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STUDIES ON THE DISTRIBUTION OF FACTOR I IN MAMMALIAN BRAIN

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Factor I, whose role as a transmitter substance of inhibitory neurones is under discussion (Florey, 1954, 1956, 1957; Florey & McLennan, 1955*a*, *b*; Edwards & Kuffler, 1957; McLennan, 1957), has been found to occur in the brains of mouse, rat, guinea-pig, rabbit, cat, cattle and horse (Florey, 1954). We may safely assume that it occurs in the brains of all mammals and that conclusions drawn from its behaviour in one type of brain may be applied to other mammalian brains as well. Recent studies have shown that Factor I is produced by brain tissue and that most, if not all, of it is present in an 'occult', inactive form from which it can be released *in vitro* by the action of hypotonic medium, heat, weak alkali or weak acid (Elliott & Florey, 1956). It seems likely that its release *in vivo* is effected by the excitation of certain (inhibitory?) neurones. For crustacean inhibitory neurones this has recently been demonstrated (Florey, 1957).

In mammals Factor I occurs in high concentration only in the central nervous system, but is absent in peripheral nerves, in contrast to crustaceans where Factor I is also found in peripheral nerves (Florey, 1954). These findings have been interpreted as an indication of a functional connexion between Factor I and inhibitory neurones, inasmuch as in mammals inhibitory neurones are found in the central but not in the peripheral nervous system, while in crustaceans they are present in peripheral nerves also. It is obvious, however, that much more detailed information is needed as to the occurrence of Factor I in the various structures of nervous systems in order to ascertain a connexion between location and function of this Factor.

In the present study we attempted to find out whether there is a differential distribution of Factor I within the mammalian central nervous system. We found that the concentrations of Factor I within the different structures and pathways of the brain show wide and consistent variations. We will try to

correlate our findings with the available information concerning the inhibitory and facilitatory function of these systems and to relate the distribution of Factor I to the already known data concerning the distribution of acetylcholine, adrenaline, noradrenaline, 5-hydroxytryptamine and substance P.

METHODS

Assays for Factor I were performed on the crayfish stretch receptor preparation according to the method described by Elliott & Florey (1956). The species of crayfish used in all tests was *Cambarus virilis* Hagen. The method permits the determination of the Factor I content of rather small samples; in certain cases we assayed tissue samples as small as 10 mg.

The procedure for the preparation of extracts was as follows: Brains from freshly killed cattle were obtained at local slaughter-houses. Dissection of the brains was usually begun 3 hr after excision. The dissections were completed within 2 hr. Control tests proved that no significant synthesis or loss of Factor I occurred during this dissection period. In one such test we processed the caudate nuclei of several brains, 3, 4, 5 and 6 hr after excision and assayed them in the routine manner. All values in this series were found to be within $\pm 1{\cdot}25\%$ of their average. During the first 2 hr after excision some synthesis may occur (Elliott & Florey, 1956).

Particular care was taken to avoid squeezing or squashing of the tissues, since destruction of cell structures might lead to synthesis or enzymic inactivation of Factor I (Florey, 1954; Elliott & Florey, 1956).

Samples of cortical areas were removed in a cool humid chamber by means of a Stadie–Riggs microtome (Stadie & Riggs, 1944) without moistening. The thickness of the slices was approximately 0·5 mm. Immediately after its dissection each sample was weighed. Larger structures were placed in 9 times their weight of potassium-free, unbuffered saline medium (composition (mM): NaCl 205, $MgCl_2$ 2·6, $CaCl_2$ 13·5). Smaller samples were placed in 49 times their weight of saline medium with half the concentration of potassium which is present in the normal crayfish saline (composition (mM): NaCl 205, KCl 2·7, $MgCl_2$ 2·6, $CaCl_2$ 13·5). All samples were boiled for 5 min in a water-bath and subsequently homogenized. The extracts were kept during the night in the refrigerator at 6° C and assayed on the following day. Dilutions of the extracts were made with the complete saline medium of Elliott & Florey (1956). This solution is made up in the following way: to 1 l. of solution, containing (g) NaCl 12, KCl 0·4, $MgCl_2.6H_2O$ 0·5 and $CaCl_2$ 1·5, are added 100 ml. of 0·11 M tris-(hydroxymethyl) amino methane-maleate buffer, adjusted to give a final pH of 6·5, and 2 ml. of $0{\cdot}4\%$ bromocresol-purple.

