Campbell, P. N., Greengard, O. & Kernot, B. A. (1960). *Biochem. J.* **74**, 107.
Campbell, P. N. & Kernot, B. A. (1959). *Biochem. J.* **73**, 24 P.
Campbell, P. N. & Kernot, B. A. (1962). *Biochem. J.* **82**, 262.
Campbell, P. N. & Stone, N. E. (1957). *Biochem. J.* **66**, 19.
Chatterjee, B. R. & Williams, R. P. (1962). *Biochem. biophys. Res. Commun.* **9**, 72.
Claude, A. (1943). *Science*, **97**, 451.
Cohn, M. (1952). *Meth. med. Res.* **5**, 271.
Cohn, P. (1959). *Biochim. biophys. Acta*, **33**, 284.
Decken, A. von der (1961*a*). *Exp. Cell Res.* **23**, 517.
Decken, A. von der (1961*b*). *Nature, Lond.*, **189**, 145.
Decken, A. von der (1962). *Biochem. J.* **84**, 109 P.
Decken, A. von der (1963*a*). *Acta chem. scand.* (in the Press).
Decken, A. von der (1963*b*). *J. Cell Biol.* **16**, 471.
Decken, A. von der (1963*c*). *Exp. Cell Res.* (in the Press).
Decken, A. von der & Campbell, P. N. (1962). *Biochem. J.* **84**, 449.
Ernster, L., Siekevitz, P. & Palade, G. E. (1962). *J. Cell Biol.* **15**, 541.
Grabar, P. & Williams, C. A., jun. (1955). *Biochim. biophys. Acta*, **17**, 67.
Hartley, R. W., jun., Peterson, E. A. & Sober, H. A. (1962). *Biochemistry*, **1**, 60.
Hoagland, M. B. (1960). In *The Nucleic Acids*, vol. 3, p. 349. Ed. by Chargaff, E. & Davidson, J. N. New York: Academic Press Inc.
Kabat, E. A. & Mayer, M. M. (1961). *Experimental Immunochemistry*, pp. 22 ff. Springfield, Ill.: Charles C. Thomas.
Kirsch, J. F., Siekevitz, P. & Palade, G. E. (1960). *J. biol. Chem.* **235**, 1419.
Korner, A. (1959). *Biochim. biophys. Acta*, **35**, 554.
Korner, A. (1961). *Biochem. J.* **81**, 168.
Kruh, J., Rosa, J., Dreyfus, J.-C. & Schapira, G. (1961). *Biochim. biophys. Acta*, **49**, 509.
Lamfrom, H. (1961). *J. molec. Biol.* **3**, 241.
Lengyel, P., Speyer, J. F. & Ochoa, S. (1961). *Proc. nat. Acad. Sci., Wash.*, **47**, 1936.
Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951). *J. biol. Chem.* **193**, 265.
Maxwell, E. S. (1962). *Proc. nat. Acad. Sci., Wash.*, **48**, 1639.
Morgan, W. S., Perlmann, P. & Hultin, T. (1960). *J. biophys. biochem. Cytol.* **10**, 411.
Nirenberg, M. W. & Matthaei, J. H. (1961). *Proc. nat. Acad. Sci., Wash.*, **47**, 1588.
Ouchterlony, Ö. (1949). *Ark. Kemi Min. Geol.* **26** B, 1.
Palade, G. E. & Siekevitz, P. (1956). *J. biophys. biochem. Cytol.* **2**, 171.
Peters, T., jun. (1958). *J. Amer. chem. Soc.* **80**, 2700.
Rendi, R. & Hultin, T. (1960). *Exp. Cell Res.* **19**, 253.
Roberts, R. B. (1958). *Microsomal Particles and Protein Synthesis*. New York: Pergamon Press.
Sober, H. A. & Peterson, E. A. (1958). *Fed. Proc.* **17**, 1116.
Takanami, M. (1960). *Biochim. biophys. Acta*, **39**, 318.
Tsugita, A., Fraenkel-Conrat, H., Nirenberg, M. W. & Matthaei, J. H. (1962). *Proc. nat. Acad. Sci., Wash.*, **48**, 846.
Vinograd, J. & Hutchinson, W. D. (1960). *Nature, Lond.*, **187**, 216.
Weinstein, I. B. & Schechter, A. N. (1962). *Proc. nat. Acad. Sci., Wash.*, **48**, 1686.
Zamecnik, P. C. (1962). *Biochem. J.* **85**, 257.
Zamecnik, P. C., Loftfield, R. B., Stephenson, M. L. & Steele, J. M. (1951). *Cancer Res.* **11**, 592.

