study on protein excretion. Earlier observations by the writer had found hemoglobin uptake in the collecting tubule of the pronephros (*Rana pipiens*). Smetena (Am. J. Path. Vol. 18, 1942) has described protein uptake in certain of the distal tubules in *Amblystoma*. In both cases nephrostomatous tubules are involved. Apparently the distal segment of the "open" tubule can absorb protein in addition to the proximal segment, a function seemingly lost in glomerular kidneys (fish, mammal). This problem is under further investigation.

Mutarotase in the Kidney of Fish and Frog

Albert S. Keston

New York University

Mutarotase, an enzyme which was discovered by this investigator in animal tissues, particularly kidney, catalyzes the interconversion of the various forms of certain reducing sugars. It has been proposed by this investigator that mutarotase is involved in the transport of sugars into cells and the reabsorption of sugars by kidney. He has previously reported evidence consistent with this point of view in that the sugars which are transported across cell membranes in response to insulin, and which are

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reabsorbed by kidney, are substrates of mutarotase while the others which are not reabsorbed or transported are not mutarotase substrates. Furthermore, mutarotase is inhibited by low concentrations of phlorizin, a drug which is known to block reabsorption of glucose.

During the stay at the laboratory experiments were carried out to determine the mutarotase content of kidneys of various amphibians and fishes. Previously mutarotase had been found in the kidneys of mammals and birds. The aglomerular fish, *Lophius*, was found to contain no detectable amounts of mutarotase in its kidney, while frog and catfish contained appreciable amounts of the enzyme in their kidneys. These data are regarded as consistent with the involvement of mutarotase in reabsorption of sugars, inasmuch as the aglomerular *Lophius* does not excrete appreciable amounts of glucose and the others reabsorb glucose.

Effect of Commercial ACTH upon Chromatophore Number

Herbert G. Langford
Medical College of Virginia

While treating a case of Addison's Disease with cortisone and DOCA, the regression of a mole, which later proved to be a junctional naevus, was noticed. It was presumed that the ACTH level had been reduced by therapy. It was, therefore, hypothesized that a substance behaving like ACTH controlled the number of pigment-bearing cells. ACTH had been shown previously to cause pigment dispersal of chromatophores, but there had been no studies of its effect upon the number of such cells. Therefore, the following study was done.

Young bull-frogs (*Rana catesbeiana*) were used as the test animal. The number of chromatophores in an area 1.5 mm. square of the web of one hind limb was counted. Alternate animals were injected in the dorsal lymph sac with 0.16 units ACTH daily. The number of chromatophores was followed daily. At the end of five days, six frogs in both series were available. The untreated animals showed a mean drop of 0.3 chromatophores while the treated series showed a mean rise of 53.1. This is a statistically significant difference.

It is felt that ACTH or a substance contaminating it controls chromatophore number as well as pigment spread in the frog. The case cited above suggests that the same phenomenon may take place in man.