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The Enzymic Inactivation of Some Physiologically Active Polypeptides by Different Parts of the Nervous System

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The pattern of enzyme distribution in tissues can sometimes give an indication of the functions of the enzyme and of the tissue in which the enzyme occurs. In the nervous system, for example, studies have been made on the distribution of enzymes concerned with protein metabolism (for references see Waelsch & Lajtha, 1961) and on enzymes which are involved, in various ways, with the transmission of nerve impulses (e.g. Feldberg & Vogt, 1948). The hypothalamus, which is generally considered to be responsible for the production of posterior-pituitary hormones, has been shown to possess enzymes capable of inactivating oxytocin, vasopressin, bradykinin and substance P (Hooper, 1962a). These peptidases may participate in some manner in the metabolism of oxytocin and vasopressin. Alternatively they may be catabolic enzymes, of low specificity and possessing widespread distribution, whose primary function is to assist in the maintenance of amino acid pools of the nervous system. To obtain information about the function of the hypothalamic enzymes the distribution of the peptidases in certain regions of dog brain and spinal cord has been studied. The present paper describes the distribution of the enzymes, and draws some conclusions about the role of the enzymes in the nervous system.

METHODS

Areas of central and peripheral nervous system examined for peptidase activity. Mongrel dogs of either sex weighing 8–25 kg. were anaesthetized with ether and killed by bleeding. In a few instances dogs previously subjected to an abdominal operation under chloralose anaesthesia were killed by bleeding and used. The brain was rapidly excised, and pial blood vessels were removed from the appropriate areas before dissection. Particular care was taken in the removal of as much blood as possible, since dog erythrocytes are known to contain enzyme(s) capable of inactivating oxytocin (Werle, Semm & Enzenbach, 1950), and plasma also contains enzymes inactivating bradykinin (Rocha e Silva, Beraldo & Rosenfeld, 1949). Samples of tissue from six regions were removed and the corresponding parts from the two hemispheres were combined and worked up together. The six regions examined were: (i) caudate nucleus, (ii) cerebellar cortex, (iii) cortex (bordering on the sulcus cruciatus), (iv) hypothalamus (excluding corpora mammillaria and median eminence), (v) thalamus (medial part) and (vi) white matter (lining of the lateral ventricle); approx. 200–500 mg. of tissue was obtained from each region per animal.

Dorsal roots, ventral roots and dorsal-root ganglia from the cauda equina were the selected regions of the peripheral nervous system. Roots and ganglia were removed as quickly as possible, and dissected free from adhering fat and membranes. The ventral roots used for enzyme study were cut off at their origin from the cord and taken as far as the

region where they joined the dorsal-root ganglion. Approx. 100–300 mg. of each region was removed per animal. All samples were weighed, homogenized in 10 vol. of water and dialysed against a total of about 10^6 vol. of water at 4° for 18 hr. The dialysed homogenates of brain tissues were diluted with water to give the same weight of tissue/ml. of homogenate. The root and ganglia homogenates, however, were adjusted to contain equal amounts of non-diffusible nitrogen, and not of wet wt. of tissue. The nitrogen content of roots and ganglia was measured by a micro-Kjeldahl method.

Determination of enzyme activity. Enzyme activity was determined by measuring the loss of substrate that occurred on incubation with homogenate under the following conditions. The dialysed homogenates were incubated at 37° with the following substrates: bradykinin, $2\mu\text{g./ml.}$, substance P, 40 Euler (1942) units/ml.; oxytocin, 1 international oxytocic unit/ml.; vasopressin, 1 international pressor unit/ml. The pH of the medium was maintained at 7.3 by 0.2 vol. of $0.4\text{M-Na}_2\text{HPO}_4\text{-NaH}_2\text{PO}_4$ buffer. Enzyme action was stopped after a 3 hr. incubation period by heating samples in a boiling-water bath for 15 min. The residual concentrations of bradykinin and substance P were measured with isolated guinea-pig ileum; the concentrations of certain samples of substance P were determined with both guinea-pig ileum and isolated fowl rectal caecum (Cleugh, Gaddum, Holton & Leach, 1961). Concentrations of oxytocin were measured with isolated rat uterus, and residual vasopressin was determined by its effect on rat blood pressure (Dekanski, 1952). A bracket type of assay was used (Hooper & Jessup, 1959). Group-comparison *t* tests have been calculated as indicated by Snedecor (1959). Fiducial limits at a probability of 0.95 were calculated as described by Burn (1952) and were similar to those obtained in previous work (see, e.g., Hooper, 1962*a*).

