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# CHOLINESTERASE AND SUCCINIC DEHYDROGENASE IN THE CENTRAL NERVOUS SYSTEM OF THE DOG

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In the assessment of the possibility of a cholinergic mode of synaptic transmission in the central nervous system it is desirable to obtain the maximum amount of information on the distribution of the content of acetylcholine, the acetycholine synthesizing system and cholinesterase in different regions. This information is useful in three main ways: first, gross inequalities of distribution might suggest that cholinergic transmission, even if applicable at some synapses, may not necessarily be applicable to all synapses: secondly, these same inequalities may indicate those synapses at which further study is most likely to provide a crucial test of the hypothesis; and thirdly, quantitative discrepancies between the respective data may call into question some of the basic assumptions which previous authors have used in devising tests of the theory.

A considerable body of data on the acetylcholine content of the brain exists (see Feldberg, 1945), of which the most extensive data on single species is that of MacIntosh (1941) on the cat and dog. In considering the information on content it must be borne in mind, however, that it is influenced to a considerable extent by the method used in estimation. The problems of accurately estimating such a labile substance in the presence of both very active anabolic and catabolic enzymes are immense, and some of these difficulties have been illustrated in recent papers by Richter & Crossland (1949), Crossland (1950) and Elliott, Henderson & Swank (1950). The values on acetylcholine content available at present must therefore be regarded as tentative and possibly subject to considerable error.

The only data on the distribution of the acetylcholine synthesizing system in a large number of areas of the nervous system are in the very thorough study which was carried out on the dog by Feldberg & Vogt (1948).

Numerous authors have studied the cholinesterase content of a few parts of the brain, but owing to a variety of minor differences of technique in estimation it is difficult to gather this information into a coherent body. Nachmansohn's

(1939) data are the most extensive to date by a single author, but even so are not comparable in range to those of Feldberg & Vogt on the synthesizing system and, further, the work was carried out before the discovery by Mendel & Rudney (1943*a*) that when 'cholinesterase' is measured with acetylcholine as substrate two enzymes, which may possibly differ in their functional significance, contribute to the value obtained. Where comparison has been possible there is excellent agreement between the results of Nachmansohn and those of the present authors.

Succinic dehydrogenase has been studied as a representative of the enzymes important in the general metabolism of the cells of the central nervous system, but which have not been shown to have any direct connexion with acetylcholine metabolism.

## METHODS AND MATERIALS

Adult dogs of various breeds ranging in weight from 10 to 35 kg. were used. The dogs were killed with coal gas and the nervous system was rapidly removed and placed in a dish containing ice-cold glucose-free Tyrode's solution. The dissection of the nervous system was then immediately commenced, taking special care over the following points: as exact identification of the anatomical sites as possible, separation from adjoining areas, e.g. dissection of the white matter off the underside of cortex with minimal removal of the deeper layers of grey matter, and stripping of the pia mater, choroid plexus and any other visible blood vessels. The pieces of tissue were dried by squeezing with moderate pressure between two sheets of Whatman's No. 1 filter-paper. If the tissue were not completely blood-free, it was squeezed a second time between filter-papers. The tissue was then transferred with a spatula to a small tared screw-capped bottle, weighed to the nearest 0·1 mg. and then placed in the refrigerator at about 4° C. until the estimation could be performed. If possible at least 50 mg. of tissue were taken; it was of little value to attempt succinic dehydrogenase determinations with less than 150 mg. of tissue. The total time taken from the death of the dog until all the required samples had been weighed and stored was usually 2–2½ hr. As Nachmansohn (1939) found, and has been confirmed by the authors, the enzyme in isolated tissue is stable and may be kept in the refrigerator until the following day without appreciable loss of activity. When succinic dehydrogenase was being estimated fewer samples were taken and the time taken for dissection was reduced to less than 2 hr.