In control tests it was seen that no loss of Factor I occurs during 48 hr of cold storage of extracts. The reproducibility of our assays was found to be within 5%. This was repeatedly ascertained by repetitive assays of one or more samples on one or more different stretch receptor preparations.

The inhibitory activity of the extracts has been determined in crayfish units (c.u.) and compared with a reference solution containing gamma-aminobutyric acid. Crayfish units reference (c.u.r.) have been used as absolute values for the Factor I content of the original tissues. For the derivation of these units see Elliott & Florey (1956). The recent identification of a large part of the Factor I of brain with gamma-aminobutyric acid (Bazemore, Elliott & Florey, 1956, 1957) allows a direct measurement of Factor I activity in terms of the activity of this known substance. We have found in a large number of comparisons that 1 c.u.r. of Factor I can be identified with the activity of 2 μg of gamma-aminobutyric acid. We have used solutions of gamma-aminobutyric acid in all bioassays for Factor I. However, since the total activity of Factor I is not yet completely identified we refrain from giving absolute values for gamma-aminobutyric acid and use the term c.u.r. as a measure of the Factor I content of brain tissue. This seems the more indicated as McLennan (1957) has recently shown that the actions of Factor I and gamma-aminobutyric acid are not always identical.

For the stretch receptors of *Cambarus virilis* it has already been shown (Elliott & Florey, 1956) that none of the neurogenic principles like adrenaline, noradrenaline, 5-hydroxytryptamine, thiamine, acetylcholine, choline, and histamine, of concentrations likely to be present in the extracts assayed, interfere with the action of Factor I. We have prepared substance P according to a method described by Lembeck (1953). Even a solution of this preparation of 1 mg/ml. was without effect on the stretch receptors. It is thus unnecessary to purify the crude extracts or to make use of pharmacological blocking agents for Factor I assays.

The inhibitory action of Factor I on the crayfish stretch receptor preparation is blocked by low concentrations of picrotoxin (Elliott & Florey, 1956). We have repeatedly made use of this fact in order to ascertain that the inhibition caused by the extracts is indeed due to Factor I.

Samples were obtained from a total of sixty-nine beef brains. The animals were not selected for sex or age. Of these, thirty-five were dissected in Montreal and thirty-four in Seattle. No obvious differences were found between the Factor I content of 'eastern' and 'western' beef brains. Such structures as the caudate nuclei which gave rather uniform values for Factor I were particularly suitable for comparisons. The values for six caudate nuclei obtained in Montreal ranged from 202 to 240 c.u.r./g, and the values for five caudate nuclei obtained in Seattle varied from 230 to 255 c.u.r./g.

The revised Nomina Anatomica, as approved by the International Nomenclature Committee, Paris 1955, are used throughout this paper with the exception of the names for cortical areas of bovine brain which have been taken from Papez (1929). Papez gives names of human brain structures which probably correspond to the structures of bovine brain described by him. In Table 2 these corresponding names are given in parentheses. They conform to the above-mentioned Nomina Anatomica. Wherever this is commonly done we have used the anglicized form of the latin names.

RESULTS

As is shown by Tables 1 and 2, there are considerable and consistent differences between the Factor I contents of the various brain structures. The values for the various areas of cerebral and cerebellar cortex are grouped around 100 c.u.r./g with the exception of the parietal cortex, the Factor I content of which was found to be significantly higher (average 177 c.u.r./g).

TABLE 1. Factor I content of bovine brain: White matter

Structure	Activity (c.u.r./g) Individual samples	Average
Inferior cerebellar peduncle	34, 35, 43	37
Middle cerebellar peduncle	11, 14, 23, 23	18
Superior cerebellar peduncle	86, 101, 105, 111	101
Pyramidal tract	16, 17	16
Pons	32, 54	43
Crus cerebri	116, 116, 160, 160, 166, 181, 210	158
Corpus callosum;		
Genu	36, 42,	
Body	14,	30
Splenium	32	
Fornix	16, 20	18
Optic tract	54, 60, 76, 76, 90, 100	76
Optic nerve	<8, <8, <8, <8	<8
Oculomotor nerve	20	20
Trochlear nerve	30	30
Trigeminal nerve	<12	<12
Dorsal spinal roots	<10, <10, <10	<10
Ventral spinal roots	<10, <10, <10	<10