Enzyme substrates. The bradykinin (lot 4447 $\times$ 116) was a pure synthetic preparation supplied by courtesy of Parke,

Davis and Co., Detroit, Mich., U.S.A. The substance P was a gift from Dr J. H. Gaddum: it was a horse-intestine preparation of potency 40 Euler units/mg., assayed with both guinea-pig ileum and fowl rectal caecum. Pitocin and Pitressin (Parke, Davis and Co., London) were the respective sources of oxytocin and vasopressin.

RESULTS

Previous work has indicated that oxytocin and vasopressin are probably inactivated by the same enzymes in the dog hypothalamus: the identity of the enzymes is based on the observations that (*a*) the rate of inactivation of oxytocin was markedly decreased by vasopressin in a mixed substrate of the two hormones (Hooper, 1962*a*), and (*b*) the intracellular distribution of enzymes destroying oxytocin (Hooper, 1962*b*) is paralleled, within the limits of the experimental methods employed, by the distribution of enzymes inactivating vasopressin (K. C. Hooper, unpublished work). Because of the probable identity of enzymes, the present study has been made on the inactivation of one hormone only, namely vasopressin, by six regions of dog brain. The distribution pattern obtained for the inactivation of vasopressin should also apply to oxytocin.

Regional distribution of some enzymes inactivating vasopressin in the dog brain. The distribution of enzymes in the brain of one male and one female animal is listed in Table 1. The recovery of vasopressin from the incubation medium is expressed as a percentage of the hormone recovered from a control medium; the control medium contained an

Table 1. *Inactivation of vasopressin by brain tissues*

Homogenates of tissues from six regions were prepared as described in the Methods section. The incubation medium consisted of vasopressin (5 international pressor units), 0.4M-phosphate buffer, pH 7.3 (1 ml.), tissue homogenate (equivalent to 60 mg. of tissue for dog 1 and 95 mg. for dog 2) in a total volume of 5 ml. The control medium contained homogenate that had been heated in a boiling-water bath for 15 min. After 3 hr. of incubation at 37° enzyme action was stopped by heating at 100° for 15 min. The residual hormone concentration was measured by the rat-blood-pressure method and units of enzyme activity were calculated as described in text.

Tissue	Recovery of vasopressin (% of control)	Units of enzyme activity	Specific activity	Enzyme distribution (hypothalamic content = 10)
Dog 1 (♀)				
Caudate nucleus	58	0.33	0.028	5.8
Cerebellum	46	0.47	0.039	8.1
Cortex	57	0.34	0.028	5.8
Hypothalamus	40	0.57	0.048	10.0
Thalamus	46	0.47	0.039	8.1
White matter	73	0.19	0.016	3.3
Dog 2 (♂)				
Caudate nucleus	21	0.96	0.051	9.1*
Cerebellum	16	1.27	0.068	12.3*
Cortex	37	0.61	0.032	5.8
Hypothalamus	18	1.04	0.055	10.0
Thalamus	50	0.43	0.023	4.2
White matter	64	0.28	0.015	2.7

* Values probably artificially high owing to the presence of vasodepressor substances in media.

