J. Physiol. (1948) 107, 372–381 547.435–292:612.8

ACETYLCHOLINE SYNTHESIS IN DIFFERENT REGIONS OF THE CENTRAL NERVOUS SYSTEM

By W. FELDBERG* and MARTHE VOGT

From the Physiological Laboratory, University of Cambridge, and the Pharmacological Laboratory, The College of the Pharmaceutical Society, University of London

(*Received* 10 *November* 1947)

In the present paper an attempt has been made to map out, in the central nervous system of the dog, the distribution of the enzyme (or enzyme system) which synthesizes acetylcholine. It has been shown that this enzyme can be extracted from acetone-dried tissue (Feldberg & Mann, 1944) and that such extracts, when incubated aerobically, form large amounts of acetylcholine in the presence of KCl, $MgCl_2$, choline, cysteine, citrate and adenosine-triphosphate (Nachmansohn & Machado, 1943; Nachmansohn, John & Waelsch, 1943; Nachmansohn & John, 1945; Feldberg & Mann, 1944, 1945, 1946; Feldberg & Hebb, 1947). Since this method is applicable to very small amounts of tissue, it was possible to compare the enzyme concentration in various quite small regions of the central nervous system.

METHODS

Dissection. The dogs were anaesthetized with ether, and bled. The brain and spinal cord were taken out as rapidly as possible. The nervous tissue was divided into several pieces and parts not immediately wanted were kept in the refrigerator whilst the dissection of the first samples was carried out. The excised samples were at once dried with cold acetone. The number of regions tested in a single experiment was limited, since care was taken that the time interval between excision of the first and the last sample did not exceed 2 hr. Samples kept in the cold for such a period did not lose much activity, certainly not more than 20%. In addition there was satisfactory agreement in control experiments in which the order of preparing the samples from different regions was varied. Samples from the tracts and horns of the spinal cord and from the region of the supraoptic nuclei were cut out with a small knife after freezing the tissue. Such freezing for a short time did not affect the ability of the tissue to synthesize acetylcholine.

Details about the dissection of those regions which are macroscopically ill-defined are as follows. Samples from the cerebral cortex and cerebellar cortex were obtained by cutting off the grey matter from the underlying fibres with fine scissors. The cortical areas were identified with the help of the maps published by Klempin (1921). The region called 'cerebellar nuclei' is that part

* With a grant from the Medical Research Council.

of the cerebellar medullary body which contains the nuclear masses but it also contains a large number of fibres. The region of the nuclei gracilis and cuneatus contains many fibres from the corresponding tracts. The position of most other nuclei (nucleus vestibularis, motor nucleus of the vagus with hypoglossal nucleus, nucleus ruber and substantia nigra) can be identified macroscopically, but their cells are scattered between fibres of varied origin. There is no sharp definition of the different parts into which the internal capsule was divided; these were obtained by sectioning the brain horizontally and taking in succession fibres from an anterior, a posterior and an intermediate region. The optic tracts were, at first, used as a whole. Later on they were split longitudinally in such a way that one-half contained the latero-ventral and the other half the mediodorsal fibres. A region comprising the supraoptic nucleus was obtained in the following way. A block from each hemisphere, containing half the chiasma and one optic tract, was frozen hard; then a horseshoe-shaped band of tissue, a few millimetres in thickness and width, and immediately adjacent to the medial part of the optic tract, was cut out of each block.

Assay of enzymatic activity. The method was essentially the same as that described by Feldberg & Mann (1945, 1946). The acetone-dried tissue was extracted with 0·9% NaCl but the extracts were incubated without first centrifuging off the insoluble material as was done in the experiments of Feldberg & Mann. Special care was taken that the different samples contained the same proportion of dried tissue and of saline solution. This is necessary since the rate of synthesis of acetylcholine in brain extracts is dependent on the concentration of some heat stable tissue constituent called the 'activator' (Feldberg & Mann, 1945, 1946) or the 'coenzyme of acetylation' (Lipmann, 1946). Thus, if different amounts of a particular tissue were suspended in the same volume of fluid, the concentration of the activator would vary and the rate of synthesis per g. tissue change accordingly.

