specific mitochondrial proteins. In this regard, it may be pointed out that of a substantial number of purified proteins examined thus far, only serum albumin, myosin, cytochrome *c*, and mitochondrial protein interacted with pentachlorophenol.

The demonstration that representative uncoupling phenols bind to mitochondrial protein suggests the following mechanism for their action: These reagents interact with the mitochondrial proteins participating in oxidative phosphorylation at sites other than the active centers and induce configurational changes, analogous to the allosteric effects proposed by Monod, *et al.* (*8*). These altered configurations result in modified enzymes whereby coupling activity not only is lost, but other latent enzymatic activities may appear. Such changes may or may not be reversible, depending on the affinity of the proteins for the uncoupling reagent.

The effect of lipid solubility is not discounted by this proposed mechanism. Rather, we envisage that uncoupling reagents must traverse a lipid barrier or a lipid-protein interface to reach the protein sites where they are bound. Consequently, at least two processes take part in their uncoupling action: the permeation of a nonspecific lipid barrier, and the binding by and affinity for specific proteins of the mitochondrion.

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## Uric Acid Transport System: Apparent Absence in Erythrocytes of the Dalmatian Coach Hound

Abstract. *The Dalmatian coach hound appears to lack a mediated transport system for uric acid, which can be demonstrated in the erythrocytes of other dogs by hypoxanthine inhibition of uric acid uptake. This transport deficiency may be a manifestation of a genetic defect which has different metabolic consequences in the liver, in the kidney, and in other tissues, depending on the direction of their differentiation.*

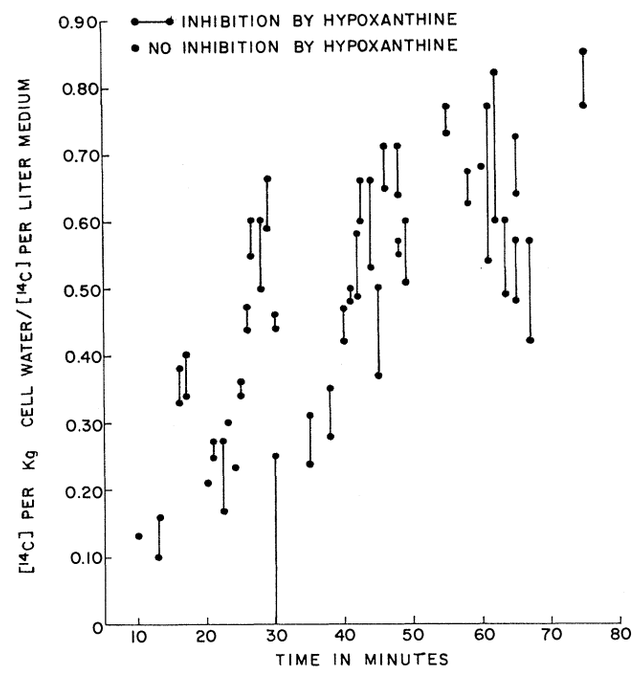
Congenital defects in the renal tubular resorption of two groups of amino acids are accompanied by related defects in their intestinal absorption (*1*). Absorption in such tissues is probably produced by special arrangements of the ordinary uptake or extrusion processes apparently present in all cells (*2*); as a consequence, genetic defects in renal and intestinal transport may be represented to some degree in the transport activity of other cells in the organism, whatever the course of their differentiation (*3*). It is in this connection that we have found that a specific transport system for uric acid, present in the erythrocytes of dogs other than the Dalmatian, appears to be largely if not entirely absent in the Dalmatian coach hound.

Uricase has long been recognized to be present in the liver of the Dalmatian (*4*). In 1938 Klemperer, Trimble, and Hastings found that, although much less uricolytic activity could be observed in surviving liver slices, the amount of uricase activity in suspensions of finely ground liver was not characteristically lower for the Dalmatian coach hound (*5*). Subsequent investigation has established two peculiarities of purine metabolism in the Dalmatian coach hound:

1) There is a defect in the renal transport of uric acid. This defect was at first supposed to be the simple absence of the characteristic tubular resorption of this substance (*6*); but subsequent investigation (*7*), including stop-flow analysis (*8, 9*), showed that the usual net resorption of uric acid under some conditions can be replaced by a net secretion, also occurring in the proximal tubule (*8*).

2) There is an additional obstacle to the oxidation of uric acid (*5, 6, 10*). The plasma uric acid rose much more steeply in the Dalmatian than in other dogs after the ureter was occluded (*6*); furthermore, the infusion of uric acid caused a larger and more persistent rise in the plasma uric acid of the Dalmatian coach hound, despite faster urinary excretion (*10*). The most plausible explanation of the metabolic abnormality lies, we believe, in the presence of a generalized defect in a specific transport process for uric acid. This defect may account not only for the decreased renal retention of uric acid, but also for a difficulty of uric acid access to the interior of various cells and to the hepatic uricase.

