Reports and Letters

Alkaline Phosphatase in the Kidneys of Aglomerular Fish

Renal tubular alkaline glycerophosphatase has been demonstrated histochemically in most vertebrate species examined. Perhaps the only exceptions are those reported by Wilmer (*1*), who was unable to find the enzyme in the kidneys of the aglomerular toadfish, *Opsanus tau,* the spotted salamander, *Amblystoma maculatum,* and the snapping turtle, *Chelydra serpentina.* The widespread occurrence of this enzyme has justified the belief that it plays some fundamental role in kidney function (*2*). Because the aglomerular kidney does not have occasion for resorptive activity and is apparently incapable of excreting sugars (*3*), Wilmer provisionally interpreted the alleged absence of alkaline phosphatase in the tubule of the toadfish kidney as valid evidence of the relation of the tubular enzyme to glucose transport (*1*). His hypothesis has gained considerable acceptance (*2, 4, 5*).

Danielli has cast some doubt on the validity of this idea by incidental references to unpublished studies by Lorch and himself revealing the presence of tubular alkaline phosphatase "in a number of species of aglomerular fishes" (*6, 7*). The present communication calls attention to this finding and reports my own demonstration of tubular alkaline phosphatase in the three particular species reported negative by Wilmer.

Freshly caught specimens of *Opsanus tau, Amblystoma maculatum,* and *Chelydra serpentina* were obtained locally in season (*8*), and the kidneys were removed as soon as possible after capture. Fixation was carried out in 65-percent alcohol for 24 hours. After the specimens were imbedded and sectioned, alkaline phosphatase was demonstrated according to the method of Gomori.

Five specimens of *Opsanus tau* were examined. Although the kidneys were removed for fixation as soon as the fish was taken from the water, the results were distinctly variable, but strong local tubular activity was demonstrated in most tissue blocks (Fig. 1). Activity tended to be least at the centers of the blocks, suggesting a fixation artifact arising from an unusual lability of the tubular alkaline phosphatase in this animal. No difficulty was encountered in demonstrating vigorous tubular activity in kidneys of two *A. maculatum* and one *C. serpentina.*

The evidence of enzyme lability noted in the toadfish seems to offer a reasonable explanation of Wilmer's negative findings. Although he does not state the source of the *A. maculatum* or *C. serpentina* examined by him, the *O. tau* had been for a long time in an aquarium. It is suggestive that Grafflin (*9*) has remarked on the lability of renal function in fishes in captivity. In addition to the experience related with toadfish, I have sometimes found it impossible to demonstrate alkaline phosphatase in the renal tubules of box turtles (*Terrapine carolina carolina*) that have been kept in the laboratory, although freshly caught specimens are well supplied with it. In any event, it is clear that acceptable evidence of the absence of this enzyme in any species can be based only on wide experience.

The general conclusions that arise from the distribution of tubular alkaline phosphatase among vertebrate species are altered by these findings and those cited by Danielli. Although the possibility cannot be excluded that this enzyme survives in aglomerular fishes as a vestigial characteristic inherited from their glomerular ancestors (*10*), its presence argues, barring this, *against* its involvement in glucose resorption. Conclusions with respect to the possible functional importance of tubular alkaline phosphatase based on its general distribution are reinforced by the reduction of the list of excepted species to none. The fact that individual animals under certain conditions can apparently exist without it does not necessarily contradict this estimate. Instead it may be that its function is of sufficiently general nature that alternative mechanisms exist which, in case of necessity, can accomplish a corresponding and vitally sufficient result.

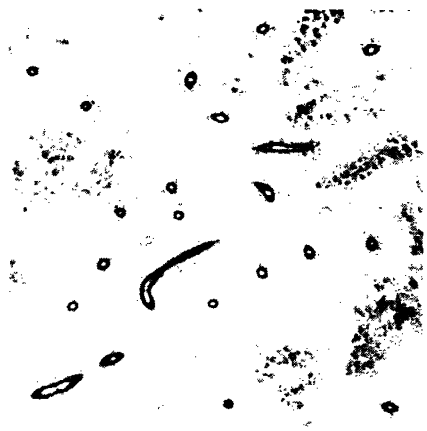

Fig. 1. Gomori alkaline phosphatase reaction in renal tubules of *Opsanus tau.* (×150)

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Ultraviolet Irradiation of Pyrimidine Derivatives

The substances identified up to the present as products of the ultraviolet irradiation of pyrimidine compounds include ammonia, urea, oxamide, parabanic acid (*1*). These clearly arise by more or less extensive disruption of the pyrimidine ring. However, in 1949 Sinsheimer and Hastings (*2*) reported that uracil, uridine and also cytidylic acid, after brief irradiation with light of wavelength 254 mμ lost their characteristic ultraviolet absorption band and yielded a product that, on treatment with acid, spontaneously regenerated the original compound. More detailed information about this reaction has been reported recently by Sinsheimer (*3*). Since this reversible photoreaction may constitute an initial step in the more drastic photodecompositions it seemed to us important to know the structure of the "reversible" product.

A survey of variously substituted pyrimidine derivatives disclosed that 1,3-

dimethyl uracil (DMU) exhibited the same reversible type of photoreaction as uridylic acid, and its physical and chemical properties made it particularly suitable for the isolation of the product. We therefore irradiated 0.2*M* aqueous DMU with 254 mμ light (G.E. 15-w germicidal lamps), fractionated the product by partition between chloroform and water, and purified it by chromatography on alumina and by repeated recrystallization from mixtures of chloroform and *n*-hexane (*4*).