There is the possibility, however, that the concentration of activator varies in different parts of the nervous system. This would introduce a source of error in our estimations, but the error would be small, because, in the presence of adenosinetriphosphate and citrate, the addition of activator, even in optimal concentration, produces a relatively small increase in the rate of synthesis. Furthermore, from the results obtained by Feldberg & Mann (1946) it appears that, if there are any variations in the concentration of activator in different parts of the nervous system, they are too small to interfere with the quantitative aspect of our assay.

When sufficient dried tissue was available, 50 mg. were ground in 4·5 c.c. of 0·9% NaCl solution to which had been added 6 mg. KCl, 2 mg. $MgCl_2$, 2 mg. NaF, 4·5 mg. cysteine hydrochloride, 0·5 mg. eserine sulphate, 3 mg. choline chloride, 12 mg. sodium citrate and 0·4 mg. adenosinetriphosphate-pyro P. When less than 50 mg. but more than 8 mg. dried tissue were available, an aliquot part of the incubation fluid was used. With less than 8 mg. dried tissue, the volume of fluid would have become too small for the assay to be practicable. Therefore the following modification was introduced. A solution of inactivated brain powder was prepared by boiling 100 mg. acetone-dried brain tissue in 4 c.c. 0·9% NaCl solution. The sample was then set up so as to contain an extract of 25 mg. of dried tissue of which 7 mg. or less were contributed by the tissue under investigation and the remainder by the inactivated brain powder. The sample was then dealt with like any other sample of 25 mg. dried tissue. In addition, a separate blank sample was incubated which contained the extract of 25 mg. inactivated brain powder and the usual constituents of the incubation mixture.

After incubation for 1 hr. at 37° C., all samples were acidified with HCl, boiled for 1 min. and stored in the refrigerator. Before assay, they were neutralized and then tested for acetylcholine on the eserinized rectus muscle of the frog with the following precautions. The larger samples were assayed against acetylcholine made up in the same volume of the test solution, from which the acetylcholine had previously been removed by short treatment with hot alkali. The smaller samples, to which extract of inactivated brain powder had been added, were assayed against acetylcholine made up in the same volume of the incubated blank sample. This was done since the test solution contained so little enzyme, and therefore so little acetylcholine, that no material could be spared to prepare a blank by boiling part of it with alkali.

RESULTS

Great individual variations were found in the synthesizing power of different brains, but the ratio between the synthesis in different regions varied little from brain to brain. For this reason, only the synthesizing power of the thalamus, which was examined in each experiment, is stated in Table 1 in absolute figures, i.e. in μg. acetylcholine formed in 1 hr. by 1 g. dried tissue, whereas the values for all other regions are expressed as percentages of this figure. The last column in Table 1 is the mean value of these percentages.

In no region of the central nervous system was the ability to form acetylcholine as high as it is in the anterior roots. This may be due to the fact that hardly anywhere in the C.N.S. do we come across a region consisting of a single kind of fibre only or of cells which are not lying between fibres of varied origin. The highest figures for the central nervous system were obtained from those regions where the spinal and cranial motor nerves originate, i.e. the anterior horns and the hypoglossal and vagal (motor) nuclei. These regions have about 70% of the activity of the anterior roots. At the lower end of the scale we find, first, the sensory nerves and tracts (optic and acoustic nerves, funiculi gracilis and cuneatus); secondly, the pyramidal fibres (both in the cord and in the cerebral peduncles); thirdly, the cerebellar cortex. The activity found in these regions is hardly greater than that of many non-nervous tissues like heart ventricle, skeletal muscle, spleen and liver.

Unlike the cerebellar cortex, the cerebellar peduncles have a moderate synthetic power; this is higher for the upper and middle than for the lower peduncle which carries the connexions with the cord. The activity of the region of the cerebellar nuclei is intermediate between that of cortex and peduncles.

The enzymatic activity in the white matter of the corpus callosum and of the internal capsule is a little higher than that of the pyramidal tracts. In the internal capsule, the most caudally situated fibres contain the smallest quantity of enzyme.