Blood samples of two unrelated pure-bred Dalmatian coach hounds were compared with samples from a number of dogs of other breeds. One of these dogs had the form and markings of a Dalmatian coach hound, but was obviously not full blooded, and lacked the peculiarity in uric acid metabolism. Because of the difficulty we have had so far in obtaining Dalmatians, the observations were repeated often on the same animals. The excretion of characteristic proportions of uric acid was established by urine analysis, and the elevated plasma levels were confirmed. Blood cells were separated by centrifugation, and washed three or four times with isotonic 0.11*M* phosphate solution, *p*H 7.0, containing 0.1 percent glucose, under conditions tending to eliminate leukocytes. The red blood cells were then incubated for periods up to 80 minutes in 20 volumes of the phosphate solution containing either 0.1 or 0.25m*M* uric acid-8-$^{14}$C. In parallel experiments 1m*M* hypoxanthine (*11*) was included in order to inhibit the mediated portion of uric acid entry. Samples of the suspension were then taken and centrifuged for 2 minutes in a refrigerated centrifuge to separate the cells. The sedimented mass



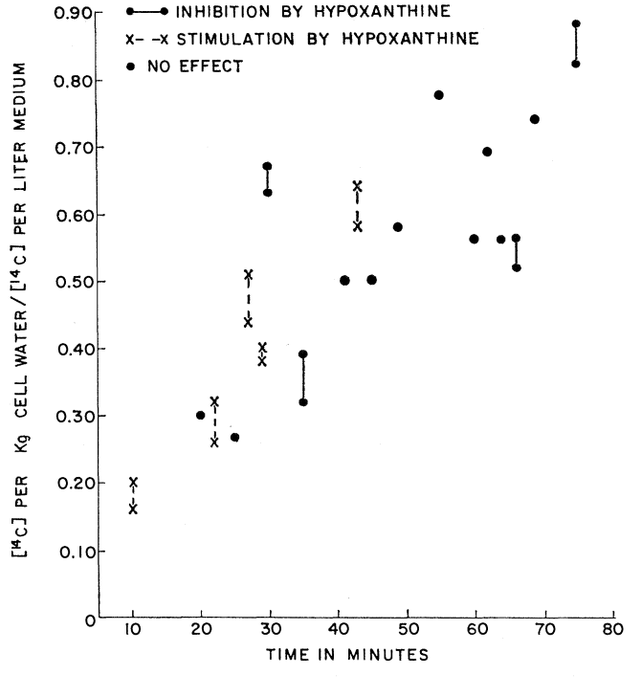




Fig. 1 (left). The action of hypoxanthine on the uptake of uric acid-8-$^{14}$C by erythrocytes of the dogs other than the Dalmatian. All results plotted between 20 and 30 minutes represent 20-minute incubations; those between 30 and 40 minutes, 30-minute incubations; and so on. The round points separated by a line show the cases where a measurable decrease in the distribution ratio was produced for the selected time interval. Round points alone show five determinations, which were randomly distributed among the experiments, where no measurable effect was observed. Fig. 2 (right). The action of hypoxanthine on the uptake of uric acid-8-$^{14}$C by erythrocytes of two Dalmatian coach hounds; $\times$- -$\times$ represents the three determinations, which occurred randomly among the experiments, where uptake in the presence of hypoxanthine was slightly *greater*. Other designations are as in Fig. 1.

of erythrocytes was freed of the last trace of supernatant medium by touching the surface with pointed wicks of filter paper. It was then treated with five portions of distilled water, followed by 2 volumes of 10 percent sodium tungstate and 2 volumes of 0.67*N* sulfuric acid. The suspending medium was treated in the same way. The radioactivity of the resulting extracts was determined by liquid-scintillation counting, with 0.2-ml portions in an alcohol-toluene solution of standard phosphors. A thixotropic agent, Cab-o-Sil, 3 percent, was used to keep the uric acid from precipitating. The distribution ratio of the counts per minute per kilogram of cell water to the counts per minute per liter of suspending medium was calculated from these results.

Figure 1 shows a factor adding to the difficulty of the present demonstration, namely the rather limited extent to which uric acid entry into the erythrocyte of the dog is slowed by the presence of hypoxanthine. The mean decrease for all time intervals studied was 13 percent. In man, the effect is much larger (*11*). Because the hypoxanthine concentration chosen appears to be nearly large enough to saturate the relevant specific transport process for uric acid and other purines (*11*), most of the residual uric acid uptake in the presence of hypoxanthine may be due to diffusion or, conceivably, to another mediated process less easily saturated. The substantial role of this hypoxanthine-insensitive pathway of uric acid entry may well be an expression of the unusual anion permeability of the red blood cell.

Figure 2 shows that the uptake of uric acid characteristically does not respond to the presence of hypoxanthine when blood cells from Dalmatian coach hounds are used (mean decrease, −1 percent). The contribution of the hypoxanthine-sensitive pathway of entry into this cell is too small to cause any observable diminution of total uric acid entry, but the absence of the hypoxanthine-sensitive component is evident.

The probability that the difference in uptake (Figs. 1 and 2) had been observed by chance and was not a real difference was less than 0.01, considering all blood samples compared. The same characteristic difference in the response to added hypoxanthine was shown also for thicker suspensions of erythrocytes when only 1 volume of the same buffer solution was used, and the uric acid uptake was measured by the loss of radioactivity from the medium.

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