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# Ultracentrifugal Characteristics of Human, Monkey and Rat Transferrins

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Electrophoretic analyses of the serum proteins of many species reveal a prominent component that has mobility of a $\beta$-globulin and the ability to bind iron fairly strongly. It has, therefore, become known generally as transferrin. Individual sera may contain several transferrins, whose presence is genetically determined, that have slightly different electrophoretic mobilities. Other differences within a group may be relatively minor.

The recent availability of rat and rhesus-monkey transferrins (Gordon & Louis, 1963) made it of interest to compare their molecular weights with those of human and pig transferrins given in the literature. As the values found for rat and monkey transferrins were substantially lower than the values that have been accepted for human and pig transferrins, a new investigation of human transferrins was carried out. This gave a molecular weight that corresponded closely with those for the rat and monkey transferrins.

## MATERIALS AND METHODS

*Transferrins.* These were isolated from serum by preliminary electrophoretic separation in a block of Pevikon C870 (Stockholms Superfosfat Fabriks A.-B., Stockholm, Sweden) followed by gradient elution from diethylaminoethyl-Sephadex A-50 (Pharmacia, Uppsala, Sweden) under

the conditions selected by Gordon & Louis (1963). Three batches of rat transferrin, two of rhesus monkey (*Macacus rhesus*) transferrin, and one of human transferrin were prepared in this way.

Two lots of commercial human transferrin (Behring, batch B1227/34 in both cases) were examined, first as received, and then after further fractionation on a column of Sephadex G-200 (Pharmacia, Uppsala, Sweden) in sodium phosphate buffer, pH $6{\cdot}8_5$ (and $I$ 0·1) containing sodium chloride (0·5M).

Sedimentation-velocity measurements only were done on two other preparations of human transferrin isolated from serum, one (TR1) by chromatography on a column of diethylaminoethylcellulose (initial buffer: sodium phosphate, pH 6·4 and $I$ 0·015) followed by starch-gel electrophoresis, and the other (TR2) by fractionation with 0·4% rivanol (6,9-diamino-2-ethoxyacridine lactate) at pH 9 and subsequently ammonium sulphate (between 50 and 60% saturation).

*Buffers.* Before ultracentrifuging, solutions were dialysed to equilibrium with buffer, generally sodium phosphate (pH 7·0, 7·5 or 8·0, and $I$ 0·1). Several other buffers were used for special purposes. One which consisted of sodium phosphate, $I$ 0·1, and 0·3M-sodium sulphite had pH $7{\cdot}4_5$. Another contained 0·1M-borax, 0·1N-hydrochloric acid and 0·6 mM-iodoacetamide, pH 8·4. Two experiments at higher ionic strength were done in the buffer used by Mahling (1963), 0·2M-sodium chloride plus sodium phosphate, pH 6·8 and $I$ 0·11.

*Sedimentation-velocity measurements.* These were done in a Spinco model E ultracentrifuge at a nominal speed of 59780 or 52640 rev./min. in either 12 mm. or 3 mm. cells, sometimes with wedge windows. Records were obtained by using schlieren optics, which included a Wratten 77 A filter to isolate light of $\lambda$ 546 m$\mu$ and a phase-plate. Measurements and calculations were done in the usual way (Charlwood, 1961), all sedimentation coefficients being corrected to water at 20° and expressed in Svedberg units (S).

