

# REVIEW ARTICLE

## CHEMICAL TRANSMISSION IN THE CENTRAL NERVOUS SYSTEM\*

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THE human brain is an assemblage of some  $10^{10}$  nerve cells. Large groups of neurones are functionally—though not anatomically—connected through the interlacing of their elongated processes, so that an impulse, starting in one cell, is presented with a choice of pathways along which it can be propagated to influence activity in other parts of the central nervous system. The actual path taken on any occasion is determined by the previous history of the brain, by concurrent events in it and by the inborn organisation of its neurones. In such ways arise the wide variations in behaviour and achievement which are the outward signs of the extreme plasticity of the human brain. The complexity of the brain, indeed, requires no emphasis and if those aberrations of behaviour which constitute mental illness had their basis solely in abnormalities of neurone organisation, there would be little immediate hope of developing a rational treatment of mental disease, the biggest challenge facing medicine today. Fortunately, a basic simplicity underlies the complexity, for all neurones are essentially alike in their structure and physiological behaviour, while the mechanism by which a nerve impulse is transmitted across the gap, or synapse, between one neurone and the next appears to be fundamentally the same throughout the nervous system. Until recently, the process of synaptic transmission was often considered to be an electrical process, analogous to that which maintains the nerve impulse. There is now, however, widespread support for the view that this transmission is achieved by chemical means: as the impulse approaches the end of one nerve, it liberates, from the fine termination of the nerve fibre, a minute jet of a chemical substance which, passing across the synapse, stimulates the next nerve cell in the chain. It is true that the nerve impulse itself, an “electrical” event in the sense that it is a change or polarisation propagated by local action currents, rests on chemical processes. It might also be argued that the arrangement of neurones in a brain is chemically determined since it is dependent on the individual’s protein organisation. But, according to current ideas, synaptic transmission is much more immediately chemical in nature since it depends on the continuing synthesis, release and destruction of specific chemical substances. It is natural to believe that such a mechanism will be more easily upset by minor biochemical changes in the brain, and more susceptible to pharmacological intervention, than one which depends on the passage of action currents from one cell to the next and is only more remotely supported by chemical events. Thus the more general acceptance

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of the hypothesis of chemical transmission has led to a biochemical view of mental illness and to a renewed hope that its rational treatment may soon become a matter of applied pharmacology. These beliefs have inspired a remarkable volume of research and the past decade has seen the recognition of neurochemistry, with which we may include neuropharmacology, as a discipline in its own right. Much of this recent work has been concerned with attempts to identify the substances involved in central transmission and this will form the chief topic of this review.

A complete survey of all recent work in this field, even were it possible, would be of doubtful value to the reader. Instead, the claims of the several candidates for transmitter status will be critically considered and attention will be focussed on those experimental results and theoretical considerations which, in the writer's opinion, are most likely to point the way to a true assessment of the parts played in central nervous function by the several active compounds which can be extracted from brain tissue.

The enthusiastic worker in any field tends to direct his critical faculty towards the work of others and away from his own, and readers of this review may reasonably complain that the writer's own results and opinions are accorded a prominence out of proportion to their importance. Those who wish to preserve a sense of balance are recommended to consult other general reviews<sup>1-3</sup> as well as those which relate to the individual compounds discussed here. These are referred to in the appropriate sections of this review.

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Biologists are prone to extend the scope of definitions beyond their legitimate limits and there is an unfortunate tendency to apply the term "transmitter" rather loosely to a variety of substances for whose transmitter status there is no real evidence. In order to clarify the nature of the transmission process, and because much of the material in later sections of this review is more easily interpreted with its aid, a brief account of the development of the chemical hypothesis of synaptic transmission is required.

Du Bois-Reymond (in 1877) seems to have been the first to consider the possibility that nerves might exert their excitatory action on muscle cells by liberating a chemical substance but it was Elliott<sup>4</sup>, in the early years of this century, who first firmly proposed the chemical hypothesis in the form we know today.

Elliott was struck by the similarity of the effects of sympathetic stimulation and adrenaline injection and he suggested that sympathetic nerves mediated their action by the liberation of adrenaline. Direct evidence in favour of this hypothesis was delayed until 1921, when there appeared the first of a series of papers by Loewi and his colleagues, the details of which are so widely known that they need no repetition here. Loewi demonstrated that the effects of vagus and sympathetic nerve stimulation in the frog were brought about by the liberation of chemical substances named, respectively, "Vagusstoff" and "Acceleransstoff". "Vagusstoff"

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was soon identified as acetylcholine but the nature of "Acceleransstoff" remained obscure for many years in spite of Elliott's original suggestion and the fact that adrenaline is liberated from the adrenal glands under just those conditions which also involve sympathetic stimulation. The parallelism between the actions of adrenaline and sympathetic stimulation was close but not sufficiently close to allow a positive identification of the sympathetic transmitter with adrenaline. The recent discovery that nor-adrenaline is an important constituent of sympathetic nerves has gone far to resolve this difficulty. Those who require a fuller account of the early development of the chemical transmission hypothesis would be well rewarded by reading the accounts by Dale<sup>5</sup> and Newton<sup>6</sup>.

The obvious interpretation of Loewi's experiments was immediately accepted by physiologists. It is important to realise, however, that his results were applicable only to the postganglionic fibres of the autonomic nervous system. These nerves control viscera and blood vessels which normally respond by slow and prolonged movements in contrast to skeletal muscle—which reacts to stimulation of its nerves by rapid twitches—and to the central nervous system in which impulses flash from cell to cell. To many, a chemical mechanism, adequate for transmission to the sluggish viscera, seemed entirely inappropriate for the rapid transmission of information which is required outside the autonomic nervous system. They regarded chemical transmission as a primitive mechanism, superseded in most parts of the body, even in the ganglia of the autonomic system, by an altogether faster, electrical transmission system in which the action currents in a nerve were the means of stimulating neighbouring muscle or nerve cells. Loewi himself did not believe that a humoral mechanism could account for the transmission of impulses from a motor nerve to striated muscle<sup>7</sup>.

The first extension of the chemical hypothesis was made by Dale and his colleagues who sought evidence for humoral transmission at the junction of motor nerve and striated muscle fibres\*. They showed that stimulation of motor nerves liberated acetylcholine, that acetylcholine, appropriately injected, caused twitches in voluntary muscle and that inhibition of muscle cholinesterase—whose physiological substrate is acetylcholine—led to changes in the response of the muscle consistent with preservation of a transmitter substance<sup>8-11</sup>. A similar series of experiments on autonomic ganglia produced suggestive evidence of the participation of acetylcholine in ganglionic transmission, too<sup>12-14</sup>. The conclusion that the results of these studies justified an extension of the humoral hypothesis of chemical transmission was strongly challenged by many authorities, led by Eccles<sup>15</sup>, and a sharp division of opinion arose and persisted for a decade.

Resolution of this dispute began when Eccles, Katz and Kuffler showed that the nerve impulse in a motor fibre gave rise in the muscle to an end plate potential (e.p.p.) which itself was the origin of the propagated

\* This junction is usually referred to as the "neuro-muscular junction" and it will be so described throughout this paper. The term should be understood to exclude the less specialised junction between autonomic nerves and smooth muscle.

muscle action potential. Thus the end plate potential is the essential intermediary between the two propagating impulses<sup>16</sup>. Kuffler demonstrated that the e.p.p. could only have been produced by a chemical substance liberated from the motor nerve and persisting transiently in the neuromuscular space<sup>17</sup>, while Eccles and MacFarlane showed that the behaviour of the e.p.p. towards a number of compounds with anticholinesterase activity was consistent with its production by acetylcholine<sup>18</sup>. Since that time abundant evidence in favour of the acetylcholine hypothesis of neuromuscular transmission has accumulated. Experimental support for the participation of acetylcholine in ganglionic transmission is perhaps not quite so complete but few would be prepared to deny its transmitter function here too.

The electrical hypothesis has been almost forgotten since its most active supporter became a fervent protagonist of the theory he once attacked. Nevertheless, it is well to remember that, however misguided their conclusions, some of the arguments of the erstwhile opponents of chemical transmission are not without their relevance today. Thus not all workers recognise clearly enough that the pharmacological effects of a naturally-occurring compound do not necessarily mirror its physiological action, nor that the effects of enzyme inhibitors are not necessarily or completely attributable to preservation of their presumed substrate.

The assertion that chemical transmission is a primitive mechanism has now lost much of its force and the idea of chemical transmission at central synapses has become more readily acceptable to those who had previously been dissatisfied with the evidence in its favour. It is, indeed, a little alarming to those (like the writer) who have always championed the cause of chemical transmission, to find that the hypothesis is now being so uncritically accepted and it falls to them to caution the newly-converted and to emphasise that the fact of chemical transmission in the peripheral nervous system does not of itself provide any solid evidence for its occurrence in the central nervous system. The neuromuscular junction is anatomically rather simple: branches of the motor nerve fibre divide into a few terminal twigs, which come into relationship with quite widely separated points on the end plate of the muscle. Action potentials travelling along these twigs could affect only a small area of the end plate but acetylcholine can diffuse over a sufficiently large area to depolarise the end plate and thus initiate a propagated action potential. At central synapses a more complicated situation exists, for a large number of fibres, derived from several nerve cells, allow a more dense collection of terminal twigs on the surface of the nerve cell body. It is theoretically possible that the action currents from this large number of fibres could produce a sufficiently large area of depolarisation to make the intervention of a chemical transmitter unnecessary or to reduce its importance at some synapses. Recently, however, direct evidence in favour of chemical transmission at central synapses has become available.

Eccles and his colleagues have used micro-electrodes to impale individual motor neurones of the spinal cord<sup>19</sup>. They have been able to show that potential changes in the nerve cell membrane accompanying reflex

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excitation and inhibition arise in the membrane itself: that is to say, they are not merely passive changes induced by action currents stemming from the stimulated afferent nerves. If they are not due to action currents, they must be due to chemical agents. Recently Grundfest<sup>20</sup> has brought together a formidable mass of evidence, derived from his own work and that of others, that the synaptic regions of central neurones are not excitable by electrical means. If this is so, and if it can be shown to be a general property of those portions of the nerve cell body which take part in the formation of synapses, there can be no possibility of anything but chemical transmission throughout the nervous system.

### CHOLINERGIC TRANSMISSION IN THE CENTRAL NERVOUS SYSTEM

The brain and spinal cord contain acetylcholine, the enzyme which synthesises it, choline acetylase, and both "true" and "pseudo" cholinesterase. Acetylcholine and choline acetylase occur together but the distribution of cholinesterase does not entirely coincide with that of choline acetylase, since it is clearly necessary for a cell to be able to dispose of acetylcholine in its neighbourhood whether it has been made there or merely transported from another site.