equal amount of tissue that had been heated for 15 min. in a boiling-water bath before incubation with hormone. The residual concentrations of polypeptide have been converted into units of enzyme activity by reference to the curve relating enzyme activity and residual concentrations of vasopressin (Hooper, 1962*a*). The concentrations of enzyme in the different regions of the brain are expressed as enzyme activity/mg. wet wt. of tissue: the relative concentrations of enzyme in the tissues, referred to a hypothalamic content of 10, are also given. The measurement of enzyme concentrations in cerebellum and caudate-nuclei fractions of dog 2 was not entirely satisfactory because the increased amounts of homogenate in the incubation media (19 mg./ml. against 12 mg./ml. for dog 1) caused difficulties in the assay of vasopressin concentrations. A depressor effect was noted on rat blood pressure before the normal pressor action of the hormone. The depression of rat blood pressure could have resulted in a low value for hormone recovery and a high value for the enzyme content of the tissues. Table 1 shows that white matter contains less enzyme than do regions rich in nerve cells. There is no great difference between the concentrations of enzyme in the remaining five regions, although the cortex appears to contain less than the hypothalamus.

The inactivation of bradykinin by brain tissues is shown in Table 2. Hooper (1962*a*) showed that homogenate of hypothalamus inactivated bradykinin more rapidly than it did oxytocin or vasopressin. This difference was also noted with other regions of brain, since very low residual concentrations of bradykinin were obtained after incubation with about one-tenth of the tissue (1.2 mg./ml.) used in the vasopressin experiments. All regions contained appreciable and equal quantities of enzyme, except for white matter, which contained less.

The inactivation of substance P by the six brain regions was not examined; however, it is well known that whole brain does contain such enzymes (Eber & Lembeck, 1956; Krivoy, 1957), and a few

specific areas of brain, e.g. basal ganglia (Gullbring, 1943) and hypothalamus (Hooper, 1962*a*), have been shown to be active.

The distribution of enzymes in brain suggested that regions consisting largely of white matter contained less enzymes inactivating bradykinin and vasopressin (and presumably oxytocin) than did areas containing nerve cells. To determine more precisely this aspect of distribution, the enzyme content of certain relatively homogeneous sections of the nervous system have been examined. The selected regions were spinal dorsal roots, ventral roots and dorsal-root ganglia. Dorsal and ventral roots consist largely of myelinated axons, and contain no cell bodies other than the Schwann cells, whereas the dorsal-root ganglia contain large numbers of nerve cells in addition to capsule cells, connective tissue and some fibres.

Action of spinal roots and ganglia on oxytocin and vasopressin. Roots and ganglia were incubated with hormones at concentrations of about 100 μ g. of N/ml. because, at this level, hypothalamus homogenate contained easily detectable amounts of enzymes inactivating the polypeptides (Hooper, 1962*a*). The results obtained from six animals are shown in Tables 3 and 4, where the recovery of substrate is expressed in terms of a control as described above. The significance of the difference between the recovery of hormone from control and test samples has been assessed by a group-comparison *t* test (Snedecor, 1959). Probability values have been obtained from the *t* values by reference to a *t* distribution table (Fisher & Yates, 1948). In these experiments, a probability of $P < 0.01$ has been chosen to indicate a significant loss of substrate.

The results shown in Table 3 indicate that oxytocin was not destroyed to any significant extent on incubation with dorsal or ventral roots. There is an indication that some oxytocin may have been lost on incubation with the dorsal-root-ganglia preparation that contained the larger amount of tissue. Table 4 shows the recovery of vasopressin after incubation with homogenates; here also the results indicate that dorsal roots do not inactivate the

Table 2. *Inactivation of bradykinin by brain tissues*

Homogenates (1.2 mg. wet wt. of tissue/ml.) were incubated with bradykinin (2 μ g./ml.) as described in Table 1. The residual biological activity was measured with isolated guinea-pig ileum.