### *Estimation of cholinesterase activity*

The estimations were carried out in the Warburg manometric apparatus at 37° C. using vessels with a volume of 18–23 ml. The tissue samples were transferred to chilled porcelain mortars and ground first with fine silver sand and then sufficient of a bicarbonate medium (NaCl 100 mM., $NaHCO_3$ 30 mM., KCl 2 mM., $CaCl_2$ 1 mM.) to give a suitable amount of tissue per ml. The tissue was then thoroughly homogenized with the medium, and 0·5–1·0 ml. was pipetted into the main compartment of the Warburg vessel. Sufficient medium was then added to make the volume up to 1·8 ml.; 0·2 ml. of substrate was placed in one of the side arms of the vessel which was then equilibrated with 95% $N_2$ + 5% $CO_2$ mixture giving a final pH of 7·6. Readings were taken at 10 min. intervals for 60–120 min. and the values calculated from the period when the gas evolution was constant, always excluding the first 20 min. In calculating the amount of tissue added to each vessel it was assumed that the specific gravity of the sample was unity. The final substrate concentrations were: acetylcholine chloride (Merck) 12·7 mM.; DL-acetyl-β-methylcholine chloride (Merck) 35·8 mM.; benzoylcholine chloride (Hoffmann-LaRoche) 10 mM. Correction was made for non-enzymatic hydrolysis of substrate, and an attempt was made to correct for the gas evolution due to glycolysis, but this was irregular and partly suppressed by substrate. Since no glycolysis

substrates were added to the medium the small amounts carried over in the tissue were soon depleted, and consequently after 20 min. the gas evolution due to glycolysis was very small. If readings were commenced at least 20 min. after temperature equilibrium was reached, the error due to glycolysis was not important.

Our results are expressed in '$Q$' units in which $Q$ is defined as '$\mu$l. $CO_2$ evolved/g. wet weight of tissue/10 min.'.

A total of seventeen dogs was used in these estimations and each mean is based on three to nine individual values. The terms ChE I and ChE II are used as by Augustinsson (1948) as physiologically non-committal terms corresponding to what other authors call true and pseudo-cholinesterase, or acetylcholinesterase and cholinesterase.

The results given in the tables are derived entirely from the manometric method. In calculating the value given in Table 1, col. 6, allowance has been made for the relative rates of hydrolysis of acetylcholine, acetyl-$\beta$-methylcholine and benzoylcholine.

As the manometric method is somewhat insensitive considerable doubt remained whether the very low values obtained for optic nerve, corpus callosum, and frontal white matter represented any activity at all, and accordingly some experiments were done by a bioassay procedure which has a considerably greater sensitivity.

*Bioassay method for determination of* ChE I

The tissue was ground with M/60 $Na_2HPO_4$: $NaH_2PO_4$ buffer (pH 7·25) and 1 ml. of this homogenate was added to 4 ml. of the same buffer containing 50 $\mu$M. of acetyl-$\beta$-methylcholine. The mixture was incubated for 2 hr. at 30°C., the reaction then stopped by the addition of 0·5 ml. 0·1 N-HCl and the sample stored in the refrigerator until assay could be carried out. The residual ester was assayed on the guinea-pig small intestine, using a method mentioned by MacIntosh & Perry (1950). The guinea-pig ileum was used, and was suspended in a modified Ringer-Locke solution of the following composition: NaCl 9 g./l.; KCl 0·42 g./l.; $CaCl_2$ 0·24 g./l.; $NaHCO_3$ 0·2 g./l.; $MgCl_2$ 0·1 g./l.; glucose 1 g./l.; and mepyramine maleate (Rhone-Poulenc) 0·5 $\mu$g./l. Under these conditions the intestine is usually quiescent and gives a brief contraction to applied acetylcholine or acetyl-$\beta$-methylcholine. Samples can be added to the bath at intervals of 1–1·5 min. and a difference of concentration of 5% can be discriminated. At this substrate concentration the velocity of hydrolysis follows the monomolecular equation and the value of the calculated velocity constant $k$ gives the enzyme activity. The correction for non-enzymatic hydrolysis is very small relative to the enzymatic hydrolysis. Correlation of these values with the manometric ones was achieved by running samples simultaneously by both tests and using the values obtained for active regions such as cerebellar cortex and caudate nucleus for conversion of the $k$ values into Warburg equivalents. These results cleared any doubt of the validity of the Warburg results and showed that the regions cited do contain ChE I in the low concentrations of the order indicated in Table 1.