The evidence that central transmission is chemical in nature strengthens the possibility that the presence of choline acetylase in the central nervous system betokens a transmitter role for acetylcholine. The work of Feldberg and his colleagues, however, has shown that not all neurones are capable of synthesising acetylcholine. There is a tendency for neurones with high choline acetylase activity to alternate with cells from which the enzyme is absent<sup>21,22</sup>. Thus sensory impulses reach the brain by a three neurone pathway, the first and third of which are deficient in choline acetylase while the second has a high acetylcholine-synthesising power. It is reasonable to believe that this alternation of neurones with different enzyme activities corresponds to an alternation of cholinergic and non-cholinergic elements. While there are many exceptions to this general concept, as its author has been prompt to point out, it is a useful one, for it emphasises that acetylcholine is probably the mediator of transmission across a large number of widely scattered synapses throughout the central nervous system.

The evidence in favour of this view has been very completely reviewed by Feldberg on several occasions during the past 14 years<sup>23-27</sup>. It must be admitted that surprisingly little new direct evidence in favour of central cholinergic transmission has accumulated over the years, but the indirect evidence summarised in these reviews has greater weight when it is assessed in the circumstances of the more favourable climate of opinion which prevails today\*.

In view of the thoroughness of the surveys by Feldberg it is not necessary here to mention more than a few relevant pieces of experimental work.

\* In the first of the reviews mentioned, an unfortunate misprint attributes to Feldberg the opinion—in 1945!—that "the role of acetylcholine in the central nervous system is all but settled". The wording intended was "the role of acetylcholine . . . is anything but settled".

It will be recalled that, as evidence in support of their claim that neuromuscular transmission is chemically mediated, Dale and his colleagues showed that acetylcholine was liberated on stimulation of the motor nerve and that it was capable of stimulating the muscle. Similar, if less definite, evidence is available for the brain. Tower and McEachern, for instance, recovered acetylcholine from the cerebrospinal fluid of patients experiencing convulsions<sup>28</sup>, and MacIntosh and Oborin<sup>29</sup> demonstrated that acetylcholine accumulated in a small saline-filled cup placed in contact with the exposed cerebral cortex of a cat. The rate of accumulation of acetylcholine fell as anaesthesia deepened. Finally, the observation of Richter and Crossland<sup>30</sup> that the acetylcholine content of brain bears an inverse relation to the degree of prevailing cerebral activity can most easily be explained by assuming that, while its synthesis remains constant, acetylcholine is released in amounts proportional to the extent of stimulation.

It has frequently been shown that acetylcholine has a stimulating action on the brain. Most of these experiments are open to the objection, insofar as they are intended to demonstrate a physiological role for acetylcholine, that very large doses have to be employed. This criticism can hardly be levelled against the work of Bonnet and Bremer<sup>31</sup>, who demonstrated excitatory actions on the cat cortex of as little as 0.1  $\mu\text{g}$ . of acetylcholine, nor against Pickford<sup>32,33</sup>, who injected acetylcholine and anticholinesterases directly into the supra-optic nucleus of the dog. Stimulation of the nucleus was inferred by the secretion of antidiuretic hormone. These experiments are of particular interest in the context of the present discussion, for not only are the cells of the supraoptic nucleus of the dog particularly sensitive to acetylcholine, but Feldberg's work on the distribution of choline acetylase in the central nervous system indicated that the fibres converging on the supraoptic nucleus are rich in choline acetylase. Thus, cells which are in contact with fibres rich in acetylcholine are themselves highly sensitive to the action of acetylcholine. This relation forms strong evidence of a cholinergic transmission mechanism at least at these synapses. In certain synapses in the spinal cord there is also evidence of a cholinergic mechanism. Collaterals of motor nerves which leave the main fibre close to its origin from the neurone return to the grey matter of the cord to synapse with small neurones (the Renshaw cells) which themselves, on stimulation, inhibit activity in motor neurones. Thus a stimulated motor neurone sets into play a mechanism which tends to inhibit its own activity, an arrangement which may serve to limit the extent of convulsions. Since the motor nerve itself is cholinergic, it is safe to assume that its collaterals are also cholinergic, that is, transmission of impulses from these collaterals to the Renshaw cells is mediated by acetylcholine. Eccles, Fatt and Koketsu<sup>34</sup> have shown that the response of the synapses to pharmacological agents is consistent with this interpretation. Thus the intra-arterial injection of eserine increased the Renshaw cell discharge while  $\beta$ -erythroidine, an acetylcholine inhibitor, inhibited it.

The central actions of acetylcholine are complicated by the fact that

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under some circumstances it shows inhibitory effects. This is sometimes due to the fact that it is stimulating cells, such as the Renshaw cells, which are themselves inhibitory and sometimes because an excessive accumulation of acetylcholine at a neurone which it has excited will prevent the cell from firing again because it holds the neurone in a depolarised state. A relatively small increase will convert a stimulating dose into a paralysing one. Crossland and Mitchell have shown in addition that a dose which is stimulant to a quiescent cortex becomes inhibitory when the background activity of the brain increases<sup>35</sup>. A striking example of the inhibitory effects of acetylcholine is provided by the experiments of Feldberg and Sherwood, who injected acetylcholine or cholinesterase inhibitors, which would presumably allow the accumulation of endogenously-liberated acetylcholine, into the lateral ventricles of a conscious cat. The injections produced a catatonic-like stupor<sup>36</sup>. Such stupors are a feature of certain types of schizophrenia in man and it is very interesting that, on the few occasions when it has been possible to use it, purified cholinesterase injected into the lateral ventricles of schizophrenics in catatonia has caused a dramatic relief of their stuporous condition<sup>37</sup>.

It is possible that, in the central nervous system, the details of the transmission process may differ from those at the neuromuscular junction. Crossland and Merrick<sup>38</sup> showed that the acetylcholine content of rat brain increased during anaesthesia and that the extent of the change was independent of the nature of the anaesthetic agent used, or the duration of the anaesthesia. These results, together with those obtained with convulsant drugs<sup>39</sup>, suggest that the brain, notwithstanding its high choline acetylase activity is not normally "saturated" with acetylcholine but that the actual acetylcholine content of its cholinergic fibres is determined by the level of activity in those fibres. The physiological significance of this adjustment of acetylcholine content to activity is not clear but it is possible that it represents a mechanism which reduces the total amount of acetylcholine liberated under conditions of increased activity. This might prevent "over-excitation" of the brain, or alternatively prevent the local development of inhibitory concentrations. It is interesting in this connection that certain forms of convulsive activity in human beings occur during sleep, or are provoked by anaesthesia, when the amount of acetylcholine in the brain is higher than in the waking state.

Notwithstanding the obvious importance of acetylcholine in the brain, and the attention which it has received over so many years, it remains a disappointing fact that it has not been possible to show any far-reaching relationship between abnormalities of acetylcholine metabolism and the incidence of mental illness or nervous disease. Some years ago, it seemed that epilepsy might be due to the impaired ability of the brain to "bind" acetylcholine<sup>40</sup> but the results on which this supposition were founded have not been confirmed<sup>41</sup>. The changes which occur in the acetylcholine content of brain during convulsive activity<sup>30,39,42</sup> arise secondarily to the convulsions themselves. Nevertheless, so much more precise knowledge

is now available concerning the acetylcholine system that there is every hope that further study of its behaviour in the brain will lead to a real understanding of the basic processes underlying the activity of the higher reaches of the nervous system. It is particularly regrettable that, just when the chemical hypothesis of nervous transmission has been generally accepted, there has been a steep decline in interest in the transmitter role of acetylcholine in the brain. Many workers, including the writer, are now engaged in the search for non-cholinergic transmitter substances, though there is no reason why they should be more likely than acetylcholine to hold the key to our final understanding of transmission in the brain. Much work will have to be done before the final role of many of the substances currently engaging the interests of physiologists is established and substantial rewards of knowledge might well await those prepared to re-direct their energies towards cholinergic transmission problems. It is interesting in this connection that evidence has recently been obtained of a link between the "intelligence" of animals and the activity of their brain cholinesterase<sup>43</sup>.

Finally, it should be noted that the presence of acetylcholine in any tissue does not necessarily pre-suppose its transmitter function. Acetylcholine is present in the nerve-free placenta; its distribution in the intestine is not related to any known disposition of nerves and there is evidence for the view that acetylcholine is responsible, quite independently of its own transmitter action, for the rhythmical activity of the heart<sup>45</sup>.

#### NON-CHOLINERGIC TRANSMISSION IN THE CENTRAL NERVOUS SYSTEM

The distribution of choline acetylase indicates, as was pointed out in the preceding section, that not all central neurones transmit their effects to neighbouring cells by the liberation of acetylcholine and there is other evidence that this is so. Sensory nerves contain no acetylcholine and Eccles<sup>46</sup> showed many years ago that acetylcholine can have no part in the transmission process across the synapses of simple monosynaptic reflexes, those, that is, in which the sensory nerve comes into immediate relationship with a motoneurone. Yet it is precisely here that, in the more recent experiments already referred to, he has demonstrated that transmission is chemical in nature. Thus at least one chemical mediator other than acetylcholine is needed and during the past few years many attempts have been made to name the non-cholinergic transmitter substance or substances.

Enthusiasm for chemical transmission has not always been combined with a critical assessment of the results being offered and there has been a tendency to lose sight of the essential qualities of a transmitter substance. The term should be applied only to a substance which, liberated from the end of a stimulated nerve, sets up an action potential in the muscle or nerve cells with which it is functionally connected. It is an essential feature of the definition that the transmitter substance actually *initiates* the action potential in the effector cell and does not merely potentiate or modify a process which could occur, in a different form, in its absence.



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There is little doubt that transmission can be modified by neuro-humoral agents which are not themselves transmitter substances. Such modifiers of transmission may be liberated with the transmitter itself from the activated nerve, they may be circulating in the blood or they may be otherwise available at the synapse. It is even possible that a substance may be a transmitter at some sites in the nervous system and a modifier of transmission elsewhere. A particularly good illustration of these points is provided by adrenaline. It, or closely related compounds, is liberated at the termination of the post-ganglionic fibres of the sympathetic nervous system, where it acts as a transmitter substance. It is also liberated, along with acetylcholine, at ganglionic synapses<sup>47</sup>, where it serves to potentiate the transmitter action of acetylcholine. Finally, adrenaline liberated into the blood stream from the adrenal medulla under conditions of intense sympathetic stimulation may potentiate the action of acetylcholine at the neuromuscular junction<sup>48</sup>.

Before any substance can be regarded as a strong candidate for transmitter status, it must be shown to be present in, and synthesised by, nerve cells; it must be liberated from these cells when they are stimulated, and it must be capable of stimulating or inhibiting other neurones standing in synaptic relationship with those from which it is liberated. The amount required to demonstrate this excitatory or inhibitory action must approximate reasonably closely to the amount liberated on physiological stimulation of the nerve after allowing for the fact that experimental injection cannot bring an active substance into such intimate contact with a cell as does physiological liberation. The nervous system must have enzymes at its disposal for destroying the transmitter substance. Finally, the material must have an appropriate action, consistent with its transmitter function, on the membrane potential of the cell it affects. Some workers have added other criteria to those listed here, but it is difficult to escape the suspicion that they have done so because the properties happen to accord with the properties of the substances they are themselves championing.