Tissue	Recovery of bradykinin (% of control)	Units of enzyme activity	Specific activity	Enzyme distribution (hypothalamic content = 10)
Dog 3				
Caudate nucleus	1.4	2.68	2.23	9.8
Cerebellum	1.1	2.81	2.34	10.2
Cortex	1.3	2.74	2.28	10.0
Hypothalamus	1.2	2.74	2.28	10.0
Thalamus	1.4	2.68	2.23	9.8
White matter	6.9	1.66	1.38	6.1

Table 3. *Action of spinal roots and ganglia on oxytocin*

The methods were as described in Table 1. The probability, *P*, of a difference between the residual concentrations of hormone in control and test samples has been calculated by a group-comparison *t* test (Snedecor, 1959) and *t* distribution tables (Fisher & Yates, 1948).

	Ventral roots			Dorsal roots			Dorsal-root ganglia		
	Tissue concn. (μg. of N/ml. of incubation mixture)	Recovery of oxytocin (% of control)	<i>P</i>	Tissue concn. (μg. of N/ml. of incubation mixture)	Recovery of oxytocin (% of control)	<i>P</i>	Tissue concn. (μg. of N/ml. of incubation mixture)	Recovery of oxytocin (% of control)	<i>P</i>
Dog 5	115	95	< 0.6	157	78	< 0.2	—	—	—
Dog 6	98	81	< 0.2	106	84	< 0.2	—	—	—
Dog 7	115	78	> 0.1	189	130	> 0.5	—	—	—
Dog 8	—	—	—	47	126	> 0.05	39	94	> 0.2
Dog 9	—	—	—	—	—	—	171	71	< 0.01

Table 4. *Action of spinal roots and ganglia on vasopressin*

The methods were as described in Table 3.

	Ventral roots			Dorsal roots			Dorsal-root ganglia		
	Tissue concn. (μg. of N/ml. of incubation mixture)	Recovery of vasopressin (% of control)	<i>P</i>	Tissue concn. (μg. of N/ml. of incubation mixture)	Recovery of vasopressin (% of control)	<i>P</i>	Tissue concn. (μg. of N/ml. of incubation mixture)	Recovery of vasopressin (% of control)	<i>P</i>
Dog 4	117	80	< 0.01	118	105	< 0.5	—	—	—
Dog 5	115	77	> 0.05	157	114	< 0.6	—	—	—
Dog 6	98	86	> 0.1	106	102	> 0.8	—	—	—
Dog 7	115	73	< 0.001	189	75	> 0.02	—	—	—
Dog 8	—	—	—	47	121	< 0.01	39	136	< 0.02
Dog 9	—	—	—	—	—	—	171	75	= 0.01

hormone. Some vasopressin was lost after incubation with the dorsal-root-ganglia preparation containing the large amount of tissue. A loss of vasopressin occurred on incubation with certain samples of ventral roots, and in two instances (dogs 4 and 7) hormone loss was significant. In none of the experiments listed in Tables 3 and 4 did the apparent loss of oxytocin or vasopressin exceed 29 %. When dorsal-root ganglia were used the loss of hormones was significant only in those experiments in which large amounts of tissue were used. When ventral roots were used a significant loss of vasopressin occurred in only two out of four experiments. The losses are most probably due to factors other than enzyme action; for example, the loss may have been caused by greater adsorption of hormone by natural than by denatured protein.