*Molecular-weight measurements.* These were made in the same ultracentrifuge in 12 mm. cells by the Archibald (1947) method of approach to sedimentation equilibrium as used by Charlwood (1961). The rotor speed was nominally 11150 and 8210 rev./min. at lower and higher protein concentrations respectively. The latter were determined either in a differential refractometer (Cecil & Ogston, 1951) at $\lambda$ 546 m$\mu$, or from the peak areas recorded in synthetic-boundary-cell or double-synthetic-boundary-cell ultracentrifuge experiments. The limits of error of molecular-weight measurements done in this way have been discussed by Charlwood (1961).

In the calculations partial specific volumes, $\bar{v}$, of 0·725 and 0·733 were used for human and rat transferrins respectively. The value for human transferrin was measured by Oncley, Scatchard & Brown (1947), by using plasma fraction IV–7, which is mainly transferrin. For rat transferrin, $\bar{v}$ was computed from the amino acid composition results given by Gordon & Louis (1963). In the absence of information $\bar{v}$ was assumed to be 0·73 for the monkey transferrin.

## RESULTS

*Quality of preparations.* The transferrins prepared by the method of Gordon & Louis (1963) were almost monodisperse in the ultracentrifuge, except that a small amount of faster-sedimenting material could just be detected in the monkey-transferrin preparations (Figs. 1 and 2*a*). Synthetic-boundary-cell experiments all gave peak areas within 2% of the values expected from the differential-refractometer measurements, and peak areas in high-speed runs, when corrected for sectorial dilution, were nearly as satisfactory, showing that the preparations were free from significant amounts of large aggregates or other impurities.

The commercial human transferrin contained approximately 8% of heavier material (Fig. 2*b*). The faster-sedimenting component that forms when

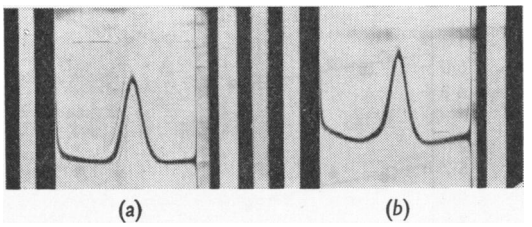


Fig. 1. Ultracentrifuge patterns obtained with: (*a*) rat transferrin; (*b*) monkey transferrin. Protein concentrations were approx. 0·8%. Sedimentation (right to left) was in sodium phosphate buffer, $I$ 0·1, for: (*a*) 97 min. at 52640 rev./min. and 23·0° (pH 8·0); (*b*) 93 min. at 59780 rev./min. and 19·0° (pH 7·0).

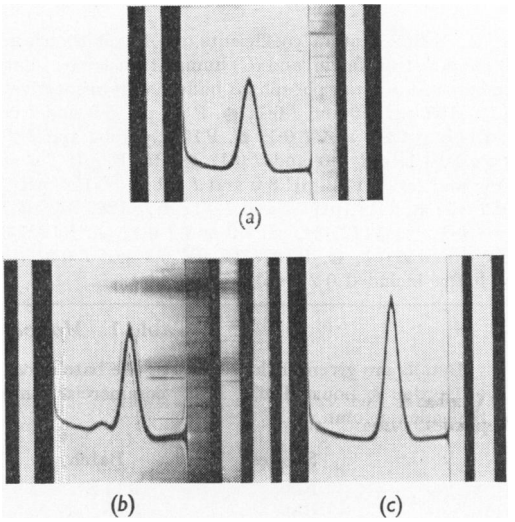


Fig. 2. Ultracentrifuge patterns obtained with human transferrins: samples (*a*) P42, (*b*) B1227/34, (*c*) S1227/34 (see Table 1). Protein concentrations were approx. 0·8%. Sedimentation (right to left) was in sodium phosphate buffer, $I$ 0·1, for: (*a*) 97 min. at 52640 rev./min. and 20·8° (pH 8·0); (*b*) 59 min. at 59780 rev./min. and 17·6° (pH 7·0); (*c*) 72 min. at 59780 rev./min. and 20·5° (pH 7·0).

bovine mercaptalbumin is stored in the freeze-dried state is mostly eliminated by the procedure for iodination, owing largely to the higher pH (Charlwood, 1963). Similar conditions, when applied to the human transferrin, failed to produce a corresponding effect. Other attempts to bring it about were made with the buffers containing sodium sulphite or iodoacetamide, but no success was achieved. The minor component was, however, removed very satisfactorily by means of Sephadex G-200 (Fig. 2c).