*Estimation of succinic dehydrogenase*

Estimation of succinic dehydrogenase was carried out by the method of Kun & Abood (1949). In the control tubes an equal amount of brain was added but the sodium succinate was replaced by an isosmotic (2%) solution of sodium chloride. It was assumed that the colour developed in the control tubes was not due to succinic dehydrogenase and the value was accordingly subtracted from the total.

It was found that in any given region the variability of succinic dehydrogenase activity from animal to animal was larger than the variations in the relative activity of different regions. For convenience the values are expressed as percentages of the activity of the cerebellum.

The cortical areas were identified with the help of the cytoarchitectonic map of Klempin (1921).

## RESULTS

In many parts of the nervous system the concentration of cholinesterase is remarkably consistent from animal to animal; this may be noted especially in the caudate nucleus where the coefficient of variation from the mean value for ChE I is only 10·1%, and in the cerebellar hemisphere where it is 14·4%. This makes it likely that the considerably larger variations encountered at some other sites are to be attributed partly to the greater difficulty encountered in obtaining anatomically identical samples and, in the case of areas with a low activity, to the lower relative accuracy of the determination at these levels. On the whole the variability of the ChE II determinations was lower than that of ChE I, probably because the total spread of the values is so much smaller that minor anatomical differences no longer constitute a serious source of error. In any case the uniformity of the results compares very favourably with the corresponding values for acetylcholine content and synthesis obtained by other authors.

Table 1 gives the collected data for the hydrolysis of acetyl-β-methylcholine (*Q* MeCh), benzoylcholine (*Q* Benz), and acetylcholine (*Q* ACh). A suitable adjustment of the values in the first two columns can be made to allow for the slower hydrolysis of the 'specific' substrates as compared with acetylcholine. Under the conditions employed acetyl-β-methylcholine is hydrolysed by ChE I at 59% of the rate of acetylcholine, and benzoylcholine by ChE II at 85% of the rate of acetylcholine. These values are means of single values derived by simultaneous equations from estimations on regions containing predominantly (*a*) ChE I and (*b*) ChE II. The agreement of the synthetic value so derived (column 4) with the *Q* ACh (column 3) is sufficiently good to make it unlikely that any enzyme unaccounted for by columns 1 and 2 contributes appreciably to the hydrolysis of acetylcholine in the dog brain.

### *Distribution of ChE I*

Turning now to the figures for *Q* MeCh, in column 1, it will be noticed first that the ratio of the highest, the caudate nucleus at 3936, to the lowest, the sub-cortical white matter at 10, is nearly 400 : 1, which is larger than the spread of values found by Feldberg & Vogt for synthesizing activity (anterior spinal roots : optic nerve, 42 : 1). It is evident then that ChE I is distributed in a most uneven fashion in the brain. In the cerebral cortex the lowest values were found in the visual cortex (area 17) and the highest in the uncinate gyrus (area 51). Otherwise there is no obvious segregation of the cortical areas into groups and it is especially noteworthy that there is no important difference in activity between the motor and sensory areas. The cerebellar cortex shows a high activity which was found to be uniform over the lateral lobes, the vermis, and the flocculus but higher values were obtained on the anterior lobe. The

TABLE 1. Distribution of cholinesterases in different regions of dog's brain and spinal cord