Of the remaining values listed in Table 1, the highest are those for the caudate nucleus, the region of the supraoptic nuclei and the cornu ammonis. The rate of synthesis of acetylcholine in these parts is usually somewhat greater than in the thalamus. In the cerebral cortex the enzyme content is always smaller. It is comparatively uniform even in areas functionally and architecturally as different as area **4** (motor), area **3** (somaesthetic), area **17** (visual) and area **51** (olfactory). The differences between the areas, however, are consistent in spite of their small size. It is noteworthy that the highest activity does not reside in the motor but in the olfactory area **51**, particularly since another olfactory centre, the cornu ammonis, was found among the more active parts of the brain.

TABLE 1. Synthesis of acetylcholine in different regions of the nervous system

Exp. ...	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	
Thalamus ... (μg. acetylcholine/g. powder)	*270*	*400*	*370*	*220*	*250*	*260*	*260*	*210*	*230*	*250*	*280*	*520*	*520*	*300*	*500*	
	Synthesis expressed as percentage of value for thalamus															Mean
Area **4**	89	60	—	68	68	81	62	69	—	68	—	—	—	—	—	71
Area **3**	59	55	—	—	—	—	—	—	—	—	—	—	—	—	—	57
Area **17**	56	45	—	59	72	54	62	60	59	—	—	—	—	—	—	58
Area **51**	—	—	—	—	—	—	—	86	74	84	—	—	—	—	—	81
Cerebellar cortex	7·4	8·7	6·7	11·4	—	—	—	—	—	—	—	—	—	—	—	8·5
Cerebellar nuclei	29	20	13·5	—	—	—	—	—	—	—	—	—	—	—	—	21
Upper cerebellar peduncle	41	34	73	73	48	69	77	—	—	—	—	—	—	—	—	59
Middle cerebellar peduncle	—	—	—	45	60	52	85	—	—	—	—	—	—	—	—	60
Lower cerebellar peduncle	—	—	—	45	28	20	52	—	—	—	—	—	—	—	—	36
Caudate nucleus	115	155	149	123	92	—	—	—	—	—	—	—	—	—	126	127
Red nucleus	—	—	—	—	—	—	—	152	83	44	61	—	—	97	—	87
Substantia nigra	—	—	—	—	—	—	—	67	57	48	52	—	—	32	—	51
Lateral geniculate body	107	87	111	115	—	—	—	—	—	—	—	—	—	—	—	105
Superior colliculus	96	97	103	118	—	—	—	—	—	—	—	—	—	—	—	104
Medial geniculate body	70	46	65	—	—	—	—	—	—	—	—	—	—	—	—	60
Inferior colliculus	56	27	49	—	—	—	—	—	—	—	—	—	—	—	—	44
Bulbus olfactorius	52	30	—	36	—	50	73	86	—	—	—	—	—	—	—	55
Cornu ammonis	—	—	—	—	—	—	—	148	74	112	97	—	—	—	—	108
Corpora mamillaria	—	—	—	—	—	—	—	—	44	—	54	—	—	40	—	46
Infundibulum	11	22	—	—	—	—	—	48	—	—	—	—	—	—	—	27
Nucleus supraopticus	—	—	—	—	—	—	104	—	130	112	—	—	—	—	—	115
Anterior horns	—	—	—	—	—	169	123	167	—	—	—	—	—	—	—	153
Hypoglossal and motor vagal nuclei	—	—	—	—	—	150	181	286	96	176	—	—	—	—	—	178
Posterior horns	—	—	—	—	—	92	92	124	—	—	—	—	—	—	—	103
Nuclei gracilis and cuneatus	—	—	—	—	—	85	88	86	—	—	—	—	—	—	—	86
Nucleus vestibularis	—	—	—	—	—	—	—	—	70	14	46	—	—	—	—	43
Pyramids	13·3	12	11·6	—	—	—	—	—	—	—	—	—	—	—	—	12·3
Pes pedunculi	—	—	—	—	—	—	—	—	13	40	12	—	—	—	—	22
Internal capsule, anterior part	—	—	—	—	—	—	—	—	35	44	34	—	—	—	—	38
Internal capsule, central part	—	—	—	—	—	—	—	—	35	48	36	—	—	—	—	40
Internal capsule, posterior part	—	—	—	—	—	—	—	—	26	14	41	—	—	—	—	27
Corpus callosum	28	30	19	—	—	—	—	—	—	—	—	—	—	—	—	26
Funiculi gracilis and cuneatus	10	6	13	—	—	—	—	—	—	—	—	—	—	—	—	10
Tractus olfactorius	—	—	—	—	—	35	50	57	—	—	—	—	—	—	—	47
Nervus opticus	—	—	2·2	6·8	—	11·5	—	—	—	—	—	—	—	—	2	5·6
Chiasma opticum	—	—	—	—	—	35	—	—	—	—	—	—	—	—	—	35
Tractus opticus, whole	43	21	41	41	—	52	—	—	—	—	—	—	—	10	—	35
Tractus opticus, latero-ventral part	—	—	—	—	—	—	—	—	—	—	6·4	21	—	—	8	12
Tractus opticus, medio-dorsal part	—	—	—	—	—	—	—	—	—	—	43	39	—	—	42	41
Pituitary, anterior lobe	—	—	—	—	—	—	—	—	—	0·6	18	0	3	4	—	6
Pituitary, posterior lobe	—	—	—	—	—	—	—	—	—	0	23	2·4	9	0	—	7
Nervus vestibularis	—	—	—	—	—	—	—	—	46	20	21	—	—	—	—	29
Nervus facialis	—	—	—	—	—	—	—	—	130	96	54	—	—	—	—	93
Anterior roots	—	—	—	—	184	258	215	286	—	—	—	—	—	—	—	236