It will be seen that, at any rate at the neuromuscular junction, acetylcholine has the transmitter properties demanded but care must be taken not to assume that the details of the transmission process are identical throughout the body. Thus the acetylcholine liberated from motor nerves is inactivated, after it has initiated the muscle twitch, by cholinesterase, which is located at the muscle end plate. At the ganglionic synapse, however, cholinesterase is present in the pre-ganglionic fibre, where it is badly placed for hydrolysing acetylcholine which has acted at receptors in the ganglion cell. It appears that, in ganglionic transmission, acetylcholine is removed from its locus of action not by hydrolysis but by diffusion or perhaps by being bound up again in the pre-ganglionic fibre<sup>49,50</sup>. This reservation does not impair the validity of the argument that the nervous system must possess enzymes capable of destroying transmitter substances, for even where enzyme destruction is not an essential step in the transmission process, the nervous system still needs a safeguard against the possible accumulation of excessive amounts of

the transmitter substance. The point to be emphasised is rather the danger of assuming that transmission at one site is absolutely analogous with that at another.

Non-cholinergic excitatory transmitter substances must satisfy a further requirement for they should be found in highest concentration in tracts of fibres of low choline acetylase activity. Acetylcholine is widely distributed and there is no reason to expect the existence of more than one, or a limited number, of other transmitters with an equally wide distribution. Table I indicates the extent to which the several substances to be

TABLE I  
DISTRIBUTION OF PHARMACOLOGICALLY-ACTIVE SUBSTANCES IN SOME PARTS OF THE NERVOUS SYSTEM

	Choline acetylase μg./g. powder	Nor-adrenaline μg./g.	5HT μg./g.	Hista- mine μg./g.	Sub- stance P units/g.	Holton's stable vasodilator substance per cent	CEF per cent
<i>Regions which would be expected to contain non-cholinergic transmitter substances</i>							
Deep roots ..	<20	0.01	0	4-11	40	10	100
Optic nerves ..	16	0	0.02	0	9 (ox)	6	100
Dorsal columns	33	—	—	0	0.3 (ox)	27	0
Internal capsule (post) ..	70	—	—	—	—	—	100
Cerebellum ..	26	90	0.07	0.01	<0.1	1.6	100
Pyramids ..	42	—	0.06	0	—	—	15
<i>Regions from which non-cholinergic transmitter substances might be absent</i>							
Ventral roots ..	573	11,000	0.06	0	6-9 (ox)	6	0
Sympathetic ganglia ..	—	—	6	0	61 (ox)	—	0
<i>Other regions</i>							
Lateral geni- culate body ..	325	2,600	0.07	—	—	—	100
Mid brain ..	170	—	0.37	0.20	—	—	0
Hypothalamus ..	—	2,000	1.03	0.28	12	70	—
Thalamus ..	323	3,000	{0.24 Med 0.28 Lat }	{0.07 Med 0 Lat }	<0.4	12.5	6
Area postrema	—	—	1.04	0.24	—	460	—
Caudate nucleus	437	13,300	0.10	0	<0.2	46	100
References ..	21 and 22	71	52	69	85 86	69	103
							111

discussed below meet this requirement; though it quotes values for only a few parts of the central nervous system, it does fairly reflect the main features in the distribution of the substances under consideration. Further details of the distribution of active substances throughout the brain are available in the review by Paton<sup>3</sup>.

A note on the sub-headings of Table I might be helpful. In the central nervous system, nerve fibres collected into tracts run in the white matter and end in synaptic relationship with the cells of other neurones in grey matter. Thus tracts of fibres containing little choline acetylase (for example, the optic nerves) would be expected to possess a high concentration of non-cholinergic transmitter substance. A similarly high concentration would be expected in areas of the brain, such as the cerebellum, whose grey matter contains a high proportion of non-cholinergic neurones. Conversely, tracts of fibres rich in choline acetylase might

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be deficient in the alternative transmitter. In those many areas of the brain, however, which show the alternation of cholinergic and non-cholinergic pathways already referred to, the synaptic regions would be expected to show a high concentration of both transmitter substances. These areas are entered in the Table under the heading "Other regions".

Mention should also be made of the differing amounts of active substances found in nervous tissue. It is usual to say, for example, that 5-hydroxytryptamine is concentrated in the hypothalamus. This is true in the sense that the hypothalamus contains more 5-hydroxytryptamine than any other part of the brain but its absolute concentration in the hypothalamus is still much below that of acetylcholine.

### *Adrenaline and Noradrenaline*

Although adrenaline has had a long and honourable association with the general hypothesis of chemical transmission, it is only recently that attention has been directed towards its possible transmitter function in the central nervous system. It would be idle to pretend that a satisfactory formulation of the central function of adrenaline and noradrenaline has yet been proposed and no service would be performed by trying to force the experimental data into any such scheme. The best comment on the situation is that of Vogt<sup>51</sup>, herself a distinguished worker in this field: "Our ignorance as regards the function of brain sympathin could not be more complete".

In the brain, as in sympathetic nerves, adrenaline and noradrenaline occur together, with a preponderance of noradrenaline. Following Vogt, it is convenient to call this mixture "sympathin" and to refer to its individual components only when referring to experiments in which they have been specifically employed. It is important to add that the term "sympathin" does not here have the significance it has when the peripheral sympathetic system is under discussion. In the latter context the word refers to the actual transmitter of sympathetic impulses, as applied to the brain—it is merely a convenient way of describing a mixture of pharmacologically-active compounds.

Vogt<sup>52</sup> has studied the distribution of noradrenaline in the central nervous system of the dog: she found it in the hypothalamus and, in smaller amounts, in the grey matter of the mid-brain and medulla. It is virtually absent from other areas of the brain and from the spinal cord. More recently a similar distribution of noradrenaline has been noted in the human brain<sup>53</sup>. This distribution, as can be seen by reference to Table I, does not suggest that sympathin is a general non-cholinergic transmitter substance, though its particular localisation in the hypothalamus, a part of the brain which contains the controlling centres of the autonomic nervous system, raises the possibility that it may be concerned in the central regulation of autonomic activity as well as in its peripheral mediation. However, if concentration in the hypothalamus is to be the sole criterion of such a function, several other substances, including acetylcholine, will have to be considered as autonomic regulators.

Vogt found that drugs which cause increased secretion of the adrenal

medulla also cause a loss of sympathin from the hypothalamus. While it is true that even intense stimulation of the post-ganglionic fibres of the sympathetic system causes no detectable depletion of their contained sympathin, it is possible that, in the brain, synthesis of sympathin cannot keep pace with its liberation during stimulation. This is not, in itself, an unlikely possibility, since a similar hypothesis is required, as has already been mentioned, to explain the observation that the amount of acetylcholine in the brain is reduced as cerebral activity increases. Thus Vogt's findings suggest that physiological stimulation of the central nuclei of the sympathetic system causes the liberation of sympathin, which may therefore be involved in transmission in this area of the brain. It is not yet possible to state whether sympathin acts as a true transmitter, whether it modifies the transmission, or whether it is rather more remotely concerned in the events at the synapse. It will be recalled, however, that adrenaline appears to act as a modulator of the undoubtedly cholinergic transmission process at the neuromuscular junction and across ganglionic synapses and that it may even be liberated, together with acetylcholine, from stimulated ganglionic fibres. There is some suggestive evidence that sympathin may have a similar function in the brain. Duke and Pickford, for instance, injected acetylcholine and adrenaline into the supraoptic nucleus of the hypothalamus and found that adrenaline potentiated the action of acetylcholine<sup>54</sup>. The interest of this observation lies in the fact that transmission from other neurones to the supraoptic nucleus is probably cholinergic in type so that the situation here appears to be analogous to that at peripheral cholinergic synapses. Bülbring and Burn also obtained experimental results that may be interpreted on the assumption that adrenaline has a similar action at cholinergic synapses in the spinal cord<sup>55</sup>. Another relevant finding is that intravenously injected adrenaline penetrates the blood-brain barrier of the hypothalamus much more readily than that elsewhere in the brain<sup>56,57</sup>. Endogenously-liberated adrenaline from the adrenal medulla might therefore reach diencephalic regions, a possibility which is more consonant with the idea of adrenaline as a modulator rather than as a mediator of transmission, for synaptic transmission is a delicately-balanced process which is likely to be upset by the sudden arrival of quantities of transmitter material from an extra-synaptic source.

The areas of the brain containing sympathin are also closely related to the brain-stem nuclei of the reticular formation. This system, which has only recently attracted the attention of physiologists, consists of a diffuse system of ascending and descending fibres in the spinal cord and brain stem. The ascending fibres arise as collaterals of sensory fibres, so that sensory information normally passes to the central nervous system along two routes: one the well-defined, anatomically discrete lemnisci and the other, the diffuse reticular pathway. Impulses travelling by the latter route reach nuclei in the brain stem, whence they are related to the cortex which is thereby kept in an awake or "alerted" condition. Animals in which sensory input to the still-living cortex has been cut off, by a spinal cord or brain stem section, which leaves the blood supply to the

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brain intact (the *cerveau isolé* and *encéphale isolé* preparations of Bremer) appear to be sleeping and show the characteristic electroencephalographic sleep pattern. Stimulation of the reticular formation in such animals has an arousal effect. The reticular system also has a descending component, which receives impulses from higher parts of the central nervous system and cerebellum and which exerts, through its two divisions, facilitatory or inhibitory effects on muscle tone. Bonvallet, Dell and Hiebel have shown<sup>58</sup> that adrenaline produces an "alerting reaction" similar to that produced by stimulation of the ascending reticular system. The same group of workers have adduced evidence that adrenaline also stimulates neurones of the descending reticular system<sup>59</sup>. Rothballer has confirmed these observations and has shown that noradrenaline behaves like adrenaline in its actions on the reticular formation<sup>60</sup>. While there is, therefore, little doubt that there are sympathin-receptive neurones in the reticular nuclei, it is again impossible to say whether they are indicative of an adrenergic mechanism of transmission. The arguments already put forward for the view that sympathin merely *modifies* transmission in the central autonomic nuclei apply equally to the reticular formation. Moreover, if an alerting response involves the stimulation of sympathin-sensitive neurones by fibres which liberate sympathin, it might be supposed that some of the reticular fibres would themselves contain sympathin. There is, however, no evidence that this is so: the spinal cord, for instance, contains no noradrenaline. On the other hand, the work of Bradley<sup>61,62</sup> seems to show that acetylcholine, which also stimulates the reticular system, affects different receptors from those stimulated by adrenaline. It is thus possible that adrenaline, if it is not itself a mediator of transmission, might here be modifying a non-cholinergic transmission process.