| Area | (1) *Q* MeCh ±S.D. | | (2) *Q* Benz | (3) *Q* ACh | (4) *Q* ChE I, II | (5) $\frac{Q\text{ MeCh}}{Q\text{ Benz}}$ | (6) Activity due to ChE II (%)‡ |
|---|---|---|---|---|---|---|---|
| Proreal gyrus 8 | 171± | 29 (5)† | — | 331 | — | — | — |
| Precentral gyrus 6*a* | 237± | 38 (4) | 35 | 371 | 442 | 6·77 | 9 |
| Postcentral gyrus 4*a* | 178± | 41 (6) | 42 | 330 | 351 | 4·23 | 14 |
| Primary sensory cortex 3 | 150± | 25 (5) | — | 282 | — | — | — |
| Sylvian gyrus 52 | 230± | 42 (6) | 32 | 407 | 427 | 7·18 | 9 |
| Postsplenial gyrus 17 | 107± | 34 (5) | — | — | — | — | — |
| Suprasplenial gyrus 18, 19 | 114± | 12 (5) | 27 | 232 | 225 | 4·23 | 14 |
| Posterior suprasylvian gyrus 20 | 162± | 81 (4) | 46 | — | — | 3·52 | 17 |
| Cingulate gyrus 23, 24 | 203± | 51 (5) | 32 | 377 | 382 | 6·34 | 10 |
| Hippocampal gyrus 28 | 238± | 52 (3) | 49 | — | — | 5·12 | 12 |
| Uncinate gyrus 51 | 466± | 148 (8) | 91 | 1372 | 897 | 5·75 | 11 |
| Cerebellar hemisphere | 1075± | 155 (7) | 24 | 1864 | 1850 | 44·8 | 2 |
| Cerebellar vermis | 1228± | 489 (3) | — | — | — | — | — |
| Cerebellar flocculus | 931± | 350 (3) | — | — | — | — | — |
| Cerebellar anterior lobe | 1756± | 670 (3) | — | — | — | — | — |
| Cerebellar dentate nucleus | 530± | 300 (5) | — | — | — | — | — |
| Cerebellar superior peduncle | 333± | 97 (5) | 90 | 654 | 670 | 3·70 | 16 |
| Cerebellar middle peduncle | 243+ | 68 (5) | 50 | 582 | 471 | 4·85 | 13 |
| Cerebellar inferior peduncle | 294± | 56 (5) | — | — | — | — | — |
| Caudate nucleus, head | 3936± | 396 (9) | 360 | 7450 | 7094 | 10·9 | 6 |
| Thalamus, dorso-lateral nucleus | 409± | 97 (7) | 161 | 808 | 883 | 2·54 | 21 |
| Thalamus, massa intermedia | 600± | 94 (5) | 194 | 1437 | 1245 | 3·09 | 18 |
| Lentiform nucleus | 2606± | 1200 (7) | 318 | 4992 | 4794 | 8·20 | 8 |
| Hypothalamus | 323± | 37 (5) | 358 | 866 | 967 | 0·90 | 43 |
| Red nucleus | 452± | 162 (5) | — | 1269 | — | — | — |
| Periaqueductal grey matter | 537± | 205 (3) | — | — | — | — | — |
| Olfactory bulb | 197± | 27 (3) | — | — | — | — | — |
| Fornix | 50± | 22 (3) | 69 | — | — | 0·73 | 49 |
| Optic nerve | 11± | 7 (5) | 222 | 283 | 280 | 0·05 | 93 |
| Optic tract | 86± | 49 (6) | 76 | 237 | 235 | 1·13 | 38 |
| Lateral geniculate | 230± | 57 (4) | — | 708 | — | — | — |
| Medial geniculate | 316± | 86 (4) | — | 938 | — | — | — |
| Superior corpus quadrigeminum | 932± | 195 (6) | 159 | 1619 | 1765 | 5·89 | 11 |
| Inferior corpus quadrigeminum | 364± | 73 (5) | 184 | 811 | 833 | 1·98 | 26 |
| **Corpus callosum | 16± | 13 (7) | 27 | 62 | 59 | 0·59 | 54 |
| *Subcortical white matter | 10± | 5 (6) | 19 | 53 | 39 | 0·53 | 57 |
| Pes pedunculi | 84± | 24 (4) | 114 | 237 | 276 | 0·74 | 49 |
| Medullary pyramids | 82± | 41 (6) | 94 | 177 | 250 | 0·86 | 41 |
| Lateral spinal columns | 50± | 20 (5) | 36 | — | — | 1·39 | 33 |
| Spinal grey matter | 611± | 132 (6) | 218 | — | — | 2·80 | 20 |
| Anterior spinal root | 149± | 57 (7) | 20 | 212 | 275 | 7·45 | 8 |
| Posterior spinal root | 34± | 21 (7) | 20 | 82 | 81 | 1·70 | 29 |
| Posterior columns | 36± | 7 (5) | 39 | — | — | 0·92 | 43 |
| Nucleus gracilis and cuneatus | 477± | 91 (4) | — | 993 | — | — | — |
| Pituitary posterior lobe | 50± | 34 (3) | — | 252 | — | — | — |

*Q* MeCh, *Q* Benz and *Q* ACh = μl. $CO_2$ evolved/g. wet wt. of tissue/10 min. with acetyl-β-methylcholine, benzoylcholine and acetylcholine respectively as substrates. For meaning of *Q* ChE I and II see text.