Action of dorsal roots, ventral roots and dorsal-root ganglia on bradykinin. The results obtained with tissues from seven animals are shown in Table 5. The pronounced inactivation of bradykinin by roots and ganglia contrasted sharply with the lack of effect on oxytocin and vasopressin. At concentrations as low as 15 μg. of N/ml., the tissues destroyed a major part of the bradykinin substrate. The quantity of enzyme present in the tissues from dogs 6, 7, and 8

was so large that the effects were near maximal. An accurate measurement of residual concentrations of bradykinin could not be made and did not allow a comparison of the enzyme content of roots and ganglia. The tissues obtained from animals 6, 7 and 8 have been incubated with the four polypeptide substrates mentioned above, and the results with bradykinin in Table 5 can be compared directly with the results with other peptides in Tables 3, 4 and 7. Much smaller concentrations of tissue homogenates were used for the material from dogs 9, 10, 12 and 13, thus enabling reliable comparisons of enzyme activity to be made. A difference in the enzyme content of the three regions is noticeable: the dorsal-root ganglia possessing the greatest and the ventral roots the least activity. Also, the specific activity of identical regions in different animals is fairly constant, being about 108 for ganglia, 60 for dorsal roots and 20 for ventral roots.

Effect of picrotoxin and strychnine as inhibitors on the inactivation of bradykinin by roots and ganglia. The unequal distribution of enzyme in dorsal and ventral roots might suggest that the peptidase is primarily concerned with afferent-nerve metabolism. Dorsal roots have not, as yet, been shown to possess a nerve-impulse transmitting agent, though

Table 5. *Action of spinal roots and ganglia on bradykinin*

The methods were as described in Table 3. Bradykinin was used at a concentration of 2 $\mu\text{g./ml.}$ The specific activity is expressed as units of enzyme activity/mg. of non-diffusible nitrogen.

	Ventral roots			Dorsal roots			Dorsal-root ganglia		
	Tissue concn. ($\mu\text{g. of N/ml. of incubation mixture}$)	Recovery of bradykinin (% of control)	Specific activity	Tissue concn. ($\mu\text{g. of N/ml. of incubation mixture}$)	Recovery of bradykinin (% of control)	Specific activity	Tissue concn. ($\mu\text{g. of N/ml. of incubation mixture}$)	Recovery of bradykinin (% of control)	Specific activity
Dog 6	98	6.9	17	106	4.2	19	—	—	—
Dog 7	115	< 1.3	—	189	< 1.6	—	—	—	—
Dog 8	—	—	—	47	< 0.5	—	39	< 0.7	—
Dog 9	—	—	—	17	16.8	66	17	4.4	115
Dog 10	—	—	—	12.5	34	54	12.5	12.8	104
Dog 12	15	76.1	12	15	25	59	15	6.2	116
Dog 13	15	51.8	27	15	21.8	64	15	10.3	95

Table 6. *Effect of picrotoxin and strychnine on the destruction of bradykinin by dorsal roots*

Bradykinin (2 $\mu\text{g./ml.}$) was incubated with dorsal roots (19 $\mu\text{g. of N/ml.}$) at pH 7.3 for 3 hr. at 37°, and the residual peptide concentration was measured with guinea-pig ileum. The effect of 1 mM-picrotoxin and of 1 mM-strychnine on the degree of inactivation was then determined. Fiducial limits at a probability of 0.95 are shown in parentheses.

	Residual peptide concentration ($\mu\text{mg./ml.}$)	Recovery (%)
Dog 14		
Bradykinin + picrotoxin	2015	100.8 (± 17.3)
Bradykinin + strychnine	1844	92.2 (± 21.5)
Bradykinin + dorsal roots	590	29.5 (± 5)
Bradykinin + dorsal roots + picrotoxin	296	14.8 (± 1.5)
Bradykinin + dorsal roots + strychnine	591	29.5 (± 3)

it has been suggested that substance P may act as such (Lembeck, 1953; Holton, 1960), and it is known that both dorsal and ventral roots contain enzymes inactivating this peptide. Certain of the effects produced by convulsant drugs such as strychnine and picrotoxin are attributed to accumulation of excess of dorsal-root-nerve-impulse transmitting agent caused by inhibition of inactivating enzymes. The high concentrations of the enzymes inactivating bradykinin in dorsal roots suggested that these enzymes might be connected in some manner with afferent-nerve-impulse transmission. However, it was found that neither strychnine (1 mM) nor picrotoxin (1 mM) inhibited the inactivation of bradykinin by a mixture of roots and ganglia (Table 6). This finding throws no light on the theory it was designed to test.