It is also interesting that the enzyme concentration in the superior colliculi and lateral geniculate bodies, which are both optic centres, is similar and higher than in the corresponding acoustic centres, the inferior colliculi and medial geniculate bodies.

The results for the optic tracts and the chiasma need some comment. The activity of the chiasma was found to be higher than that of the optic nerve and lower than that of the tracts (Exp. 6). Since no other instance is known in which an uninterrupted tract changes its enzymatic activity during its course, it seemed likely that the increase in enzymatic activity on approaching the geniculate bodies was due to an admixture of cholinergic fibres not belonging to the optic tract. Dr J. D. Green and Dr G. W. Harris, from the Anatomy School, Cambridge, whose advice we sought on the subject, suggested the supraoptic commissural fibres as a possible source of contamination. They further advised us to split the tracts longitudinally, so as to separate the dorso-medial from the ventro-lateral fibres. If the commissural fibres, most of which run in the dorso-medial part of the optic tracts, were responsible for the high enzymatic activity of the tracts, such a procedure should produce two bundles of which the ventro-lateral one should have the lower activity of the two and one which approaches that of the optic nerve. The figures of Exps. 11, 12 and 15 confirm this interpretation and suggest that the commissural fibres are cholinergic.

The figures in Table 1 show further that the nuclear masses in which sensory fibres end (the posterior horns, nuclei gracilis and cuneatus and nucleus vestibularis) have a much higher enzymatic activity than the fibres themselves but that the activity is far smaller than that of the motor nuclei of either cord or medulla.

Finally, the anterior and posterior lobes of the pituitary are found to contain very little if any enzyme for the synthesis of acetylcholine.

DISCUSSION

The examination of the different parts of the central nervous system for their power to form acetylcholine was carried out in the hope of obtaining new evidence about the role of acetylcholine as transmitter at central synapses. Different authors hold different views on this subject and the question is anything but settled. The opposing views have been reviewed by Feldberg (1945*b*). In his opinion acetylcholine may well be the transmitter at some central synapses but not at others. The findings of the present experiments point in the same direction.

The interpretation of our results will be based on the following conceptions which are largely derived from our knowledge of the properties of cholinergic neurons in the peripheral nervous system:

(1) A cholinergic neuron is characterized by the fact that it is able to synthesize acetylcholine, not only at its ending but along the whole course of

its axon. Thus a tract composed of cholinergic fibres will be identified by a high concentration of the enzyme which synthesizes acetylcholine. Although there is at present no evidence that the enzyme has any function to fulfil along the course of the fibre, recent observations of Weiss (1947) offer an explanation for its uniform distribution in the neuron. Weiss showed that the axoplasm of nerve fibres migrates in a slow but constant flow from the cell body to the nerve endings, presumably carrying down to the synapse all enzymes manufactured in the cell.