* No significant difference was found between frontal and occipital white matter.

** No significant difference was found between different parts of the corpus callosum.

† Number of animals used for values in columns 1.

‡ Percentage of total acetylcholine hydrolysed that is split by ChE II, derived by the formula

$$\frac{59 \times Q\text{ Benz}}{0{\cdot}85\ Q\text{ Mech.} + 0{\cdot}59\ Q\text{ Benz.}}.$$

caudate nucleus showed outstanding activity and it is probable that parts of the lentiform nucleus are no less active, but we were unable to obtain sufficiently consistent results with the latter to be able to give a reliable value. The thalamus was considerably less active than either of these, but it is interesting that a representative part of the intralaminar system, the massa intermedia, was somewhat more active than the dorso-lateral nucleus.

The moderate activity of the hypothalamus was unexpected in view of the accumulating evidence of responsiveness to acetylcholine in this region (Emmelin & Jacobsohn, 1945; Pickford, 1947). The optic pathway shows an interesting sequence, a very low value for the optic nerve, a somewhat greater value for the optic tracts, a still higher value in the lateral geniculate body and the highest value in the superior corpus quadrigeminum, then a sharp fall to a very low value in the occipital subcortical white matter (including the optic radiations) and a low value in the visuosensory cortex (areas 17–19). The motor tracts also yield an interesting sequence, moderate in the motor cortex, very low in the subcortical white matter, higher in the pes pedunculi, medullary pyramids and lateral columns, high in the spinal grey matter and moderately high in the anterior spinal roots. It is unfortunate that in some of these regions the motor tracts are perforce diluted with other structures. In the sensory pathway the ChE I concentration is low in the posterior roots and columns, shows a sharp rise in the nuclei gracilis and cuneatus, remains at about the same level in the thalamus and then falls off in the sensory cortex. In contrast is the comparative uniformity of the olfactory bulb, medial geniculate body, inferior corpus quadrigeminum and uncinate gyrus.

### *Distribution of ChE II*

When we consider the distribution of ChE II we are struck by the much smaller range of the values; the ratio of highest to lowest value is only 18 : 1. In the cortical areas the ChE II bears a fairly constant relationship to the ChE I values. In the hypothalamus the *Q*Benz is actually higher than the *Q*MeCh. It will be seen that in general the fibre tracts contain relatively large amounts of ChEII. The most strange results are those for the optic nerve and tract; the former is very low in ChEI and very high in ChEII, the latter contains moderate amounts of both enzymes. In view of Feldberg & Vogt's evidence that the high choline acetylase activity of the optic tracts relative to the optic nerves was due to the high activity of the commissural fibres, it seems possible to explain our results by assuming that the optic nerve fibres are low in both cholinesterases but are accompanied by an extraneural structure very high in ChE II, this might be the ophthalmic artery or structures associated with it. At the chiasma this structure is no longer present and the optic fibres are joined by a commissural component rich in both enzymes.

*Proportion of cholinesterase activity of the nervous system due to ChE II*

On the basis of the rather slight evidence provided by Mendel and co-workers (Mendel & Rudney, 1943*b*; Hawkins & Mendel, 1947), it has been widely accepted that the amounts of ChE II in the nervous system are negligible as compared with the ChE I. The final column of Table 1 shows that this is not true. In 80% of areas examined ChE II contributed more than 10% to the rate of hydrolysis of acetylcholine, and in 30% of areas more than 40%. It is therefore not sound to assume that in the special case of brain, the use of acetylcholine as a cholinesterase substrate effectively estimates only ChE I. It would seem unwise without further evidence to dismiss a possible functional significance for the ChE II present in the brain.

*Distribution of succinic dehydrogenase*

The values for succinic dehydrogenase are seen in Table 2. It will be seen that the values for cellular parts of the nervous system are fairly uniform, although the caudate nucleus is the most active area. The fibre tracts are about one-eighth as active as this and the nerve trunks show a very low activity.