TABLE 2. Factor I content of bovine brain: Grey matter

Structure	Area	Activity (c.u.r./g) Individual samples	Average
Cerebral cortex			
Motor cortex: Anterior and posterior sygmoid gyri (=inferior and supracentral gyri)	4, 6	110, 142	126
Sensory cortex: Coronal gyrus (=postcentral gyrus)	3	78, 102, 123	110
Postcruciate gyrus (=superior postcentral gyrus)		147	
Anterior ectosylvian gyrus	—	100, 104	
Inferior postcentral gyrus	—	100, 123	
Temporal lobe: Posterior ectosylvian gyrus (=superior temporal gyrus)	41, 42, 22	76, 101, 105	97
Posterior suprasylvian gyrus (=middle temporal gyrus)	21	105	105
Temporal pole	38	75, 85	80
Frontal cortex: Superior and middle frontal gyri	8, 9, 10	63, 86	75
Parietal lobe cortex	—	106, 172, 240, 190	177
Occipital lobe cortex	—	115, 118, 121, 133	122
Insula	—	88, 94	91
Hippocampal gyrus	—	111	111
Cingulate gyrus	—	68, 88, 11	89
Basal ganglia			
Caudate nucleus	—	202, 212, 215, 230, 233, 240, 240, 245, 246, 250, 255, 255	235
Putamen	—	134, 166, 171, 178, 195	169
Globus pallidus	—	205, 333, 350	296
Amygdaloid nucleus	—	107	107
Thalamus			
Whole	—	72, 72, 78	74
Anterior thalamic nucleus	—	80, 74	77
Metathalamus			
Lateral geniculate body	—	106, 127, 150, 182	141
Medial geniculate body	—	85, 133, 153, 162	133
Epithalamus			
Pineal gland	—	10	10
Hypothalamus			
Mamillary body	—	125, 133, 136, 140, 158	138
Infundibulum and tuber cinereum	—	100, 115	107
Hypophysis	—	22	22
Subthalamic nucleus	—	255, 309, 333	299
Mesencephalon			
Inferior colliculi	—	121, 190, 230	180
Superior colliculi	—	230, 277, 300, 307	281
Substantia nigra	—	317, 383, 360, 424, 428, 444, 446, 464	406
Red nucleus	—	60, 77, 117, 182	109
Substantia grisea centralis, random samples	—	100, 105, 187, 225, 240, 240, 380, 454, 454, 477	234
Level of inferior colliculi	—	154, 182	168
Level of posterior border of superior colliculi	—	146, 281	213
Level of anterior border of superior colliculi	—	100, 211	155
Cerebellum			
Cerebellar cortex: Posterior lobe	—	60, 63	62
Anterior lobe	—	65, 116	91
Parafloccular lobe	—	95	95
Anterior lobe: Whole	—	145	145
Dentate nucleus	—	202, 205, 210, 295, 333, 367, 420, 490	315
Rhombencephalon			
Reticular formation:			
Central portion, level of nucleus of XII nerve	—	90, 97	94
Lateral portions, level of nucleus of XII nerve	—	125, 133	129
Central portion, level of exit of VII nerve	—	133, 267	200
Lateral portions, level of exit of VII nerve	—	92	92
Central portion, upper pons	—	183, 177	180
Inferior olive	—	41, 66, 97	68
Dorsal cochlear nucleus	—	133	133
Spinal cord			
Posterior horn	—	150	150
Anterior horn	—	75	75

Concentrations similar to those found in most cortical areas are present in the lateral and medial geniculate bodies, the thalamus, the amygdaloid nucleus, inferior olives and the dorsal cochlear nucleus. A number of other grey structures contain conspicuously high amounts of Factor I. In order of increasing Factor I content they are: mamillary bodies (125–158), rhombencephalic reticular formation (90–267), putamen (134–190), inferior colliculi (100–200), mesencephalic substantia grisea centralis (100–477), caudate nuclei (202–255), superior colliculi (230–307), globus pallidus (205–350), subthalamic nucleus (255–333), dentate nuclei (202–490), hypothalamus (375–450) and substantia nigra (316–464 c.u.r./g).

For the grey matter of the spinal cord we found 75 c.u.r./g for the anterior, and 150 c.u.r./g for the posterior horns. Both samples were taken from the cervical region of the cord. Assays of ventral and dorsal spinal roots (lumbar region) indicated activities of less than 10 c.u.r./g (see also Florey, 1954; Florey & McLennan, 1955*a*).