Action of dorsal roots, ventral roots and dorsal-root

ganglia on substance P. The loss of substance P after incubation with roots and ganglia from six animals is shown in Table 7. Residual substrate concentrations were assayed with guinea-pig ileum and the results calculated as described for bradykinin. The results show that dorsal roots, ventral roots and dorsal-root ganglia contain comparable amounts of enzyme activity. The inactivation of substance P by roots has been described by several workers (Eber & Lembeck, 1956; Krivoy, 1957; Inouye, Kataoka & Tsujioka, 1961), and the present results confirm that dorsal and ventral roots contain comparable amounts of peptidase activity. The fact that dorsal-root ganglia contain similar quantities of activity suggests that the enzyme is fairly uniformly distributed throughout the cauda equina.

Incubation of crude preparations of substance P with trypsin or dorsal-root enzymes has been reported to produce a 'kinin-like' substance termed 'component T' stated to be very similar to, if not identical with, bradykinin (Inouye *et al.* 1961). In the present work, the possibility of a pharmacologically active material being formed during incubation of dog roots and ganglia with substance P was examined by means of parallel bioassays (Gaddum, 1955). Component T and substance P may be assayed with guinea-pig ileum or fowl rectal caecum, the latter being three times more sensitive to substance P than it is to component T (Inouye *et al.* 1961). If component T had been formed during incubation, the residual biological activity measured on the ileum would have been larger than that indicated by the rectal caecum. In experiments on dogs 7, 8 and 9, the residual concentrations of substance P were measured by both bioassay methods. In two out of six experiments the guinea-pig ileum indicated a larger residual biological activity than did the fowl rectal caecum. This could easily be accounted for by impurities in the substance P,

Table 7. *Action of roots and ganglia on substance P*

The methods were as described in Table 3. Substance P was used at a concentration of 40 Euler units/ml. The specific activity is expressed as units of enzyme activity/mg. of non-diffusible nitrogen. The residual substance P was measured with guinea-pig ileum.

	Ventral roots			Dorsal roots			Dorsal-root ganglia		
	Tissue concn. (μ g. of N/ml. of incubation mixture)	Recovery of substance P (% of control)	Specific activity	Tissue concn. (μ g. of N/ml. of incubation mixture)	Recovery of substance P (% of control)	Specific activity	Tissue concn. (μ g. of N/ml. of incubation mixture)	Recovery of substance P (% of control)	Specific activity
Dog 5	115	33	5.6	157	29	4.5	—	—	—
Dog 6	98	21	9.3	106	34	5.6	—	—	—
Dog 7	115	51	3.0	189	28	3.7	—	—	—
Dog 8	—	—	—	47	79	2.1	39	41	12.0
Dog 9	—	—	—	171	27	4.4	171	31	3.9

whereas the results should have been consistent if component T occurred under the conditions of these experiments.

DISCUSSION

In comparing the peptidase activities of various regions of the nervous system it has been assumed that enzymes effecting an inactivation are similar to those found in the hypothalamus. Although the identity of the enzymes has not been proved, it would appear that many other proteolytic enzymes known to occur in nerve tissues would be inactivated by the extensive dialysis of the homogenates [e.g. the unstable neutral proteinase described by Ansell & Richter (1954), and the many peptidases with diffusible cofactors], or would be inoperative during incubation owing to the unfavourable conditions of the medium (e.g. cathepsins and neutral proteinases inhibited by phosphate). The small amount of blood remaining in the tissues does not contain enough enzymes to react with the substrates. Bradykinin is known to be rapidly destroyed by blood (Rocha e Silva *et al.* 1949); however, dialysed dog blood at a concentration of 40 μ g. of N/ml. did not inactivate bradykinin to any significant extent, whereas roots and ganglia were active at a concentration of 15 μ g. of N/ml. Substance P is not inactivated on incubation with blood (Euler & Gaddum, 1931).

