The octopamine, dopamine and noradrenaline content of locust brain and regions is shown in Table I. Octopamine and dopamine are present in substantial quantities but noradrenaline is barely detectable. The octopamine values are similar to those reported for cockroach brain[11] and the dopamine and noradrenaline values compare favourably with those obtained for locust brain using fluorimetric methods[5]. Octopamine is concentrated in the optic lobes of the brain while dopamine is more uniformly distributed between the optic lobes and the remainder of the brain. The optic lobes constitute about half the weight of a 4 mg locust brain. Table II shows the effect of reserpine (60 μg/g) and fusaric acid (100 μg/g) on the octopamine levels in the locust central nervous system (brain and ventral nerve cord). Both reserpine and the dopamine β-hydroxylase inhibitor cause a significant decrease in the level of octopamine in the nervous system. These treatments also produce a large decrease in locomotory activity. Reserpine is known to reduce dopamine content in the cockroach brain[6]. The fact that inhibition of dopamine β-hydroxylase leads to a decrease in octopamine levels adds weight to the suggestion that octopamine is synthesized from tyramine which has also been demonstrated in the locust central nervous system[24] (and ROBERTSON, PHILIPS, WU and DYCK, unpublished observations). Locust brain homogenates can decarboxylate tyrosine and DOPA (3,4-dihydroxyphenylalanine)[25] and they also exhibit tyrosine hydroxylase and dopamine β-hydroxylase activity[26]. In view of the high levels of octopamine and dopamine and the low noradrenaline content in the insect brain, it would appear that distinct catecholaminergic and octopaminergic systems are present. The octopaminergic system would be characterized by the enzymes tyrosine decarboxylase and dopamine β-hydroxylase and the dopaminergic system by the enzymes tyrosine hydroxylase and DOPA decarboxylase. Similar octopaminergic and dopaminergic systems have been proposed and largely substantiated for another arthropod, the lobster[18].

The role of octopamine in the insect central nervous system, as in other nervous systems, remains obscure. This report demonstrates that insect nervous tissue contains large amounts of both octopamine and dopamine and, at least on the basis of the very low content, relegates noradrenaline to a lesser role in the functioning of insect neuronal systems. It has been proposed that the effects of certain neurotransmitters such as octopamine, dopamine, noradrenaline and 5-HT may be mediated by cyclic AMP which is formed intraneuronally by the membrane-bound enzyme, adenylate cyclase[27]. The presence of octopamine and dopamine, together with specific octopamine- and dopamine-sensitive adenylate cyclases in the insect central nervous system suggests that these amines may fulfill a neurotransmitter role.

[24] H. A. ROBERTSON, Studies on the distribution and biosynthesis of octopamine in the insect nervous system, Abstr. Commun. 5th Int. Meet. Int. Soc. Neurochem., Barcelona (1975).
[25] L. L. MURDOCK, R. A. WIRTZ and G. KOHLER, Biochem. J. *132*, 681 (1973).
[26] C. E. SEKERIS and P. KARLSON, Pharmac. Rev. *18*, 89 (1966).
[27] P. GREENGARD, J. A. NATHANSON and J. W. KEBABIAN, in *Frontiers in Catecholamines and Research* (Eds. E. USDIN and S. SNYDER; Pergamon, New York and London 1973), p. 377.

Isolation of Sodium-Complexing Polypeptides from Mammalian Blood and Cardiac Muscle

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Summary. Two distinct polypeptides have been isolated from rat heart and ox blood. They are both found to be effective in forming complexes with sodium ions, and it is suggested that they may have a function in stabilizing sodium ion activity.

In connection with the observations of LAICO et al.[5] that tissues contain considerable amounts of small molecular weight polypeptides, the following experimental findings may be of interest.

Following the demonstration of osmotically inactive sodium ions in the perfused rat heart[6], we instituted a search for those constituents responsible for depressing the activity of sodium ions in cardiac muscle. On the basis of previous work[7,8], it was evident that it would be necessary to look beyond simple metabolic intermediates for the agent required, and we therefore sought other constituents of cardiac muscle which had the required property.

Extracts of rat heart were fractionated by passage through Sephadex columns, using a dilute aqueous solution of sodium chloride as eluant. By the use of a flame photometer in conjunction with a sodium-responsive glass electrode, fractions were located which had the property of depressing the activity of sodium ions below the level to be expected for that ionic strength.

The investigation by these techniques was extended to mammalian blood, which was found to be a more convenient source of larger quantities of sodium-complexing agent. The substances isolated from these active fractions

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[5] M. T. LAICO, E. I. RUOSLAHTI, D. S. PAPERMASTER and W. J. DREYER, Proc. natn. Acad. Sci. *67*, 120 (1970).
[6] J. A. BARCLAY, E. H. HAMLEY and K. WHITE, Biochem. J. *88*, 14P (1963).
[7] M. J. HICKLING and K. WHITE, Biochem. J. *96*, 52P (1965).
[8] J. A. BARCLAY, M. J. HICKLING and K. WHITE, Biochem. J. *99*, 11P (1966).

were found to be polypeptide in nature, and the amino-acid compositions were determined by the use of an automatic amino-acid analyzer.