The technique used by Feldberg and Sherwood enables drugs to be introduced into the ventricular system of conscious, unrestrained cats. Material introduced into animals in this way comes into immediate contact with the very regions of the brain rich in sympathin. In view of the results just described it is, at first sight, puzzling to find that intraventricular adrenaline produces, instead of an alerting response, a condition resembling light anaesthesia. It is, however, a common experience in pharmacology to find that a substance which in low concentrations is excitatory becomes inhibitory when its concentration increases (this is a very fortunate circumstance, for it can be used to explain away all inconsistencies in drug action) and there is evidence that adrenaline can have depressant actions, even when it is administered intravenously. Thus in Rothballer's experiments<sup>60</sup> "de-activation" as well as activation of the cortex was observed. Though very small doses of adrenaline (5-80  $\mu\text{g}$ . per cat) were used in the experiments of Feldberg and Sherwood<sup>36</sup>, it is possible that the effective concentration of the drug was increased by reason of its virtually direct application to the neurones upon which it was acting.

Although there is suggestive evidence that brain sympathin may play a part in transmission processes in the diencephalic region, it is clear

that insufficient information is available to enable a comparison to be made of its characteristics with those listed above as being necessary attributes of a chemical mediator of synaptic transmission. It is perhaps necessary to add that it is not yet known whether brain sympathin is contained in nervous or merely in glial (connective) tissue.

Marrazzi has put forward a rather different view of adrenaline action in the brain<sup>63</sup>. He stimulates the cerebral cortex of one side of the brain and records the potentials evoked on the contralateral side. These evoked potentials are potentiated by acetylcholine and inhibited by adrenaline and on this basis Marrazzi proposes a general inhibitory function for adrenaline throughout the central nervous system. It should be mentioned that Marrazzi also believes that adrenaline exerts a primary inhibitory action at the neuromuscular junction and at ganglionic synapses. Other authorities, however<sup>47,48</sup>, maintain that the primary action of adrenaline at these sites is potentiation of transmitter action and that the inhibition which frequently occurs is due to excessive potentiation, the adrenaline having converted the stimulant dose of acetylcholine into an inhibitory one. Adrenaline, however, can hardly be a transmitter in the cerebral cortex, where it is present in minute amounts if at all, while in view of what is known about the permeability of the blood-brain barrier it seems unlikely that circulating adrenaline can affect cortical transmission. Moreover, it is not easy to see how such circulating adrenaline, even if it reached the cortex, could exert the very localised and nicely-graded effect that would be required for co-ordinated central inhibition.

Finally, mention should be made of the fact first reported by Osmond and Smythies<sup>64</sup> and since developed by others, that oxidation products of adrenaline cause psychosis-like states on administration to man. This has led to the view that some types of mental disorder may be due to abnormalities of adrenaline metabolism. Discussion of this problem can more conveniently take place in the section which follows.

#### *5-Hydroxytryptamine (Serotonin)*

Erspamer detected a pharmacologically-active substance, to which he gave the name Enteramine, in acetone-extracts of the intestine. It attracted little attention until it was shown to be identical with 5-hydroxytryptamine, which Page and his colleagues extracted from blood serum. Under certain experimental conditions, 5-hydroxytryptamine has pressor effects and its discoverers proposed that its trivial name should be serotonin. Most British writers use instead the abbreviation 5-HT (or just HT) but as a protest—which is likely to prove quite ineffectual—against the tendency to allow neurochemical papers to take on the appearance of a reporter's notebook, the abbreviation will be avoided in this discussion. Those who feel, with Page<sup>66</sup>, that the repeated use of the word 5-hydroxytryptamine produces the irritating sound of fat spitting in the frying pan, might prefer to return to the use of the inoffensive "Serotonin".

During the past ten years 5-hydroxytryptamine has attracted far more

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attention than any other pharmacologically-active substance and in his review, covering work published during only four recent years, Page found it necessary to quote 529 references<sup>66</sup>. Serotonin is not confined to the nervous system and it has actions on a wide variety of tissues: general accounts of its occurrence, metabolism and properties are to be found in two exhaustive reviews by Page<sup>65,66</sup>, in a shorter one by Lewis<sup>67</sup> and in the proceedings of a recent symposium<sup>68</sup>.

In the brain the distribution of 5-hydroxytryptamine is very similar to that of noradrenaline<sup>69,70</sup> though the latter is present in considerably larger amounts (Table I). There is a close, though not perfect, correlation between the distribution of 5-hydroxytryptamine and 5-hydroxytryptophan decarboxylase, the enzyme responsible for its final elaboration<sup>72</sup>. The presence of 5-hydroxytryptamine in areas of the brain particularly concerned in emotional responses has quickened interest in the psychogenic effects of substances which are believed to interfere with its metabolism. Among those who have been particularly interested in the central function of serotonin, two general schools of thought have emerged and these will be dealt with separately.

Lysergic acid diethylamide (LSD) antagonises the action of 5-hydroxytryptamine on some pharmacological preparations: administered to human beings in very small doses it produces hallucinations and a psychotic-like state which in some ways resembles schizophrenia<sup>73</sup>. Woolley and his colleagues have shown that a number of naturally occurring drugs, such as yohimbine and harmine also antagonise the action of serotonin and cause abnormalities of behaviour in experimental animals suggestive of a disturbance of their mental state<sup>74</sup>. These observations suggest that a link exists between 5-hydroxytryptamine and hallucinogenic activity but this has not been established beyond doubt. Some substances which antagonise the actions of 5-hydroxytryptamine are not hallucinogens (the best known example is 2-bromo(+)-lysergic acid diethylamide<sup>75</sup>) and some compounds with hallucinogenic properties are inactive against 5-hydroxytryptamine. Among this latter group are included, as mentioned earlier, certain oxidation products of adrenaline.

Even if we provisionally accept the proposition that the effects of the hallucinogens on the brain can be related to 5-hydroxytryptamine metabolism, it is still difficult to say whether they operate by mimicking its actions, by inhibiting the enzyme (amine oxidase) which normally destroys it or by blocking its effects and thus creating a virtual deficiency. The structural similarities between these compounds and 5-hydroxytryptamine are such as to make all three actions theoretically possible and indeed they can be demonstrated on the appropriate pharmacological preparations in very much the same way that ephedrine can be shown both to simulate and to block the action of adrenaline. It ought to be possible to solve the problem easily by studying the effects on the brain of 5-hydroxytryptamine alone and in combination with a hallucinogen. Unfortunately, an increased brain content of 5-hydroxytryptamine does not follow its parenteral injection, due presumably to the obstruction offered by the blood-brain barrier.

Feldberg and Sherwood found that intraventricular injection of 5-hydroxytryptamine in the cat caused muscular weakness, sometimes accompanied by profuse salivation<sup>66</sup>; Woolley used very much larger doses and noted signs of violent central stimulation sometimes accompanied by convulsions<sup>74</sup>. Boydanski, Weissback and Udenfriend<sup>76</sup> ingeniously avoided the blood brain barrier to 5-hydroxytryptamine by intravenously administering large amounts of 5-hydroxytryptophan, which does pass the barrier and is then decarboxylated to 5-hydroxytryptamine in the brain. The increased amounts of 5-hydroxytryptamine in brain were again accompanied by signs of central stimulation. These apparently excitatory actions of 5-hydroxytryptamine are similar, though by no means identical, to those of lysergic acid, whose actions on the brain may be partly due to its mimicking the actions of 5-hydroxytryptamine. On the other hand, Olds and his collaborators demonstrated an antagonism of lysergic acid and 5-hydroxytryptamine in the brain<sup>77</sup> and Lewis maintains that the predominant action of 5-hydroxytryptamine is a depressant one<sup>67</sup>.

Woolley does not, apparently, believe that 5-hydroxytryptamine acts as a transmitter substance. Instead, he has turned his attention to the oligodendrocytes of brain. These components of glial tissue show a pulsating movement which persists in cells in tissue culture. They contract strongly under the influence of 5-hydroxytryptamine and normal pulsation can be restored by the addition of antagonists of 5-hydroxytryptamine; some of the actions of lysergic acid are similar to those of 5-hydroxytryptamine. Woolley believes that the normal pulsation of oligodendrocytes serve to stir the extracellular fluid in the relatively poorly-vascularised brain and thus help to maintain the exchange of metabolites<sup>74</sup>. If this is so, interference with their normal action might produce general impairment of cerebral metabolism. This is an attractive hypothesis, if only because it is a brave attempt to escape from the all too common assumption that the only function of humoral substances in the nervous system is to act as mediators of synaptic transmission. Against the hypothesis has to be placed the doubts which many have expressed whether oligodendrocytic movements are rapid or powerful enough to exert any significant agitatory action. Moreover, if the normal function of 5-hydroxytryptamine is bound up with the activities of cells which have so general an effect, it is difficult to explain the localisation of 5-hydroxytryptamine in restricted areas of the brain.

The other approach to the problem of the function of 5-hydroxytryptamine in brain is provided by Brodie and his associates. Brodie and Shore have suggested that 5-hydroxytryptamine is a synaptic transmitter in the central levels of the parasympathetic system<sup>78</sup>. They have drawn up a list of the qualities that should be exhibited by a transmitter substance and they have attempted to show that 5-hydroxytryptamine meets these requirements. In the writer's opinion the list is an inadequate one and is insufficient to establish the transmitter status of 5-hydroxytryptamine.

Brodie and his associates have been particularly interested in the actions of reserpine, a tranquillizing drug which appears to impair the



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ability of the brain to bind 5-hydroxytryptamine. It is suggested that reserpine, by liberating 5-hydroxytryptamine from its binding sites, allows its accumulation in small amounts which stimulates the parasympathetic centres and thus cause sedation. If the amine oxidase of brain is inhibited by the administration of iproniazid, a subsequent dose of reserpine causes violent central stimulation rather than sedation. Brodie and Shore explain this effect by suggesting that the iproniazid has allowed the accumulation of 5-hydroxytryptamine to an inhibitory concentration: inhibition of a sedating system would be expected to lead to excitation. However, these results could just as easily be explained on the hypothesis that 5-hydroxytryptamine has an excitatory effect on the sympathetic centres. Reserpine, by depleting these centres of their transmitter, would lead to depression and iproniazid, by preserving 5-hydroxytryptamine, would allow it to accumulate and exert an effect which represented an exaggeration of its normal physiological role, just as a cholinesterase inhibitor may precipitate convulsions. It is difficult to see how 5-hydroxytryptamine can persist in the brain after its liberation by reserpine in the presence of uninhibited amine oxidase. The problem is not made any easier by the facts that reserpine also leads to a loss of sympathin from the brain<sup>79</sup> and that it, as well as 5-hydroxytryptamine, is hydrolysed by amine oxidase. Thus the explanation just offered for the action of reserpine and iproniazid would be equally valid if the central sympathetic transmitter were sympathin.

