was not phenylacetylglycine and has not yet been identified.

These initial studies indicate that certain marine animals handle phenylacetic acid differently from most mammalian species. Parenterally administered phenylacetic acid is excreted slowly by fish in urine and to a lesser extent in bile as an unidentified metabolite. Metabolism of phenylacetyl-coenzyme A as measured by the formation of ethyl acetate soluble products occurs most readily in kidney mitochondria in the presence of exogenous glutathione. We hope to identify the unknown compounds which are presumably amino acid or peptide conjugates, and to study further peptide conjugation in marine species.

1973 #25

THE EVOLUTION OF A FACILITATED DIFFUSION PATHWAY FOR AMIDES IN THE VERTEBRATE ERYTHROCYTE

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There is increasing evidence that urea movement across cell membranes may involve more than simple diffusion. This applies not only to urea reabsorption by the kidney and toad bladder but to urea movement across the erythrocyte membrane. Macey and Farmer have shown for example that phloretin blocks the entry of urea into the human erythrocyte but has no effect on osmotic water flow or the entry of a number of other solutes (Biochim. Biophys. Acta, 211, 104-106, 1970). They concluded that urea movement across the human erythrocyte membrane was by facilitated or carrier-mediated diffusion.

We have surveyed the erythrocytes of representative vertebrates, from hagfish to man, to determine whether amide movement takes place by simple diffusion or facilitated diffusion. Two criteria were used in this study: 1) the relative rates of entry of urea and its more lipophilic analogue, acetamide; 2) the presence or absence of an inhibitory effect of 6×10^{-4} M phloretin. A more rapid rate of entry of acetamide and absence of an inhibitory effect of phloretin would be consistent with simple diffusion, while more rapid entry of urea and inhibition of entry by phloretin would suggest facilitated diffusion.

Osmotic hemolysis of erythrocytes at 5°C was used to determine the rate of amide entry in the presence and absence of phloretin. 0.02 to 0.1 ml of an erythrocyte pellet was introduced into a solution containing urea or acetamide at concentrations of 0.15 to 2.0 M and after a time interval of 0.5 to 40 minutes the suspension was centrifuged and the extent of hemolysis of the supernatant was read in a spectrophotometer at 510 mμ. Under the conditions of these experiments the rate of hemolysis is determined by the rate of entry of urea or acetamide (Am. J. Physiol., 224, 1109-1115, 1973).

Our findings are summarized in Table 1. We would conclude from this study that amide movement across the erythrocyte membrane occurs by simple diffusion in representatives of the Agnatha, Osteichthyes, Chondrichthyes, and Aves. Facilitated diffusion develops at the level of the Amphibia

TABLE 1

EFFECT OF AMIDES AND PHLORETIN ON HEMOLYSIS

	Time (min)	% Hemolysis		Phloretin Inhibition
	Time (min)	Urea	Acetamide	
Hagfish, *Myxine glutinosa*	41.00 (7)[a]	34±6	76±10	0
Dogfish, *Squalus acanthias*	7.00 (3)	21±4	70±7	0
Little skate, *Raja erinecea*	2.00 (1)	5	28	0
Winter flounder, *Pseudopleuronectes americanus*	1.25 (3)	13±3	62±10	0
Goosefish, *Lophius piscatorius*	21.00 (1)	3	78	0
Frog, *Rana clamitans*	16.00[b]	61	58	+
Tadpole, *Rana clamitans*	2.00[c]	50	17	+
Toad, *Bufo marinus*	1.25 (4)	85±2	34±2	+
Turtle, *Terrapene carolina*	1.25 (3)	98±6	83±4	+
Rooster, *Anas platyrhynchos*	3.00 (2)	0	44.3	0
Duck, *Gallus domesticus*	4.00 (3)	0	59±4	0
Dog, *Canis familiaris*	1.25 (4)	99±1	25±1	+
Human, *Homo sapiens*	1.25 (4)	100±1	17±1	+

[a] Numbers in parentheses indicate number of animals studied.

[b] Pooled blood from three frogs

[c] Pooled blood from 10 tadpoles

with their emergence to terrestrial life. When it develops it may be a transport system for all amides since phloretin inhibits the movement of both urea and acetamide. The transport system is present in the erythrocytes of the tadpole indicating that it is present at the early aquatic stage, rather than developing at the time of conversion to an amphibious stage. The loss of the system in the birds cannot be explained; it may be related to a shift from urea to uric acid and ammonia as the major excretory products of nitrogen metabolism. This work was supported by Grants AM-03858 and HL-05928 from the U.S. Public Health Service.