*Sedimentation coefficients.* These are shown in Fig. 3 with the calculated regression lines, which show in all cases small concentration-dependence.

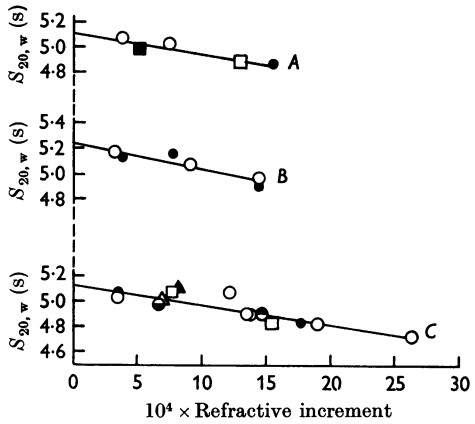


Fig. 3. Sedimentation coefficients of (*A*) rat transferrin, (*B*) monkey transferrin, and (*C*) human transferrin. Batch numbers and sodium phosphate buffers were respectively: (*a*) ○, P35, pH 7·0 and *I* 0·1; ●, P35, pH 8·0 and *I* 0·1; □, P11B, pH 8·0 and *I* 0·1; ■, P19B, pH 6·4 and *I* 0·2; (*b*) ○, P64/2, pH 7·0 and *I* 0·1; ●, P58B, pH 7·0 and *I* 0·1; and (*c*) □, P42, pH 8·0 and *I* 0·1; △, TR2, pH 7·5 and *I* 0·1; ▲, TR1, pH 7·5 and *I* 0·1; ◓, S1227/34, pH 7·0 and *I* 0·1; ○, B1227/34, pH 7·0 and *I* 0·1; ◒, B1227/34, pH 8·0 and *I* 0·1; ●, B1227/34, pH 6·8 and *I* 0·31 (this last buffer included 0·2 M-sodium chloride).

$S^0_{20,w}$ was close to 5·1 s for the rat and human transferrins and about 5·2 s for monkey transferrin. There was no perceptible influence on human transferrin when the ionic strength was increased from 0·1 to over 0·3. The minor component in the commercial transferrin had a sedimentation coefficient of about 7·2 s.

*Molecular weights.* Apart from the sample of human transferrin that contained 8 % of a second component, all preparations gave molecular weights close to 68000 (Table 1). The slightly higher value for monkey transferrin could be a consequence of its content of a trace of heavier material (Fig. 1*b*), although the departure from 68000 is within the limits of experimental error. The high value for the commercial transferrin is clearly to be attributed to its minor component, removal of which leads to a value almost as low as for the column product P42.

## DISCUSSION

Although sedimentation coefficients of rat and monkey transferrins do not seem to appear in the literature, there are several references to human transferrin, and one to pig transferrin, which are summarized in Table 2. Some of the values are based on a small number of measurements and some were done in earlier types of ultracentrifuge before it was appreciated that temperature errors leading to high results might arise unless special precautions were taken to maintain standard conditions of operation (Cecil & Ogston, 1948). The buffers used also differed considerably in ionic strength, but Fig. 3 indicates lack of dependence on this factor. The method of plotting sedimentation coefficients, against either initial concentration of solution or mean concentration during ultracentrifuging, affects the apparent concentration-dependence by a factor that is essentially constant for experiments of comparable length (and is 0·87 in the present case). However, $S^0_{20,w}$ is independent of the abscissa convention. The present work gives