In an effort to include in one theory the effects of reserpine on both 5-hydroxytryptamine and sympathin, Olds has suggested<sup>80</sup> that sympathin is the excitatory transmitter of impulses in the central nuclei of both the sympathetic and parasympathetic systems, while 5-hydroxytryptamine is the inhibitory transmitter. On this view, reserpine depletes both systems of their transmitter substances and leads to a general depression of central activity; iproniazid and reserpine together allow the accumulation of both mediators but, presumably, the excitatory system is prepotent. Marrazzi has taken this idea of 5-hydroxytryptamine action a stage further. It has already been mentioned that he assigns a general inhibitory role to adrenaline. Since, in his preparations, 5-hydroxytryptamine is more potent than adrenaline<sup>63</sup> he supposes that it too, plays the part of inhibitory transmitter and that it may be more important in this respect than adrenaline itself. Criticisms which apply to Marrazzi's theory of adrenaline inhibition are no less valid against his theory of 5-hydroxytryptamine inhibition.

The brain certainly possesses inhibitory neurones, and apparent sedation can arise either from stimulation of an inhibitory system or inhibition of an excitatory one. To this must be added the fact that a substance which in low doses, or under special circumstances, stimulates neurones may in higher doses or other circumstances inhibit these same neurones. While these are undoubted facts, it is clear that they allow experimental evidence to be fitted into almost any theory the investigator chooses, as the foregoing discussion of the actions of 5-hydroxytryptamine amply illustrates. If 5-hydroxytryptamine is a transmitter substance it

would seem to exert its action over a very narrow area of the central nervous system. It can hardly be responsible for general non-cholinergic transmission. Indirect experiments which attempt to decide the function of a substance by studying the effects of inhibitors and analogues cannot lead to unequivocal conclusions and a more direct attack on the 5-hydroxytryptamine problem is needed. A convincing demonstration that it is liberated when specific tracts of fibres are stimulated or that it can alter the membrane potential of a neurone to which it is applied would do more for its neurophysiological respectability than any number of studies with hallucinogenic agents, important as they will no doubt become when they can be interpreted in the light of a tenable theory.

The most embarrassing feature of 5-hydroxytryptamine is its relationship with adrenaline. The similarity of their structures, distribution and metabolism makes it difficult to decide which is involved in any experimental situation, and also raises the question whether 5-hydroxytryptamine and adrenaline may not reflect different aspects of a common process. On the other hand, it must be remembered that 5-hydroxytryptamine is found in many tissues of the body, that it acts on many organs and that it is widely distributed in the animal and vegetable worlds, where it tends to be associated with those substances and processes we look upon as fundamental. Its importance can hardly be questioned and it is possible that it may ultimately be shown to be concerned in the brain in some basic cellular regulation.

### *Histamine*

Until recently, few were prepared to accept the idea of chemical transmission in the central nervous system and those who were, thought in terms of acetylcholine, then the only substance with obvious transmitter potentiality. It is a measure of his remarkable prescience that, as long ago as 1935, Sir Henry Dale not only foresaw the possibility of non-cholinergic transmission but also indicated a way in which its chemical mediator might be identified<sup>81</sup>.

Stimulation of sensory fibres to the skin—as, for example, by scratching—causes vasodilatation in the stimulated area. The mechanism involved is an unusual one, stemming from the fact that the sensory nerve fibres from the skin also receive branches from the neighbouring blood vessels. Impulses from the skin pass along the sensory fibres into the spinal cord in the usual way but, on reaching the point of branching they also pass, in the “wrong” direction, *down* the branch, to the blood vessel. Arriving at the vessel, they cause vasodilatation, probably by the liberation of a chemical substance. Because the vasodilatation is effected by impulses which travel in the opposite direction to that normally taken in sensory nerves, it is spoken of as an antidromic vasodilatation. Dale pointed out that a nerve is unlikely to liberate different materials from its two ends. Thus the material which causes antidromic vasodilatation might also appear at the termination of the fibre in the spinal cord on the arrival there of sensory impulses. In the cord, however, it would act as a chemical transmitter to the next neurone—a non-cholinergic transmitter,

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since sensory fibres contain no acetylcholine. Dale's suggestion has been acted upon by many workers who have sought vaso-active material in extracts of sensory nerves or dorsal roots. It has proved much easier to find vasodilator substances than it has to provide evidence that any one of them is a transmitter substance. This may be because the power of causing vasodilatation is a characteristic of so many substances that it is not easy to say whether any particular constituent of nervous tissue is more likely than another to be the mediator of antidromic vasodilatation. A rather more detailed examination of Dale's view and of the experiments which justify the candidature of histamine, substance P and adenosine triphosphate (ATP) as mediators of antidromic vasodilatation is presented in an earlier review by the writer<sup>82</sup>.

The possibility that histamine may be involved in antidromic vasodilatation was suggested, many years ago, by the experiments of Lewis<sup>83</sup>, and this view was developed by Kwiatkowski<sup>84</sup>, who detected histamine in quantity in sensory nerves. The evidence against histamine as a mediator of antidromic vasodilatation is now considerable, but since it is a constituent of nervous tissue, it still warrants consideration in any discussion of chemical transmission.

Kwiatkowski studied the distribution of histamine in the central nervous system; though the amounts he found were generally small, he reported a much higher concentration in the cerebellum<sup>84</sup>. This was a particularly interesting finding, since, though the cerebellum seems to employ non-cholinergic transmission, it has yielded little of interest to the pharmacologist. Unfortunately, in a subsequent investigation, Harris, Jacobsohn and Kahlson<sup>85</sup> were unable to detect histamine in the cerebellum, though they agreed with Kwiatkowski's estimate of the histamine content of the brain as a whole. The latter group of workers found particularly large amounts of histamine (up to 30  $\mu\text{g./g.}$ ) in the hypothalamus but little elsewhere. Since our own experiments had shown that histamine has an excitatory action on the electrical activity of the cerebellum (see below) it seemed advisable to try and resolve the discrepancy between Kwiatkowski's results and those of Harris and his colleagues. This was the more important since other groups of workers had estimated the histamine content of whole brain with widely divergent results. Accordingly, Dr. Garven and myself studied the histamine content of the brains of several species of animals. We took animals of different ages and in different physiological states. We found that the usual methods of extraction, so well tried for other tissues, were not applicable, without modification, to nervous tissue. We uncovered several factors which may have led other workers to obtain too high an estimate of the histamine content of nervous tissue, but none of our experiments produced any evidence for the presence of histamine in the cerebellum, though we were able to confirm its presence, in quantity, in the hypothalamus.

Yet another compound, therefore, has to be added to the list of those found in largest amounts in the hypothalamus. The localisation of histamine in this region of the brain suggests that it may be involved in the regulation of sympathetic activity, a possibility that is strengthened

by its occurrence in large quantities (up to 100  $\mu\text{g./g.}$ ) in postganglionic sympathetic fibres<sup>86</sup> and by Trendelenburg's demonstration that histamine will stimulate both the hypothalamus<sup>87</sup> and the superior cervical ganglion<sup>88</sup>. It is possible that the sedating actions of antihistamine drugs is related to this effect of histamine.

Histamine has also been found in dorsal roots and the optic nerve<sup>89</sup>. These are both fibres of low choline acetylase activity but there is no confirmed evidence that cells standing in synaptic relationship with these fibres can be stimulated by histamine while all other central areas of low choline acetylase activity contain no histamine. Apart from the hypothalamus, the cerebellum is the only portion of the central nervous system which is stimulated by histamine in a way that might follow the administration of pharmacological doses of a transmitter substance. Since the cerebellum itself possesses no histamine, however, there is little likelihood that transmission across its synapses is mediated by histamine. The significance of its occurrence in optic nerves and dorsal roots remains a mystery and it is impossible to state precisely its relationship with the processes of transmission in the peripheral sympathetic nervous system which already seems to have a satisfactory complement of proved transmitters and modulators of transmission.

In many parts of the body histamine is found in the mast cells of connective tissue: if it is similarly located in the nervous system its release might serve as a local regulator of blood flow, either in response to injury or, perhaps, to changing physiological conditions. Such a regulation would be particularly valuable in the hypothalamus whose normal function depends on the maintenance of a large blood supply. Mast cells also contain 5-hydroxytryptamine, whose actions are in many ways complementary to those of histamine. It may be that their functions in the nervous system are similarly related, an interesting possibility in view of Woolley's hypothesis of 5-hydroxytryptamine action.

#### *Substance P*

Hellauer and Umrath found that the vasodilator activity of saline extracts of dorsal roots was greater than that of ventral root extracts, similarly prepared<sup>90,91</sup>. The vasodilator activity appeared to be due to a substance similar to substance P, the polypeptide found in brain and intestine and first described by von Euler and Gaddum<sup>92</sup>. Hellauer and Umrath also showed that the vasodilator activity of dorsal roots could be abolished, presumably by enzymic action, on incubation of the extracts with nervous tissue. This enzymic inactivation could be prevented by the addition of strychnine and a variety of other convulsant drugs to the incubation mixture<sup>93</sup>. If the mediator of antidromic vasodilatation is also a chemical mediator of non-cholinergic central transmission these last-noted results are interesting in that they suggest that substances which precipitate convulsions may do so by virtue of their ability to allow the accumulation of excitatory transmitter substance. Holton has denied that strychnine has any effect on antidromic vasodilatation<sup>94,95</sup> but this does not entirely invalidate Hellauer and Umrath's

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results since, although both used the rabbit ear preparation, the technical details of their respective methods differ so much that the two groups may well have been measuring different substances. Hellauer and Umrath do not directly attribute the activity of their dorsal root extracts to substance P. They believe that the substance P of dorsal roots holds the sensory transmitter in a "bound" form and that acetylcholine is similarly bound to the substance P of ventral roots. In support of this view Umrath has reported that incubation of substance P from dorsal roots releases an active vasodilator substance<sup>96</sup> and he has provided some evidence that substance P from ventral roots differs from that of dorsal roots<sup>97</sup>. The attraction of this hypothesis lies in its avoiding the necessity of postulating a transmitter role for a substance with as high a molecular weight as substance P. Against it must be laid the criticism that, if substance P is the "anchor" substance for both acetylcholine and the sensory transmitter, it ought to be widely distributed in the nervous system. Its distribution in fact is rather patchy.

Many of those who believe that substance P is involved in nervous activity think of it as a transmitter substance in its own right<sup>98</sup>. They regard its vasodilator activity and its presence in the dorsal roots and the dorsal columns of the spinal cord as evidence of its participation in transmission from the first sensory neurone<sup>99</sup>.

The central actions of substance P are not, however, very striking; there is no substantial evidence that it is liberated from stimulated sensory nerves and there is doubt whether its vasodilator activity matches that produced by antidromic stimulation of sensory nerves (see below).