TABLE 2. Succinic dehydrogenase in different regions of dog's brain and spinal cord

| | % | | % |
|---|---|---|---|
| Cortex area 4 | 88 | Caudate nucleus | 134 |
| Cortex area 6 | 80 | Thalamus | 90 |
| Cortex area 18, 19 | 93 | Superior corpus quadrigeminum | 68 |
| Cortex area 8 | 72 | Inferior corpus quadrigeminum | 80 |
| Cortex area 51 | 44 | Corpus callosum | 13 |
| Cerebellar hemispheres | 100* | Posterior columns | 7 |
| Cerebellar middle peduncle | 10 | Subcortical white matter | 14 |
| Anterior spinal roots | <2 | Optic nerve | 3 |
| Posterior spinal roots | <2 | | |

* Corresponds to an absolute activity of 166·7 $\mu$g. succinic acid dehydrogenated/g. wet wt./hr.

The importance of these results is that they show that an enzyme believed to be of key importance in the general metabolism of the brain is distributed in a manner radically different from that of ChE; it is distributed in a comparatively uniform manner amongst areas of comparable cellularity. This supports the view that the cholinesterase distribution is not connected with the ordinary cell metabolism, but subserves some specific function.

*Comparison with acetylcholine synthesis*

In Table 3, columns 3, 5 and 6, the results obtained by Feldberg & Vogt (given as percentages of the activity of the thalamus) for acetylcholine synthesis distribution are compared with our results for cholinesterase. In most cases the ratio (acetylcholine synthesis)/($Q$ MeCh) $\times$ 100 falls within a comparatively

small range—in 85% of areas the ratio falls into the range 10–55. The areas outside these limits are the cerebellar cortex and the caudate nucleus with ratios of 0·8 and 3·2 respectively, and the corpus callosum and the anterior spinal roots with ratios of 163 and 158. The correlation with the *Q* Benz is equally good, and in this case the only values that fall outside a compact range are the optic nerve at 2·5, and the anterior spinal roots which give the very high ratio of 1180. It should be emphasized that the figures in the table have no absolute significance, and allowing for the different methods of calculation in the two groups of experiments the mean rate of acetylcholine breakdown is about 800 times greater than the rate of synthesis under the conditions used.

TABLE 3. Comparison of cholinesterase distribution, acetylcholine content and acetylcholine synthesis of different parts of dog's brain and spinal cord

| Area | (1) *Q* MeCh | (2) *Q* Benz | (3)* ACh synthesis | (4)† ACh content | (5) ACh synth. / *Q* MeCh | (6) ACh. synth. / *Q* Benz | (7) ACh synth. / ACh content | (8) *Q* MeCh / ACh content |
|---|---|---|---|---|---|---|---|---|
| Cortex area 3 | 150 | — | 57 | 2·8 | 38 | — | 20 | 54 |
| Cortex area 4 | 178 | 42 | 71 | 4·5 | 40 | 169 | 16 | 40 |
| Cortex area 17 | 107 | — | 58 | 2·2 | 54 | — | 26 | 49 |
| Cortex area 51 | 466 | 91 | 81 | — | 17 | 89 | — | — |
| Cerebellar hemisphere | 1075 | 24 | 8·5 | 0·18 | 0·8 | 35 | 47 | 5970 |
| Superior peduncle | 333 | 90 | 59 | — | 18 | 66 | — | — |
| Middle peduncle | 243 | 50 | 60 | — | 25 | 120 | — | — |
| Inferior peduncle | 295 | — | 36 | — | 12 | — | — | — |
| Caudate nucleus | 3936 | 360 | 127 | — | 3·2 | 35 | — | — |
| Thalamus | 409 | 161 | 100 | 3·0 | 24 | 62 | 33 | 136 |
| Red nucleus | 452 | — | 87 | — | 19 | — | — | — |
| Lateral geniculate | 230 | — | 105 | — | 46 | — | — | — |
| Medial geniculate | 316 | — | 60 | — | 19 | — | — | — |
| Superior corpus quadrigeminum | 932 | 159 | 104 | 1·65 | 11 | 65 | 63 | 565 |
| Inferior corpus quadrigeminum | 364 | 184 | 44 | — | 12 | 24 | — | — |
| Olfactory bulb | 197 | — | 55 | 1·3 | 28 | — | 42 | 151 |
| Hypothalamus | 323 | 358 | 115 | 1·4 | 36 | 32 | 82 | 231 |
| Spinal grey matter | 611 | 218 | 128 | 2·6 | 21 | 59 | 49 | 235 |
| Nucleus gracilis and cuneatus | 477 | — | 86 | — | 18 | — | — | — |
| Medullary pyramids | 82 | 94 | 12·3 | *c.* 0·2 | 15 | 13 | 62 | 410 |
| Pes pedunculi | 84 | 114 | 22 | — | 26 | 19 | — | — |
| Corpus callosum | 16 | 27 | 26 | 2·1 | 163 | 96 | 12 | 8 |
| Posterior spinal columns | 36 | 39 | 10 | *c.* 0·05 | 28 | 26 | 200 | 720 |
| Optic nerve | 11 | 222 | 5·6 | 0·3 | 50 | 2·5 | 19 | 37 |
| Optic tract | 86 | 76 | 35 | — | 41 | 46 | — | — |
| Anterior spinal roots | 149 | 20 | 236 | 15 | 158 | 1180 | 16 | 10 |