(2) The concentration of acetylcholine-forming enzyme in a tract of fibres will be interpreted as a measure of the number of cholinergic fibres contained in the tract, and not as an indication that some fibres can produce more and others less acetylcholine per unit of time.

(3) A bundle of non-cholinergic fibres may be identified by the nearly complete lack of the enzyme which synthesizes acetylcholine. A small concentration of the enzyme need not necessarily be taken as proof of contamination with some cholinergic fibres, but may be due to the fact that practically every tissue has a slight ability to synthesize acetylcholine under the conditions of our experiments.

(4) The presence, in regions of grey matter, of a high concentration of the enzyme which synthesizes acetylcholine may indicate one of three possibilities. Either the neurons of which the nerve cells form a part, or the fibres terminating at these cells, or both, may be cholinergic. Therefore, a high concentration of enzyme in a nucleus does not necessarily imply that the enzyme is produced by the cell body or its axon. For the peripheral nervous system, this fact is illustrated by the sympathetic ganglia. They are rich in enzyme which synthesizes acetylcholine, but the enzyme is provided by the preganglionic endings and disappears after their degeneration.

(5) In interpreting the results we occasionally make use of our knowledge of the central actions of acetylcholine. It is necessary to emphasize that the sensitivity of a nerve cell does not depend on whether or not it gives rise to a cholinergic axon. Again the cells of the sympathetic ganglia demonstrate this fact. Though sensitive to acetylcholine, these cells are part of an adrenergic neuron. The reverse conclusion, however, that cells which respond to acetylcholine are cells upon which cholinergic neurons impinge, is more likely to be generally true.

With these facts in mind an interpretation of our results has been attempted.

In the voluntary motor pathway, the finding of a high enzyme concentration in the anterior horns and the motor nuclei of the cranial nerves is in agreement with the conception of a cholinergic nature of the lower motor neuron. On the other hand, the low values encountered in the pyramidal tracts indicate that the upper motor neuron is not cholinergic. In the motor area of the cerebral cortex, an intermediate value was found. This might suggest that cholinergic

neurons converge on the pyramidal cells, a possibility which is strengthened by the fact that both eserine and acetylcholine, locally applied to the motor cortex, initiate impulses in the pyramidal tracts (Miller, Stavraky & Woonton, 1940). It is, however, possible that the acetylcholine, when applied artificially to the cortex or when released and allowed to accumulate after eserine, does not act on Betz's cells directly but on another type of cells, the axons of which end on the pyramidal cells.

We have seen that the voluntary motor pathway consists of a non-cholinergic upper and a cholinergic lower neuron. The fibres of the pyramidal tract, however, are not the only ones to end on the anterior horn cells. Various fibres from other systems, some of which may be cholinergic, terminate at these cells. The same cell may therefore be impinged upon by cholinergic and non-cholinergic fibres.

The succession of alternating non-cholinergic and cholinergic neurons is not confined to the motor pathway but may occur more generally in the central nervous system. Another example is provided by the optic pathway. The retina contains large amounts of the enzyme which synthesizes acetylcholine, whereas the optic nerve is practically free from it (Feldberg & Mann, 1945, 1946). This suggests that acetylcholine is the chemical transmitter at one or more of the synaptic junctions in the retina. Among these junctions may be that to the ganglionic layer from which the non-cholinergic optic nerve originates. The optic fibres end mainly in the lateral geniculate body, and the high values found for this region may indicate a cholinergic nature of the third neuron in this pathway. Further evidence on this point would have required the analysis of samples from the tract connecting the geniculate body with the visual area. This was not attempted on account of technical difficulties.

Some fibres of the optic tracts end in the superior colliculi. It may be that the high values found in this region again indicate a change-over from the second non-cholinergic to a third cholinergic neuron, but they may also result from the presence of fibres belonging to other systems.