Again, the distribution of substance P in the central nervous system is an obstacle to its acceptance as a non-cholinergic transmitter substance for, apart from its presence in the first sensory neurone, it is not found extensively in areas of low choline acetylase activity. The other areas of high substance P activity are the hypothalamus, mid-brain, caudate nucleus and the floor of the fourth ventricle. Zettler and Schlosser<sup>99</sup> have adopted an ingenious manoeuvre in an effort to prove that central transmission is shared between acetylcholine and substance P, but the argument which leads to this conclusion is of doubtful validity<sup>82</sup>.

### *Adenosine Triphosphate (ATP)*

Holton and her colleagues have made use of a sensitive photoelectric method to follow changes in the calibre of blood vessels in the ear of the living rabbit<sup>94,100</sup>. This refined technique enabled them to draw a much more complete picture of the course of antidromic vasodilatation than had hitherto been possible and they demonstrated that the character of the vasodilatation produced by histamine and substance P differed from that evoked by antidromic impulses. On the other hand, suspensions of acetone-dried powders of both dorsal and ventral spinal roots were found to contain material capable of producing a vasodilatation similar in its character and time course to that following stimulation of cutaneous sensory nerves. Of the various known compounds tested, only adenosine di- and tri-phosphates had a vasodilator activity resembling

that of the root extracts and with the aid of a very convincing differential bioassay technique, Holton and Holton<sup>101</sup> were able to present strong evidence that the activity of root extracts was indeed due to their contained ATP and ADP. ADP appears only in tissue extracts as a post-mortem breakdown product of ATP and the Holtons therefore tentatively suggested that, in the living animal, antidromic vasodilatation is mediated by ATP. Recently Holton has been able to demonstrate the actual liberation of ATP from stimulated sensory nerves<sup>102</sup>.

ATP is well-known as a high energy phosphate donor and, as such, it is found throughout the nervous system, which has a constantly high rate of energy expenditure. The idea that ATP may serve two entirely independent functions in the body is not, in itself, unacceptable: calcium is an important structural constituent of bone and is also involved in the maintenance of the excitability of muscle and nerve; acetylcholine seems to have non-transmitter as well as transmitter functions. The fact that, for metabolic reasons, ATP is required throughout the central nervous system makes its distribution irrelevant to the question of its transmitter function but Holton has indicated a simple means of differentiating between neurones which make use of ATP as a transmitter substance and those which do not.

Watery extracts of acetone-dried powders of both dorsal and ventral roots were shown to possess an enzyme capable of destroying their vasodilator material. On the other hand, simple saline extracts of fresh dorsal roots at room temperature—prepared without preliminary drying of the nerves—had a much higher vasodilator activity than had extracts of ventral roots prepared in the same way<sup>95</sup>. The evidence suggested that the loss of vasodilator power in the ventral root extracts was due to the activity of an enzyme. But the behaviour of extracts of acetone powders indicated that this enzyme was present in dorsal roots, too. The superior vasodilator activity of extracts of fresh dorsal roots had thus to be attributed to some acetone-soluble material which either inhibited the enzyme or annulled its effects by promoting the rapid synthesis of the vasodilator substance. It seemed a possible conclusion that, if ATP is a transmitter substance, those nerve fibres from which it is liberated in the performance of this function might be characterised by the presence of an inhibitor—or of a stimulant to synthesis—of the kind proposed for dorsal roots. Accordingly, Harris and Holton measured the vasodilator activity of fresh saline extracts of different parts of the central nervous system<sup>103</sup>. These results gave no evidence of a pattern of distribution of vasodilator activity regular enough to support the idea of widespread transmission by ATP, though the persistence of vasodilator activity throughout the first sensory neurone, from dorsal roots to the gracile and cuneate nuclei is evident (Table I). These results themselves, it should be added, form insufficient grounds for rejecting the notion of transmission by ATP since the error may lie simply in the test proposed for detecting neurones which use this mechanism.

Torda maintains that ATP has an excitatory action, similar to that of

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acetylcholine, on the cerebral cortex of the rat<sup>104</sup>, though Robinson and Hughes found that, on the cortex of the cat, the two substances had antagonistic effects<sup>105</sup>. There is evidence that ATP can stimulate some neurones in the spinal cord: although large doses have to be used to provoke it, the excitatory action is certainly more obvious than that exerted by substance P, histamine, noradrenaline or 5-hydroxytryptamine<sup>106</sup>. Nevertheless, the central actions of ATP are not striking.

Two questions have to be answered. Is ATP the substance responsible for antidromic vasodilatation and, if so, is it also a central synaptic transmitter substance? If the obvious interpretation of Dale's proposition is correct, the answers to both questions should be the same. Yet while most of the available evidence suggests that ATP is indeed the mediator of antidromic vasodilatation, substantial experimental support for a central transmitter function is lacking. It was this difficulty of accepting ATP as a synaptic transmitter substance that made the writer, when last reviewing this problem, reject it also as a mediator of antidromic vasodilatation. A way out of this particular intellectual difficulty has now been suggested by Holton<sup>102</sup>. There is evidence that, in sympathetic nerves, ATP binds noradrenaline<sup>107</sup> and it is possible that the two substances are liberated together during sympathetic activity. If this is so it is equally possible that ATP binds the transmitter substance in sensory nerves. Stimulation of the nerves would then release both substances at both the peripheral and central ends of the nerves: at the periphery, ATP could then exert its undoubted vasodilator action, though the actual transmission of the nerve impulse to the next neurone in the spinal cord would be effected by the transmitter substance. Although this view is admittedly speculative, it does point a way not inconsistent with Dale's proposition, of accepting ATP as a mediator of vasodilatation, even if it has to be rejected as a likely candidate for transmitter status.

### *The Cerebellar Factor*

The work summarised in the foregoing discussion has led to the recognition of substances which may well serve an important function in a few discrete regions of the central nervous system, but it is difficult to believe that any one of them has a transmitter function at non-cholinergic synapses outside these restricted areas. None of them exerts widespread excitatory effects such as are produced by acetylcholine and they are absent from many of those parts of the central nervous system which contain little acetylcholine and where transmission is presumably non-cholinergic in type. Since it seemed that the methods hitherto used had failed to detect at least one transmitter substance we decided to approach the problem of its identification from a new direction.

The cerebellum is an important organ accounting for some 5 per cent of the brain's total weight. It contains acetylcholine in a concentration of no more than one-tenth of that found in the rest of the brain<sup>38</sup> though its neurone density is high. Thus there is, in the cerebellum, apparently, no tendency to an alternation of cholinergic and non-cholinergic neurones:

synaptic processes would seem to be almost exclusively mediated by substances other than acetylcholine. There is evidence, too, that the cerebellum has no inhibitory neurones<sup>108</sup> though there is no reason to believe that the basic transmission process is otherwise different from that at other central synapses. The weight of the cerebellum, its high neurone density, its lack of inhibitory neurones and of cholinergic fibres, all point to the likelihood of its being a particularly rich source of non-cholinergic excitatory transmitter material. Such material, when extracted from the cerebellum, should have an excitatory action, when suitably administered, on the cerebellum of another animal.

We therefore made extracts of three large portions of the brain of the rabbit—the cerebral hemisphere, the mid-brain and the cerebellum—and injected small amounts of the extracts, by way of the carotid artery, into the brains of decerebrate rabbits, the electrical activity of whose cerebellum was being continuously recorded. In the early studies it was necessary to use crude brain extracts, since more elaborate extraction processes might have involved the loss of active material. Simple, ether-washed, trichloroacetic acid extracts were therefore employed. These suffer from the obvious disadvantage of being chemically heterogeneous and the effects of irrelevant material had to be carefully controlled. Full details of the experimental methods adopted to overcome this and other difficulties are to be found in the paper by Crossland and Mitchell<sup>109</sup>, from which two sets of records are reproduced in Figures 1 and 2.

It can be seen (Fig. 1*b*) that the injection of an extract of as little as 20 mg. of cerebral hemispheres was sufficient to evoke an outburst of increased electrical activity in the cerebellum. This effect of the cerebral extract was probably due to its contained acetylcholine, for it was mimicked by the injection of an amount of acetylcholine equal to that known to be

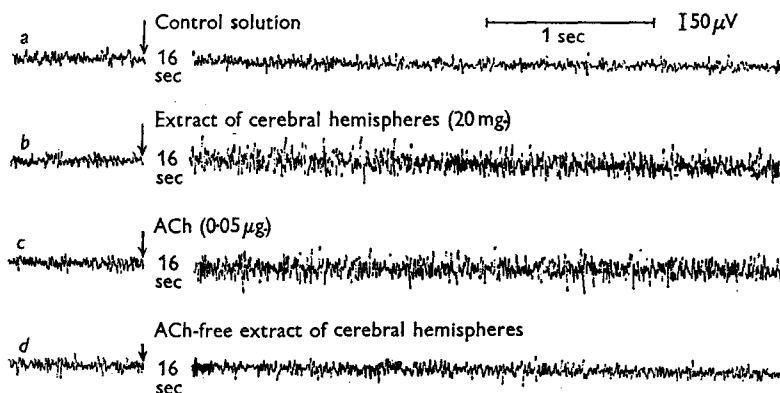


FIG. 1. Pen-oscillograph records of the electrical activity of the cerebellum of a decerebrate rabbit before and 16 sec. after the intra-carotid injection of (a) 0.1 ml. control solution; (b) a trichloroacetic acid extract of 20 mg. of rabbit cerebral hemispheres; (c) the ACh equivalent (0.05  $\mu$ g.) of the extract; (d) trichloroacetic acid extract of 20 mg. of cerebral hemispheres after removal of ACh by brief boiling at pH 11.

(From Crossland and Mitchell by permission of the *J. Physiol.*)



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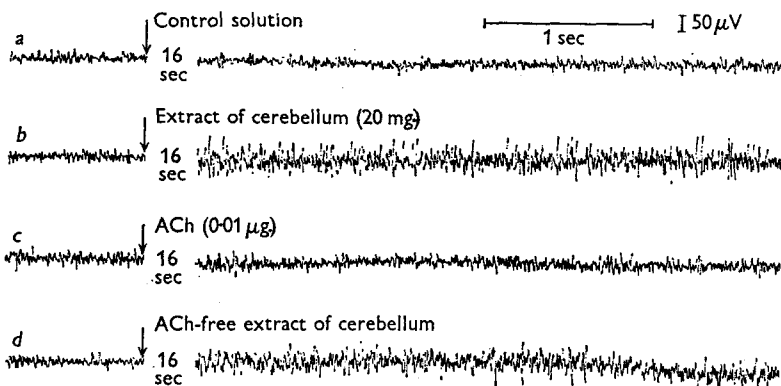


FIG. 2. Same rabbit as in Fig. 1. Effects of an extract of cerebellum. Note the lack of any effect of the ACh equivalent of 20 mg. of cerebellum (c) and the activity of the alkali-treated cerebellar extract (d). (From Crossland and Mitchell by permission of *J. Physiol.*)

present in the extract (Fig. 1c). Moreover, brief alkali boiling of the extract destroyed its acetylcholine and abolished its excitatory action (Fig. 1d). Similar results were obtained with extracts of mid-brain. Extracts of cerebellum exerted a similar excitatory action on cerebellar activity (Fig. 2b), but this could not be due to acetylcholine, for the amount of acetylcholine present in excitatory doses of the extract was too small to modify cerebellar activity (Fig. 2c) and alkali treatment of cerebellar extracts left its excitatory action unimpaired (Fig. 2d). The excitatory action of cerebellar extracts was therefore attributed to an alkali-stable substance which, it soon became clear, could not be identified with any known chemical component of brain. Until it can be finally identified, this material has been called the cerebellar excitatory factor (CEF).