Table 1. *Molecular weights of transferrins*

Details are given in the text. For the batch nos., an initial letter P signifies material isolated by the method of Gordon & Louis (1963), B the commercial sample and S the commercial sample after passage through the Sephadex column.

| Species | Batch | pH | Approx. concn. (%) | Mol.wt. |
|---|---|---|---|---|
| Rat | P11B | 8·0 | 0·7 | 66 100 |
| Rat | P35 | 8·0 | 0·8 | 67 200 |
| Rat | P35 | 7·0 | 0·4 | 67 000 |
| Monkey | P58B | 7·0 | 0·8 | 68 500 |
| Monkey | P58B | 7·0 | 0·4 | 70 800 |
| Human | P42 | 8·0 | 0·8 | 66 000 |
| Human | P42 | 8·0 | 0·4 | 66 400 |
| Human | B1227/34 | 7·0 | 0·8 | 84 100 |
| Human | B1227/34 | 8·0 | 0·4 | 86 000 |
| Human | S1227/34 | 7·0 | 0·8 | 69 600 |
| Human | S1227/34 | 7·0 | 0·4 | 67 900 |

values at the higher concentrations which correspond well with the findings of Schultze, Schönenberger & Schwick (1956), but the concentration-dependence observed is much less than theirs, and $S^0_{20,w}$ was 5·1 s compared with their value of 6·1 s. There is no evidence from Fig. 3 that the method of isolation of the protein or the buffer composition is likely to be responsible for these discrepancies, so that a check on possible temperature anomalies in the air-turbine machine would seem to be desirable. The measurements of Mahling (1963), done in a Spinco machine and partly on a sample of one of the batches of transferrin (B 1227/34) used here, are definitely higher than the results shown in Fig. 3. There is no obvious explanation for this difference. It is interesting that Phelps & Cann (1956) found $S^0_{20,w}$ to be 5·1 s for conalbumin, which Williams (1962) has shown to differ only by a carbohydrate prosthetic grouping from chicken transferrin.

The present work is at variance with all previous publications with respect to molecular weights. Again there is no comparison possible for the rat and monkey transferrins, but several values of about 90 000 have been published for human transferrin and a similar one for pig transferrin. Osmotic measurements of Oncley *et al.* (1947) on human-plasma fraction IV–7 gave a molecular weight of 93 000, from which Koechlin (1952), who had obtained a minimum value of 45 000 from iron-binding, deduced that the molecule must take up 2 atoms of iron and have a molecular weight of 90 000. The reliability of iron-binding measurements does depend on all molecules having the same binding capacity, which must not be permanently affected by the conditions used in isolating the protein. Koechlin (1952) cited unpublished light-scattering results in support of a molecular weight of 90 000, and Schönenberger (1955) obtained a value of 88 000 by the same technique.

By combination of sedimentation and diffusion coefficients Laurell & Ingelman (1947) obtained a molecular weight of 88 000 for pig transferrin, and Schultze, Heide & Müller (1957) 89 000 for human transferrin. Both groups of workers took $\bar{v}$ to be 0·725 (as also assumed here) in their calculations. The higher sedimentation coefficients found by them, whatever the reasons, are partly responsible for the molecular weights being higher than those in the current work, but the observations on the commercial transferrin suggested another possible reason for high values, the presence of a proportion of higher molecular-weight material. This could be an extraneous protein, or more likely, and taking into account its sedimentation coefficient, a dimer, possibly formed in freeze-dried material. It is known that albumin may aggregate when stored in this form (Halwer, Nutting & Brice, 1951; Christiansen, Jensen & Marcker, 1957; Charlwood, 1963). The possibility of dimer formation through protein thiol groups, which exists with mercaptalbumin, would be excluded if human transferrin contains no free thiol groups as claimed by Koechlin (1952). Some evidence that may be to the contrary (Harris, Penington & Robson, 1960) makes this point uncertain, but conditions that largely eliminate albumin dimer (Charlwood, 1963) have no similar effect on the transferrin impurity. Moreover, the lack of response to sodium sulphite or iodoacetamide suggests that it is linkages other than disulphide which are involved. It is again worth mentioning the results of Phelps & Cann (1956) on conalbumin. By osmotic measurements and by sedimentation and diffusion they obtained molecular weights of 75 000–77 000, although there were tendencies for aggregation to occur in some circumstances.