From rat heart, an active polypeptide of molecular weight approximately 700 was isolated, with an amino-acid composition as follows, the figures in parenthesis indicating the number of amino-acid residues per molecule of polypeptide: – alanine (1), aspartic acid (1), glutamic acid (1), glycine (3), lysine (1). From ox plasma, an active polypeptide was isolated of molecular weight approximately 5000, with an amino-acid composition as follows: – alanine (3), arginine (2), aspartic acid (3), cystine (2), glutamic acid (4), glycine (2), histidine (2), isoleucine (1), leucine (3), lysine (5), phenylalanine (2), proline (2), serine (2), threonine (2), tyrosine (1), valine (2).

The polypeptide from blood proved to be a much better complexing agent than the smaller cardiac polypeptide, and results indicate that it is capable of complexing approximately 50 sodium ions per molecule of polypeptide in 0.15 *M* sodium chloride solution. This is an order of magnitude greater than would be in accord with the number of acidic side chains available, which seem to be the most obvious source of complexing ability, but a possible mechanism for the complexing of relatively large numbers of alkali metal cations was proposed by MUELLER and RUDIN[9]. This involves the replacement of water molecules in the cation hydration shell by carbonyl oxygen atoms, which, of course, are plentiful in polypeptide structures.

It was felt desirable to confirm the existence of sodium ion complex formation with an independent method. The studies of PALATY[10] indicated that the presence, even in relatively small amounts, of a cation with a high surface charge density inhibits the close approach of sodium ions to binding sites, and that lanthanum is the best available ion for this purpose. We therefore investigated the effect of low concentrations of lanthanum ion on the apparent depression of sodium ion activity by the isolated plasma polypeptide and found that the presence of lanthanum ions did indeed prevent the plasma polypeptide from lowering sodium ion activity.

It seems possible that one function of the polypeptides isolated by us and reported here could be that of a sodium ion buffer. The concentration of sodium ions in plasma is known to be precisely controlled. According to PITTS[11], for mammalian plasma the sodium ion concentration lies in the range 138–146 m*M*. The presence of an effective sodium ion buffer would lead to an even narrower range for the sodium ion activity. The precision of control which this represents may be appreciated from the calculation that this sodium ion concentration range, when expressed on a logarithmic scale, corresponds to a range of only 0.02. By comparison, the normal range of hydrogen ion concentration, expressed on the pH scale, is 0.10 (i.e. 7.35–7.45, cf. PITTS[12]). Thus the precision of control exerted on the sodium ion concentration is remarkable; by implication, the presence of a sodium ion buffer must exert an additional control with a precision even more remarkable.

[9] P. MUELLER and D. O. RUDIN, Biochem. biophys. Res. Commun. *26*, 398 (1967).
[10] V. PALATY, Nature, Lond. *211*, 1177 (1966).
[11] R. F. PITTS, *Physiology of the Kidney and Body Fluids*, 3rd edn. (Year Book Medical Publishers Inc., Chicago 1974), p. 19.
[12] R. F. PITTS, *Physiology of the Kidney and Body Fluids*, 3rd edn. (Year Book Medical Publishers Inc., Chicago 1974), p. 178.

Is there a Recycling of Hydroxyproline?

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Summary. Rats can produce glycine from hydroxyproline and vice versa. After the injection, hydroxyproline is rapidly converted into glycine and then incorporated into collagen. Later the labelled amino acids in collagen are hydroxyproline, proline, serine, threonine, alanine, and glutamic acid. We suppose that all these labelled amino acids come from glycine.

It is generally held that hydroxyproline, in contrast to the large amounts of proline, cannot be recycled. This means that hydroxyproline liberated in collagen catabolism would have to be eliminated[1,2]. On the other hand, we have shown in previous experiments that remarkable quantities of labelled glycine can be found in collagen after the application of ^{14}C-proline[3]. The best explanation for this is the assumption that this glycine was derived from hydroxyproline directly and not from glutamic acid. Therefore hydroxyproline must be catabolized in the rat's organism.

Methods. L-4-hydroxyproline-^{3}H from NEN (5 mCi/0,148 mg) with a purity grade of > 98% (scan of thin layer chromatography in phenol (75 g): H_2O (25 g): NaCN (20 mg)) was injected into the peritoneum of male Sprague-Dawley rats weighing ca. 100 g each (ca. 400 µCi/animal). The animals were sacrificed 1 or 24 h later. Tail skin, and tendon collagen was extracted, dialyzed, and isolated in fibres by dialysis against phosphate buffer (0.02 *M* Na_2HPO_4)[5]. The fibres were also washed many times. Purification was stopped when there was no longer any radioactivity in the washing water. The collagen was then hydrolyzed and its amino acids isolated by column chromatography as described previously[6] (Figure). All the isolated ^{3}H-amino acids again underwent chromatography and were identified separately.

Results. 1 h after the injection of L-4-hydroxyproline-^{3}H radioactivity can be detected in the collagen of skin. This radioactivity comes almost exclusively from glycine (Table).24 h after application, the radioactivity of skin

[1] S. H. JACKSON and J. A. HEINIGER, Biochim. biophys. Acta *381*, 358 (1975).
[2] K. I. KIVIRIKKO, Acta physiol. scand. Suppl. *60*, 219 (1963).
[3] A. RUIZ-TORRES and I. KÜRTEN, Experientia *29*, 968 (1973).
[4] M. R. STETTEN, J. biol. Chem. *153*, 113 (1944).
[5] P. M. GALLOP, Arch. Biochem. *54*, 486 (1955).
[6] A. RUIZ-TORRES and I. KÜRTEN, Z. Geront. *7*, 133 (1974).