The cerebellar factor and acetylcholine are equipotent in their action on cerebellar activity, in the sense that an extract of, say, 20 mg. of cerebral hemisphere has an excitatory action which is indistinguishable from that produced by an extract of 20 mg. of cerebellum. Yet the action of the one extract is due to acetylcholine and that of the other to the cerebellar factor.

The intra-carotid injection of acetylcholine increases the electrical activity of the cerebral cortex, though its action is fleeting compared with that evoked in the cerebellum. The cerebellar factor behaves in exactly the same way and here again it is equipotent with acetylcholine<sup>110</sup>. On the other hand, it is quite inactive on a wide range of pharmacological preparations, many of which are sensitive to acetylcholine. This serves to underline the individuality of the cerebellar factor and probably explains why it has hitherto escaped detection, since most workers examine extracts of nervous tissue on smooth muscle preparations. At the same time this inactivity towards conventional pharmacological test objects has seriously hindered attempts to develop a simple, rapid and

reliable assay method on which the final purification and identification of the cerebellar factor will depend.

Although the cerebellar factor has not been identified, a few of its chemical and physical characteristics have been established<sup>111</sup>. Though stable to brief boiling in alkali, it is unstable at 100° in an acid medium. It is insoluble in acetone and acetone-dried powders form a useful starting point for an extraction process which results in a pharmacologically "pure" solution, one, that is, which contains no known active material other than the cerebellar factor. It is readily dialysable and its behaviour on adsorbent columns of various kinds suggest that it is basic in nature. It is not hydrolysed by trypsin or chymotrypsin. Although this information is scant enough, it serves to distinguish the cerebellar factor from other components of nervous extracts.

The cerebellar factor quickly loses its activity on incubation at 37° with homogenates of nervous tissue. The inactivation appears to be enzymic in nature. It is interesting, in view of Hellauer and Umrath's experiments discussed above, that this enzymic destruction of the cerebellar factor is prevented by the addition of strychnine (40  $\mu$ M) to the incubation mixture. Picrotoxin, however, seems to have no inhibitory action.

Although the original experiments were carried out with rabbit brain extracts, the active material has since been extracted from the cerebellum of the cat, dog, sheep and horse and there is no reason to doubt its presence in species as yet unexamined.

The investigation which established the existence of the cerebellar factor led simply to the conclusion that it was present in the cerebellum in a higher concentration than in the other large sub-divisions of the brain. Although extracts of extra-cerebellar tissue had no excitatory action after removal of their acetylcholine, this did not exclude the possibility that the cerebellar factor was present in small amounts. Further, a low overall concentration in a part of the brain as large as the mid-brain, for example, is not incompatible with a high concentration in localised regions of this larger area. We therefore looked for the cerebellar factor in other parts of the central nervous system. Since the identity of the excitatory material is unknown, we provisionally defined it as being "an alkali-stable, acid-unstable material, present in aqueous extracts of acetone-dried powders of nervous tissue and capable, on intra-arterial injection, of increasing the electrical activity of the cerebellum". This definition is quite adequate to exclude all pharmacologically-active material, other than the cerebellar factor, hitherto detected in tissue extracts, though we cannot yet be certain that any material which behaves in accordance with the definition given is necessarily chemically identical with the cerebellar factor. A roughly quantitative assay method was used. The minimum quantity of an extract required to cause a detectable augmentation of the electrical activity of the cerebellum in a decerebrate rabbit was expressed as a percentage of the minimum amount of cerebellar extract (pooled from all the dogs used) required to produce a similar cerebellar effect in the same rabbit. The very nature of the cerebellar response

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—a burst of activity of about 30 seconds' duration which seems to be self propagating once it has been "triggered" by the active extract—makes it unsuitable as a basis for serious quantitative work and the results quoted in Table I should perhaps be regarded as simple indications of the presence or absence of the cerebellar factor in the tracts and nuclei mentioned.

We found excitatory material in the optic nerves, dorsal roots, spino-cerebellar tracts, cerebellar peduncles and internal capsule and in grey matter (such as the lateral geniculate bodies, thalamus, sensory cortex, etc.) in which these fibres arise or terminate. These are some of the areas in which the non-cholinergic transmitter may be expected to occur (Table I). The cerebellar factor was not, however, found in all areas of low choline acetylase activity. Thus it was absent from the dorsal columns of the spinal cord and present in only small amounts in the pyramidal tracts. Nevertheless, it does seem to be more widespread, and its distribution coincides more closely to that of the postulated non-cholinergic transmitter substance, than any of the other substances so far considered in this review.

The absence of the cerebellar factor from the dorsal columns of the cord is interesting in relation to the fact that these tracts, and dorsal root fibres, contain substance P whose distribution does not otherwise coincide with that expected of a non-cholinergic transmitter substance. It should also be remembered that the dorsal columns consist of dorsal root fibres which have passed into the spinal cord without synaptic interruption. It follows that these fibres contain no cerebellar excitatory factor, though dorsal roots as a whole are rich in the material. Thus it seems that dorsal roots are not chemically homogeneous: some (those which end in synaptic relationship with the spino-thalamic and spino-cerebellar tracts) contain the cerebellar factor while others (those which are destined to participate in the formation of the dorsal columns) do not. It may well prove that the substance P content of dorsal roots is due to those latter fibres only and that substance P and the cerebellar factor may have complementary roles in the process of sensory transmission.

The cerebellar factor is absent from the peripheral fibres of the autonomic nervous system, which one would expect to be deficient in transmitter substances other than acetylcholine and noradrenaline. It has, however, been found in ventral roots whose choline acetylase activity, related to the known cholinergic nature of the neuro-muscular transmission mechanism, is very high indeed. It is not yet known whether all ventral roots contain both acetylcholine and the cerebellar factor or whether ventral roots, like dorsal roots, are chemically inhomogeneous. There is no reason why structures grouped on an anatomical basis should be chemically identical, though it must be admitted that there is no evidence known to the writer which suggests that neuro-muscular transmission does not everywhere involve the intervention of acetylcholine.

Such facts as are known about the cerebellar factor encourage us to believe that it may have an important part to play in the processes of non-cholinergic transmission: it is a low molecular weight compound,

found in at least some parts of the nervous system deficient in acetylcholine ; it has well-marked excitatory properties and it is destroyed by an enzyme found in nervous tissue. The fragmentary nature of the information we possess, however, prevents any final assessment, at this stage, of its function in the central nervous system.

The sensitivity of the electrical activity of the cerebellum to acetylcholine and to the cerebellar factor suggested that it might be equally easily affected by other active compounds. This expectation was not fulfilled, for a range of substances of pharmacological interest—including 5-hydroxytryptamine, adrenaline, substance P and ATP—were without effect on the cerebellum, even in relatively large doses. The one exception was histamine which, in amounts of as little as 0.05  $\mu$ g. stimulated cerebellar activity, although a delay of about 70 seconds intervened between the injection of histamine and the appearance of a change in the cerebellar record. When this observation was first made, it seemed possible that the cerebellar factor might itself be related to histamine. For this reason, the report by Kwiatkowski, already referred to, that the cerebellum contains appreciable quantities of histamine, was of particular interest. Kwiatkowski's finding could not, however, be confirmed and it soon became clear that the properties of the cerebellar factor were such as to make it unlikely that it could be in any way related to histamine. Nevertheless, the action of histamine on cerebellar activity remains interesting. Removal of certain parts of the cerebellum completely protects the dog—normally a notoriously susceptible animal—against motion sickness<sup>112</sup>. It is now well known that antihistamine compounds can also offer a certain amount of protection against motion sickness and it may be that this distressing condition is initiated by the action of circulating histamine on the motion sickness “centre” of the cerebellum. Recently we have found that the cerebellum contains an enzyme—not “histaminase” (diamine oxidase)—which rapidly destroys small amounts of histamine though it is very much less active against larger quantities. This enzyme, which is not found in the cerebral cortex, may protect the cerebellum against the stimulating effect of exogenous histamine. It is of interest, in this connection, that the cerebellum contains cholinesterase<sup>113</sup>, even though it seems to be but sparsely provided with cholinergic neurones. This cholinesterase might similarly protect the cerebellum against the stimulating action of circulating acetylcholine.

It is probable that not all the compounds so far discussed will be found to play equally important parts in central synaptic transmission; it is also likely that other active components of nervous tissue will be identified. Nevertheless, it is surprising that an area like the hypothalamus contains so many pharmacologically interesting substances while some other parts of the brain, the cerebellum for instance, seem to contain so few. It is difficult to imagine that future research will redress the balance by revealing a number of new substances restricted to the pharmacologically arid regions of the central nervous system. If we are to accept the idea of universal chemical transmission, how can we reconcile ourselves to this unbalanced distribution of chemical agents?

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The hypothalamic region of the brain is of extraordinary importance in the regulation of the emotional and affective state of the individual; it contains the autonomic centres whose activity produces the overt manifestations of emotion; it has extensive connections with the cerebral cortex, which it influences in response to signals from the viscera (not without reason did the ancients localise the soul in the diaphragm) and it is itself affected by those cortical events which represent the physical basis of changes of mood. It is closely linked with the pituitary and hence with the whole endocrine system and it now seems likely that the hypothalamus itself elaborates some of the hormones liberated by the pituitary, as well as manufacturing others more peculiar to itself. The old view of the hypothalamus as an endocrine organ is at last coming into its own. Finally, it is connected with the reticular formation, whose activity regulates the general tone of both higher and lower levels of the nervous system. It is not surprising, then, that interference with the metabolism of compounds localised to the hypothalamus should have such profound effects on the psychic state of an individual. Nor is it surprising that the hypothalamus, so closely involved with the main organ of regulation of the body's chemistry, should take on the appearance of a chemical factory. But a factory does not necessarily make use of its own products and its very activity is likely to result in the accumulation of side products, which are themselves of relatively small importance. The close similarities between adrenaline and 5-hydroxytryptamine, to which attention has been drawn, may be significant in this connection. Even those hypothalamic compounds which are of functional importance are not necessarily transmitter substances in the sense in which the words should be properly used; there is indeed no apparent reason why the most important transmitter substance in the hypothalamus should not, after all, be acetylcholine. It should not be forgotten that this region of the brain contains seven times as much acetylcholine as 5-hydroxytryptamine.