Our results also give some information on the sensory pathway which starts in the posterior roots. These fibres are non-cholinergic as they contain practically no enzyme which synthesizes acetylcholine (Feldberg & Mann, 1945, 1946). This is in keeping with the low values now found in the central continuation of these fibres, the funiculi gracilis and cuneatus. However, the relatively high values obtained for the nuclear masses in which these sensory fibres as well as those from the eighth cranial nerve end (the posterior horns, the nuclei gracilis and cuneatus and the nucleus vestibularis) might be evidence for the cholinergic nature of the second neuron in the sensory pathway. The fibres originating from the nuclei gracilis and cuneatus terminate in the thalamus. The third neuron originating from here and leading to the cortex is probably non-cholinergic, as suggested by the low values found for the fibres

of the internal capsule. In fact, the lowest values were found in its posterior part which, at least in man, is said to contain the sensory thalamic radiation.

From the low values found in the cerebellar cortex we may conclude that this region contains few, if any, cholinergic neurons. The values for the cerebellar peduncles are higher than those for the cortex and those for the region of the cerebellar nuclei are intermediate between those for the cortex and for the peduncles. Since the upper cerebellar peduncles, which largely consist of axons from the cerebellar nuclei, contain a certain amount of cholinergic fibre, it is probable that the latter fibres also originate in the central nuclei. Under those circumstances we must assume that certain of the neurons originating from the cerebellar nuclei are able to form acetylcholine. That the enzyme concentration in the nuclear region is none the less lower than in the upper peduncles can obviously largely be attributed to the ending, in the central nuclei, of the many non-cholinergic axons from the Purkinje cells.

The enzyme concentration in the caudate nucleus which belongs to a group of basal ganglia which inhibit involuntary impulses to the skeletal muscles is one of the highest encountered. The fact that administration of atropine in Parkinsonism can partly compensate for the loss of these centres is interesting in this respect, although it is not possible at the moment to offer any explanation, since the mechanism of the inhibitory action of these centres is anything but understood.

The low values found for the posterior pituitary show that this nervous structure contains only non-cholinergic nerve fibres. The high figures for the supraoptic nucleus therefore do not suggest that cholinergic fibres originate from the cells in this region but that such fibres terminate in synaptic connexion with these cells. Their sensitivity to acetylcholine, demonstrated by direct physiological evidence (Pickford, 1947), is an illustration, in the central nervous system, of acetylcholine-sensitive cells from which non-cholinergic fibres emerge. In contrast, the hypoglossal nuclei provide an instance of central nerve cells, which also have been shown to be sensitive to acetylcholine (Miller, 1943), but are the origin of cholinergic fibres. In the peripheral nervous system, these two cell types are illustrated by the cells of the sympathetic and parasympathetic ganglia, both kinds of cells being sensitive to acetylcholine, the former being the origin of non-cholinergic (adrenergic), the latter of cholinergic fibres.

The distribution of the enzyme which synthesizes acetylcholine corresponds, as far as data for comparison are available (see MacIntosh, 1941; Feldberg, 1945*b*), to the distribution of acetylcholine in different parts of the nervous system. The low acetylcholine content of the pyramids, the afferent fibres in the dorsal tracts, the cerebellum, the posterior roots and the optic nerve, and the high values for the retina and basal ganglia, suggest that the acetylcholine content of a tissue is related to, and dependent on, its ability to synthesize

acetylcholine, a conclusion also reached by Feldberg & Mann (1945, 1946). There is no such striking correspondence between the distribution of cholinesterase and the enzyme for the formation of acetylcholine. This may be due to the fact that there are no reliable data for comparison. In those data which are available, no distinction has been made between true and pseudo-cholinesterase although the former alone appears to be concerned with the acetylcholine metabolism (Hawkins & Mendel, 1947). In addition, many of the subdivisions used in the examination of the distribution of cholinesterase are of no great value for a neurological approach of the kind attempted in this paper. Nevertheless, there are some data which are comparable and, in fact, show some parallelism. The high values obtained for the cholinesterase activity of the caudate nucleus and the retina correspond to the high values found in the same structures for the enzyme which synthesizes acetylcholine. On the other hand, the cholinesterase values for the anterior roots and the thalamus are only about twice as high as those found for the optic nerve and the posterior roots.