The general outcome of this investigation is that the molecular weights of human, monkey and rat transferrins are all close to 68 000, and that previous estimates for human transferrin are high for reasons discussed. This finding indicates that transferrin is closely similar in molecular size to both albumin and haemoglobin.

Table 2. *Sedimentation coefficients of transferrins*

| Species | $S^0_{20,w}$ (s) | Observers |
|---|---|---|
| Human | 5·5 | Mahling (1963) |
| Human | 5·38 | Nagler, Kochwa & Wasserman (1962) |
| Human | 5·1* | Katz (1961) |
| Human | 6·1 | Schultze, Schönenberger & Schwick (1956) |
| Human† | 5·5 | Oncley, Scatchard & Brown (1947) |
| Pig | 5·8 | Laurell & Ingelman (1947) |

* $S_{20,w}$ at 1% protein concentration.
† Human-plasma fraction IV–7.

## SUMMARY

1. Human, monkey and rat transferrins have been studied in the ultracentrifuge at high speed to measure sedimentation coefficients and at low speed to determine molecular weights.

2. The sedimentation coefficients (and dependence on concentration) were closely similar for all species, $S^0_{20,w}$ being 5·1–5·2 s, but rather lower than the values available for human and pig transferrins. This difference may be due partly to temperature errors in some of the older work, but there is a residual, fairly small, discrepancy difficult to account for.

3. The molecular weights of all three transferrins were close to 68 000, in comparison with previous values of about 90 000 for human and pig transferrins. The reasons for this are discussed.

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## REFERENCES

Archibald, W. J. (1947). *J. phys. Chem.* **51**, 1204.
Cecil, R. & Ogston, A. G. (1948). *Biochem. J.* **43**, 592.
Cecil, R. & Ogston, A. G. (1951). *J. sci. Instrum.* **28**, 253.
Charlwood, P. A. (1961). *Biochem. J.* **78**, 163.
Charlwood, P. A. (1963). *Analyt. Biochem.* **5**, 226.
Christiansen, J. A., Jensen, C. E. & Marcker, K. (1957). *Acta chem. scand.* **11**, 402.
Gordon, A. H. & Louis, L. N. (1963). *Biochem. J.* **88**, 409.
Halwer, M., Nutting, G. C. & Brice, B. A. (1951). *J. Amer. chem. Soc.* **73**, 2786.
Harris, H., Penington, D. G. & Robson, E. B. (1960). *Biochem. J.* **74**, 44P.
Katz, J. H. (1961). *J. clin. Invest.* **40**, 2143.
Koechlin, B. A. (1952). *J. Amer. chem. Soc.* **74**, 2649.
Laurell, C. B. & Ingelman, B. (1947). *Acta chem. scand.* **1**, 770.
Mahling, A. (1963). *Z. Naturf.* **18***b*, 1.
Nagler, A. L., Kochwa, S. & Wasserman, L. R. (1962). *Proc. Soc. exp. Biol., N.Y.*, **111**, 746.
Oncley, J. L., Scatchard, G. & Brown, A. (1947). *J. phys. Chem.* **51**, 184.
Phelps, R. A. & Cann, J. R. (1956). *Arch. Biochem. Biophys.* **61**, 51.
Schönenberger, M. (1955). *Z. Naturf.* **10***b*, 474.
Schultze, H. E., Heide, K. & Müller, H. (1957). *Behringwerk-Mitteilungen*, **32**, 25.
Schultze, H. E., Schönenberger, M. & Schwick, G. (1956). *Biochem. Z.* **328**, 267.
Williams, J. (1962). *Biochem. J.* **83**, 355.

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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, 1962*a*). 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