The isolated material is a white, crystalline solid, (mp 102°C) readily soluble in water and in ethanol, slightly soluble in chloroform and insoluble in *n*-hexane. Its aqueous solutions show only end-absorption in the ultraviolet (Fig. 1). On treatment either with acid (*p*H 1) or alkali (*p*H 10) it is rapidly and quantitatively reconverted to DMU, identifiable both by the detailed agreement of its ultraviolet absorption curve with that of known DMU (Fig. 1) and by its characteristic distribution coefficient of 3.47 between chloroform and water at 20°C.

Analysis of the isolated product of irradiation gave the results shown in Table 1. The mean value found for its molecular weight by the cryoscopic method was 158.3. This may be compared with an estimated equivalent weight of 157 with respect to reversion to DMU (mol. wt. 140).

The molecular weight, which differs by 18 from that of DMU, and the analytic data of Table 1 point to the conclusion that the product of irradiation arises by the addition of a molecule of water to DMU. The disappearance during irradiation of DMU of the characteristic reaction with bromine water (*5*), which is known to involve addition at the 5 : 6 double bond, together with the loss of the absorption band at 265 mμ, which

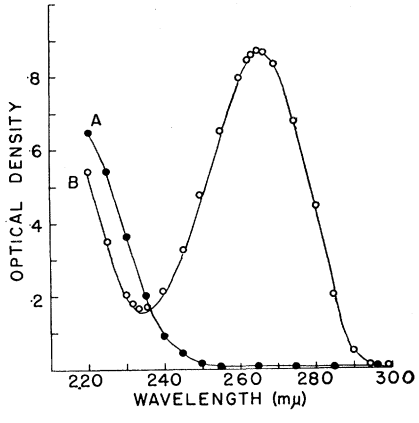

Fig. 1. Absorption curves. *A*, product of irradiation, $10^{-4}M$ in water. Points on *B*, product of irradiation, $10^{-4}M$, after heating at 100°C for 5 min at *p*H 1.0. Solid curve *B*, $10^{-4}M$ DMU in water.

Table 1. Analysis of the product of irradiation of DMU.

	Calculated for DMU $C_6H_8O_2N_2$ (%)	Calculated for $C_6H_{10}O_3N_2$* (%)	Observed (mean values)
Carbon	51.4	45.58	45.55
Hydrogen	5.71	6.33	6.38
Nitrogen	20.00	17.72	17.69

* That is, DMU plus a molecule of water.

also involves this double bond as part of the chromophore (*6*), suggests that the product is either the 6- or 5-hydroxy-1,3-dimethyl hydrouracil. The presence of an —OH group is further indicated by the observation of a strong band at 2.98 μ in the infrared absorption spectrum of the isolated product in alcohol-free chloroform, a band that is absent from similar solutions of DMU. This is also in keeping with the increased polarity of the product over DMU, as is evidenced by its much lower distribution coefficient (0.03) in a chloroform-water system.

Attempts to synthesize 6-hydroxy-1,3-dimethyl hydyrouracil have so far been unsuccessful. Reduction of 6-hydroxy-5, 5-dibromo-1,3-dimethyl hydrouracil catalytically with platinum and hydrogen, with continuous neutralization of the hydrobromic acid liberated, has yielded mainly DMU. However, it was found that the platinum catalyst itself also catalyzed the recovery of the isolated irradiation product to DMU, even in neutral solution, so that this result is in keeping with the postulated structure, although it does not prove it. Reduction methods involving the use of strong acid or alkali are ruled out because of the lability of the desired compound at extremes of *p*H.

That uracil and uridylic acid react similarly to DMU on irradiation is indicated by a number of parallels in their behavior. (i) The product of irradiation in each case can regenerate the original compound on treatment with either acid or alkali. [It should be noted that with respect to the alkaline conditions our finding differs from that of Sinsheimer (*3*).] (ii) Simultaneously with the loss in ultraviolet absorption during irradiation, all these compounds lose their ability to react rapidly with bromine water, indicating loss of the 5 : 6 double bond. (iii) The product of irradiation of uracil is more polar than uracil itself, as is indicated by its lower R_f on a paper chromatogram employing *n*-butanol saturated with water as mobile phase. The product in this case is easily located by the ultraviolet photographic method of Markham and Smith (7) after the developed chromatogram has been exposed to HCl vapor to regenerate the uracil.

If our conclusion regarding DMU is correct, the analogous product for uracil should be either 6- or 5-hydroxy hydrouracil. The 5-hydroxy compound is known (*8*) and does not have the required properties. The 6-hydroxy compound, on the other hand, does not appear to have been described. We believe that this omission is a result of the extreme facility with which it loses water to form uracil.

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Tartronate as a Possible Coenzyme of Oxalosuccinic Carboxylase

Oxalosuccinic carboxylase catalyzes the oxalosuccinate⟶α-ketoglutarate decarboxylation in the citric acid cycle and is known to be activated by Mn^{++} in acid solution (*1*). In the work reported here, tartronate (hydroxymalonate) was found to act as coenzyme of oxalosuccinic carboxylase at physiological *p*H. This was indicated by two methods: (i) titration of the carbonate formed by the decarboxylation and (ii) measurement of the optical activity of the reaction mixture.

Method A, titration of the carbonate. Veal heart was ground, extracted with acetone, and dried in a vacuum (*1*). Water (2 ml) added to the sample (0.5 g) was hard-frozen and melted; the sample was pressed and washed (4 × 1 ml water) with a 10-minute interval between washings. This extract was dialyzed 18 hours against cold water as a thin (2- to 3-mm) layer in a closed cellulose sack from which most of the air was expelled before it was tied off, and which was clamped between two sheets of 10-mesh,