If our assumptions based on the distribution of the enzyme which synthesizes acetylcholine in the nervous system are correct, i.e. that cholinergic neurons form only a fraction of the central neurons, that along one pathway there is often a succession of alternating cholinergic and non-cholinergic neurons, and that, furthermore, the same cell may be impinged upon by endings from cholinergic and non-cholinergic neurons, it is not surprising that the attempts at demonstrating the role of acetylcholine for synaptic transmissions within the central nervous system by electrical and other methods have yielded contradictory results.

How far the interpretation of our findings is justified can only be found out by further experiments and particularly by an even more detailed analysis of the distribution of the enzyme which synthesizes acetylcholine. Degeneration experiments may help in such an analysis. One difficulty, however, will not easily be overcome, i.e. the interpretation of the intermediate values found in so many regions and the possibility that cells and fibres of a different nature intermingle in the same region. There are some other facts not easily explained at present, as, for example, the individual variations and the variations between different species. When comparison is made of the power of the nervous tissue to synthesize acetylcholine in different dogs, individual variations amounting to over 100% are found. They may in part be associated with the fact that dogs of any race were used for our experiments. They may also be related to the age of the animals. Feldberg (1945*a*) has found, for instance, that in guinea-pigs the concentration of acetylcholine in the brain tissue appears to increase with age. The species differences are even more difficult to explain, particularly the diminution in the ability to form acetylcholine shown by the more highly developed brains. We do not know how far this

decrease can be accounted for by a greater proportion of supporting tissue in the more differentiated brains, or by a greater proportion of non-cholinergic neurons, or how far it could be explained on the assumption that the transmission by acetylcholine is a more primitive type of central transmission, the importance of which recedes in higher animals where it is retained in certain synapses only. These considerations emphasize the need of great caution in drawing far-reaching conclusions from data derived from one species only.

SUMMARY

1. The distribution of the enzyme or enzyme system which forms acetylcholine in the central nervous system of the dog has been determined by examining over forty separate regions with the use of a very sensitive quantitative method for the estimation of acetylcholine synthesis.

2. An attempt has been made to differentiate between cholinergic and non-cholinergic neurons in the central nervous system.

3. The results indicate that some at least of the motor and sensory pathways consist of chains of neurons which are alternately cholinergic and non-cholinergic in character.

REFERENCES

Feldberg, W. (1945*a*). *J. Physiol.* **103**, 367.
Feldberg, W. (1945*b*). *Physiol. Rev.* **25**, 596.
Feldberg, W. & Hebb, C. (1947). *J. Physiol.* **106**, 8.
Feldberg, W. & Mann, T. (1944). *J. Physiol.* **103**, 28 *P*.
Feldberg, W. & Mann, T. (1945). *J. Physiol.* **104**, 8.
Feldberg, W. & Mann, T. (1946). *J. Physiol.* **104**, 411.
Hawkins, R. P. & Mendel, B. (1947). *Brit. J. Pharm.* **2**, 173.
Klempin (1921). *J. Psychol. Neurol., Lpz.,* **26**, 229.
Lipmann, F. (1946). *Advances in Enzymology,* **6**, 231.
MacIntosh, F. C. (1941). *J. Physiol.* **99**, 436.
Miller, F. R. (1943). *Proc. Soc. exp. Biol., N.Y.,* **54**, 285.
Miller, F. R., Stavraky, G. W. & Woonton, G. A. (1940). *J. Neurophysiol.* **3**, 131.
Nachmansohn, D. & John, H. M. (1945). *J. biol. Chem.* **158**, 157.
Nachmansohn, D., John, H. M. & Waelsch, H. (1943). *J. biol. Chem.* **150**, 485.
Nachmansohn, D. & Machado, A. L. (1943). *J. Neurophysiol.* **6**, 397.
Pickford, M. (1947). *J. Physiol.* **106**, 264.
Weiss, P. (1947). *Abstracts XVII Int. Physiol. Congr., Oxford,* p. 101.