While not attempting to deny or minimise the importance of the hypothalamic products, the writer is of the opinion that those searching for non-cholinergic transmitter substances should turn to areas of the nervous system whose metabolic activities are more modest, for in such areas chemical activity is likely to be more obviously related to the transmission process. It is well to remember, too, that many of the substances considered in this review are active on smooth muscle preparations but behave in a much less impressive fashion when they are tested on nerve cells. The use of the central nervous system itself, or of isolated nervous elements, might be more satisfactory for the initial detection of central nervous transmitters. For these reasons, it is felt that the cerebellar excitatory factor deserves further study.

Most of the substances dealt with in this article do not show the wide distribution to be expected of the non-cholinergic transmitter substance and, in spite of the interest they have attracted, none of them has satisfied the few simple requirements demanded of transmitter substances. The nature of the transmitter substance at non-cholinergic synapses remains

as obscure as it was when Dale first sensed its existence, nearly a quarter of a century ago.

#### INHIBITORY TRANSMISSION IN THE CENTRAL NERVOUS SYSTEM

Discussion so far has centred on the several substances which may have to be considered as excitatory transmitters and inhibition has been mentioned only parenthetically as a possible alternative function of some of these substances. Yet inhibition of activity in neurones is as important an aspect of the integrative action of the nervous system as is their excitation. Even the simplest movement of a muscle can be successfully carried out only if the neurones which control its antagonists are simultaneously inhibited, while the inhibitory action of the higher centres of the nervous system is reflected in the increased muscular tone of the decerebrate animal and in the unrestrained behaviour of the drunk.

It is theoretically possible for a substance to mediate excitatory transmission at some synapses and inhibitory transmission at others (acetylcholine, after all, inhibits heart muscle and stimulates intestinal muscle) but it is now generally believed that the central nervous system utilises specific inhibitory transmitters. Mention has already been made of one of the mechanisms by means of which excessive motor neurone activity is restrained. Impulses in collaterals of motor nerves do not directly inhibit their cells of origin but stimulate Renshaw cells which presumably liberate an inhibitory substance, which is certainly not acetylcholine. Eccles has shown that the action of the inhibitory transmitter is inhibited by strychnine and in his opinion strychnine convulsions are due to inhibition of inhibitory transmission. Considerations such as this have led neurophysiologists to believe that neurones liberating a specific inhibitory transmitter substance are a feature of all synapses where inhibition occurs.

During the past few years, the experiments of several groups of workers have independently directed attention to a class of substances which seem to be important regulators of inhibitory activity. Florey found in 1953 that extracts of mammalian brain contained both excitatory and inhibitory substances which he named Factor E and Factor I respectively<sup>114</sup>. Factor E may well be identical with substance P and need not be considered further. Factor I inhibits the discharge of crustacean stretch receptors and antagonises the action of acetylcholine on pharmacological preparations. Florey and McLennan observed inhibition of the stretch reflex on topical application of Factor I to the spinal cord of the cat: the flexor reflex was augmented and the hypoglossal nucleus excited<sup>115</sup>. It was particularly interesting, in the light of Eccles' concept of strychnine action, that Factor I seemed to be able to protect mice against the convulsant action of strychnine.

The crayfish stretch receptor preparation forms the basis of a very convenient method of assaying Factor I and by 1956 Bazemore, Elliott and Florey were able to obtain crystalline material from beef-brain extracts. This material possessed Factor I activity and was identified as  $\gamma$ -aminobutyric acid<sup>116</sup>. As soon as it seemed that the intriguing

pharmacological effects of an impure extract of brain could be explained in terms of an identifiable chemical compound, widespread interest was aroused and a considerable mass of information concerning the action of  $\gamma$ -aminobutyric acid has already been published: it has been well reviewed by Elliott<sup>117</sup> and by Elliott and Jasper<sup>118</sup>.

McLennan has criticised the conclusion that the Factor I activity of brain extracts is due to their contained  $\gamma$ -aminobutyric acid. He bases his objections on the grounds of discrepancies between the properties of Factor I and  $\gamma$ -aminobutyric acid<sup>119</sup> and on the fact that a brain extract which still showed Factor I activity apparently contained no  $\gamma$ -aminobutyric acid<sup>120</sup>; more recently, he has advanced chromatographic evidence that Factor I activity is due in part to  $\gamma$ -guanidinobutyric acid<sup>121</sup>, which is known to be a normal constituent of brain<sup>122</sup>.

Another approach to the problem of inhibitory transmission comes from the work of Hayashi and his group in Japan, who studied the protective effect of various substances, applied directly to the cortex or injected into the carotid artery, against chemically induced seizures in the dog. They found that  $\gamma$ -amino- $\beta$ -hydroxybutyric acid had a more potent anti-convulsant activity than  $\gamma$ -aminobutyric acid<sup>123</sup> and Hayashi proposes it as the "real inhibiting factor in brain"<sup>124</sup>.

From Hungary, Lissak and Endrocizi have also reported the preparation of an inhibitory factor, whose action appears to be due to the conjoint operation of two or more substances, one of which may be  $\gamma$ -aminobutyric acid<sup>125</sup>. Finally, another group of Japanese workers has obtained evidence for the presence of  $\gamma$ -aminobutyryl choline in brain and has shown that it exerts some anti-acetylcholine action<sup>126</sup>.

Thus at least four different compounds have been named as responsible for the inhibitory action of brain extracts. It seems to the writer that the inhibitory action may well be due to the operation of more than one of these compounds and that the relative amounts of each may vary according to the method of preparation of the extracts. Preparation of Factor I does involve long chemical manipulations and the final concentration of the inhibitory components of the extract may bear little relationship to that in the living brain. Thus it may easily happen that samples of Factor I may contain  $\gamma$ -aminobutyric acid on some occasions but not on others; if all the related compounds have similar actions, the loss of  $\gamma$ -aminobutyric acid may well be overlooked in the absence of a chemical check on the nature of the extract. A much more important question concerns the underlying physiology of this action. The rest of this discussion will therefore concern  $\gamma$ -aminobutyric acid with but an occasional sideways glance at the other compounds. It is chosen in preference to the other substances named, because it has been investigated in more detail and because its actions are sufficiently close to those of Factor I as originally described.

$\gamma$ -Aminobutyric acid is present in the brain in large amounts (about 200  $\mu\text{g./g.}$  in the cat) where it seems to be formed from glutamate. The glutamine-glutamic acid system has long been suspected of exerting an anti-convulsant action.

There is suggestive evidence that  $\gamma$ -aminobutyric acid acts as a transmitter of inhibitory impulses to the crustacean stretch receptor. Nerves to this receptor which are known to have inhibitory fibres also contain Factor I.  $\gamma$ -Aminobutyric acid has a hyperpolarising effect on the membrane of the stretch receptor, as has stimulation of the inhibitory nerves and the membrane change due to  $\gamma$ -aminobutyric acid is indistinguishable in its properties from that due to inhibitory nerve stimulation<sup>127</sup>. This evidence is admittedly incomplete, but it is strikingly more powerful than any that can be offered on behalf of the possible excitatory transmitter substances, save acetylcholine, discussed earlier.

The role of  $\gamma$ -aminobutyric acid in synaptic events in the mammalian spinal cord and brain is less clear; since nervous transmission in invertebrates differs in many respects from that in mammals, the likelihood that  $\gamma$ -aminobutyric acid has a transmitter function in crustacea is of little value as presumptive evidence of its function elsewhere. Florey's original finding that Factor I offers a measure of protection against strychnine convulsions has not been confirmed, either for brain extracts known to contain Factor I<sup>128</sup> nor for  $\gamma$ -aminobutyric acid itself<sup>129</sup>. However, since the latter does not apparently cross the blood brain barrier, this is not surprising and does not necessarily suggest that  $\gamma$ -aminobutyric acid does not have the anti-strychnine action that would be expected of an inhibitory transmitter. On the other hand, experiments on mammalian cortex indicate that  $\gamma$ -aminobutyric acid and strychnine act at different loci<sup>130,131</sup>. Moreover, Curtis, Phillips and Watkins<sup>132</sup> have shown that, in the spinal cord, strychnine does not antagonise the action of  $\gamma$ -aminobutyric acid on motor neurones, in those very circumstances in which Eccles has shown that the inhibitory transmitter is antagonised by strychnine<sup>34</sup>. They have also shown that the inhibitory post-synaptic potential in these neurones, which seems to represent the fundamental neuronal response underlying inhibition, is not augmented by  $\gamma$ -aminobutyric acid. Instead,  $\gamma$ -aminobutyric acid depresses *all* membrane responses, excitatory and inhibitory alike and this action is shared by a large number of other compounds, including at least one, and probably more, of those mentioned above as possibly contributing to Factor I activity. Thus some doubt must arise as to whether  $\gamma$ -aminobutyric acid is a transmitter substance in the mammalian central nervous system, though it must be added that our knowledge of the basic mechanism of inhibition is still fragmentary. Nevertheless, the most impressive work in this field, so far, has linked inhibitory transmission with the development of the inhibitory post-synaptic potentials which do not seem to be attributable to  $\gamma$ -aminobutyric acid or its congeners. Attempts have been made to place the inhibitory action of  $\gamma$ -aminobutyric acid elsewhere (by blocking impulses in excitatory fibres for instance) but there is as yet little sound evidence that this could form an inhibitory transmission system. Yet a deficiency of  $\gamma$ -aminobutyric acid does seem to be a factor in causing the convulsions of pyridoxine deficiency, as would be expected if it were indeed a mediator of central inhibition. This dilemma might ultimately be resolved by the demonstration that the true inhibitory



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substance is a derivative of  $\gamma$ -aminobutyric acid or, perhaps more likely, that  $\gamma$ -aminobutyric acid itself is being continually liberated into the nervous system to exert a general controlling effect on nervous activity, quite outside the operation of the normal inhibitory system. It seems inevitable that, as with excitatory transmission, many more substances will be put forward—and most of them rejected—as inhibitor transmitter substances before the chemical background of synaptic transmission can be fully described.

### CONCLUSION

In the interests of keeping this review and its bibliography within reasonable bounds, much important work has inevitably been ignored; it is hoped that the omissions have not led to a serious misrepresentation of the current trends of neurochemical opinion. If the writer's views seem over-critical, it is because he feels that, in a situation where too many experiments are chasing too few basic ideas, a deflationary policy is of more value to the ultimate good of the subject than one which merely encourages the haphazard accumulation of experimental information.

A summary adjudication of the claims of the several candidates for transmitter status is quite impossible. There is powerful circumstantial evidence that acetylcholine is a transmitter substance at central synapses but the identity of the mediators and modulators of non-cholinergic transmission remains obscure. If this review helps the reader to recognise significant contributions to the solution of the problem among the increasing load of papers with which he will be confronted, it will have served its purpose. It will have done far more than this—and more than its author dare hope—if in addition it advances in any small way the day on which the roll of transmitter substances can be finally called.

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