*Q*MeCh and *Q*Benz are as defined in Table 1

* Feldberg & Vogt (1948). Percentage of activity of thalamus, whose mean synthesizing power was 323 μg./g. acetone powder/hr.

† MacIntosh (1941). μg. acetylcholine/g. brain.

### *Comparison with acetylcholine content*

There are no figures available on the acetylcholine content of the nervous system from the work of a single author that are complete enough to offer a really satisfactory comparison with the figures given above. However, MacIntosh's (1941) figures for cat brain and dog spinal cord are the best

available, and it seems more valid to use such figures than to jumble together results from a number of workers. From Table 3, columns 4, 6 and 8, it can be seen first that the correlation between the acetylcholine content and the acetylcholine synthesis is quite good. The correlation between acetylcholine content and cholinesterase is less good, the cerebellar hemispheres, corpus callosum, and anterior spinal roots showing the main discrepancies.

## DISCUSSION

The reasonable degree of correlation found in most areas between the three components of what may be called the acetylcholine system are gratifying and support the suggestion put forward by Feldberg & Vogt that there may be two types of neurone in the nervous system, one rich in the acetylcholine system and the other lacking in this system, and that these two may be correlated with a cholinergic and non-cholinergic mode of synaptic transmission respectively.

The discrepancy between the anterior spinal root values for cholinesterase and those for synthesis and content may be due to a peculiar situation of the synaptic cholinesterase. At the neuromuscular junction almost all the cholinesterase is on the post-synaptic cell surface (Couteaux, 1947; Koelle & Friedenwald, 1949; Brooks, 1951) and it is affected little by degeneration of the motor nerve. It may be that this is an evolutionary change and has been associated with a corresponding loss of cholinesterase in the motor nerve fibre. This is in marked contrast to the autonomic ganglia where almost all the ChE I disappears on degeneration of the preganglionic nerve (Sawyer & Hollinshead, 1945). It would be interesting to know whether the synapses in the central nervous system resemble the neuromuscular junction or the sympathetic ganglion in their distribution of cholinesterase and experiments to test this are now in progress.

At present it does not seem possible to offer an adequate explanation of the high ChE I of the cerebellar hemispheres. It may be however that one of the several unique cell types of the cerebellum is unusually rich in this enzyme.

The gross distribution of the enzymes described in this paper forms a basis for further study of the more detailed anatomy of the neurones containing the acetylcholine system by the histochemical procedures currently being developed in this and other laboratories. This appears to be the most profitable approach to the further understanding of the functions of these enzymes.

## SUMMARY

1. The amounts of cholinesterases I and II and of succinic dehydrogenase have been measured in a large number of areas of the dog's brain.

2. The amounts of both cholinesterases are widely different in distinct anatomical sites, although consistent from animal to animal.

3. In many parts of the nervous system, ChEII is present in important amounts.

4. Succinic dehydrogenase is present at a fairly uniform level in all cellular areas examined, but smaller amounts are found in the fibre tracts.

5. The correlation between the amount of cholinesterase, acetylcholine synthesis and acetylcholine content is good, the main exceptions being the cerebellar hemispheres with a disproportionately high cholinesterase content and the anterior spinal roots with a low cholinesterase content.

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