The inability of spinal roots and ganglia to inactivate oxytocin and vasopressin shows that certain hypothalamic enzymes are not normal constituents of all nerve tissue; it seems as if these enzymes are either present in low concentrations or totally absent from white matter. The restricted distribution also suggests that these enzymes have a special function, but it does not indicate what the function may be.

Enzymes inactivating bradykinin and substance P have been detected in several types of nerve tissue, and they would appear to have a wide distribution;

the distribution is not universal, however, for Gullbring (1943) reported that horse-spinal-cord extracts did not contain enzymes inactivating substance P. The concentrations of enzyme inactivating substance P in dorsal and ventral roots were similar; however, dorsal roots contained more enzyme inactivating bradykinin than did ventral roots. These findings suggest that the peptides are substrates for different enzymes. The unequal distribution of the enzyme inactivating bradykinin in roots and ganglia may be indicative of the site of enzyme production. Certain axonal proteins of nerve fibres appear to be synthesized in the perikarya and flow down the axons (Weiss, 1947; Waelsch & Lajtha, 1961), and gradients in cholinesterase and choline-acetylase concentrations have been noted along peripheral nerves (Lubinska, Niemierko & Oberfeld, 1961; Hebb & Silver, 1961). The concentration of the bradykinin-inactivating enzyme was greater in the dorsal-root ganglia than in the dorsal roots, and was lowest in the ventral roots. A difference of this nature would suggest that the enzyme is unlikely to be produced by Schwann cells, but that it is probably synthesized and flows in a manner similar to that of choline acetylase and cholinesterase.

SUMMARY

1. The distribution of enzymes inactivating bradykinin and vasopressin in six regions of dog brain, and of enzymes inactivating bradykinin, substance P, oxytocin and vasopressin in dog spinal roots and ganglia, has been studied.

2. In dog brain, regions consisting largely of white matter contained less enzymes inactivating bradykinin and vasopressin than did regions rich in cell bodies.

3. Dorsal roots, ventral roots and dorsal-root ganglia did not contain detectable quantities of enzymes acting on oxytocin and vasopressin.

4. Roots and ganglia appear to contain comparable amounts of enzyme inactivating substance P.

5. In low concentrations, roots and ganglia rapidly inactivate bradykinin; the enzyme concentration is greatest in dorsal-root ganglia and least in the ventral roots.

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Lactate Dehydrogenase as a Cytoplasmic Marker in Brain

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The glycolytic enzymes except hexokinase are found predominantly in the soluble cytoplasmic fraction of brain homogenates. However, about 20% of the total is recovered in a particulate fraction sedimenting at 13 000g for 15 min. after the removal of nuclei and cell debris. The particulate fraction of one of these glycolytic enzymes, lactate dehydrogenase, was largely separated from a typical mitochondrial enzyme (succinate dehydrogenase) by centrifuging in a dense medium (Johnson, 1960). It was suggested that the particulate lactate dehydrogenase represented cytoplasm entrapped in incompletely broken axons.

A large proportion of brain acetylcholine exists in bound form in the sucrose homogenate and is

present mainly in a fraction sedimented at 16 000g for 60 min. In this case also a substantial separation of bound acetylcholine from succinate dehydrogenase can be achieved by density-gradient centrifuging (Whittaker, 1959). Electron-micrographs have shown that the subfraction richest in bound acetylcholine contains a high proportion of particles that have been identified as pinched-off nerve endings (Gray & Whittaker, 1962). These structures would be expected to contain cytoplasm and therefore lactate dehydrogenase also, if this enzyme has a cytoplasmic localization. This expectation has now been confirmed by experiments in which similar intracellular distribution patterns have been obtained for particulate lactate dehydro-