Most of Factor I of the central nervous system thus appears to occur within the grey matter. Only small amounts are found in white matter, cranial nerves, pineal body and hypophysis. This would indicate a close association with the nerve cell bodies or synaptic regions. In three instances, however, conspicuous concentrations of Factor I were found in 'white' fibre tracts. This indicates that this factor is also present within the axons of certain neurones. That these axons must be of a specific functional type is demonstrated by the fact that they are found in and restricted to certain pathways, in contrast to neighbouring tracts which contain almost no Factor I. Of the three cerebellar peduncles only the superior cerebellar peduncle contains relatively large amounts of Factor I, and it is this tract that is known to carry 'inhibitory' fibres from the dentate nucleus (rich in Factor I) to the red nucleus, thalamus, etc. The optic tract is another example of a fibre tract containing larger quantities of Factor I. That the sensory fibres contribute practically nothing to its Factor I content is shown by the fact that the optic nerves contain no detectable quantity of it. We must thus conclude that the optic tract contains non-sensory fibres which are extremely rich in Factor I. It is likely that they represent at least part of the commissural fibres.

The third case of relatively high Factor I content of white matter is that of the crus cerebri. The mass of this structure consists of the cortico-pontine fibres, but it is unlikely that these fibres are responsible for the Factor I content because they connect structures of relatively low Factor I content. We suspect that the Factor I content of the crus cerebri can be ascribed to the pallido-nigral fibres which pass through this structure, connecting the globus pallidus with the substantia nigra, both of which have a high Factor I content. The available anatomical data are unfortunately not detailed enough to rule out the possibility that the area dissected contained cell groups belonging to

the neighbouring substantia nigra, which has the highest Factor I content of any brain structure. The pyramidal fibres are unlikely to contribute to the Factor I content of the crus cerebri because the same fibres, dissected from the level of the medulla, contain almost no Factor I.

DISCUSSION

Technical considerations

In spite of a highly accurate assay method there is a wide spread of the values obtained for the Factor I content of a number of brain structures. It is obvious that the variations are particularly great with those structures which are difficult to dissect, like the dentate nuclei. One can assume that the lower values can be attributed to admixed white matter. The variations in the Factor I content of cerebral cortex might, at least in part, be due to the fact that we made slices of uniform thickness but the various cortical areas differ in thickness. If this is so, we must, of course, also assume that cortical areas with seemingly equal Factor I content might in reality contain different amounts of this Factor.

The more compact nuclear masses of the brain, such as caudate nucleus, mamillary bodies and putamen, which are easy to dissect and have well-defined borders, show only 20–30 % variation of Factor I content. In contrast to these uniform values we find 60–80 % variation for dentate nucleus and for mesencephalic substantia grisea centralis. The convolute outline of the dentate nucleus makes it impossible to isolate this structure without contamination by neighbouring white matter. For this reason it seems likely that the higher values are more representative of the Factor I content of this nucleus than the lower ones. A similar reasoning applies to the rhombencephalic reticular formation. Here we are dealing with ill-defined structures consisting of minute cell groups scattered among various fibre tracts. Again it seems reasonable to assume that the higher values are more representative than the lower ones if we are interested in a 'chemical' characterization of the neurones originating in this structure. With the possibility of wide variation, it becomes difficult to judge the difference found between the different areas of the reticular formation. They might represent genuine differences in the Factor I content of these areas, but they might also reflect the irregular distribution of Factor I throughout the reticular formation, a distribution which makes it a matter of chance whether a dissected sample includes more or less of tissue rich in Factor I.

We have tried to analyse the conspicuous variability of the Factor I content of the substantia grisea centralis by assaying samples of successive areas of this structure. Again no correlation between Factor I and area was found and we have to assume that, with regard to neurones containing Factor I,

the situation here is similar to that found in the rhombencephalic reticular formation.

Functional considerations

All those centres concerned with the correlation and integration of motor activity contain conspicuously high concentrations of Factor I. Practically all centres that comprise the extra-pyramidal system are known to be involved in excitatory as well as inhibitory actions. If one assumes that there are extra-pyramidal pathways which include synaptic stations in a number of extra-pyramidal nuclei, then all these synapses must be considered excitatory in nature, since they have to activate their particular post-synaptic neurone if the inhibitory impulses are to arrive at their destination. If Factor I is an *inhibitory* transmitter, it should only be released at the end of these inhibitory pathways. If Factor I is at all a transmitter substance, its high concentration within the 'extra-pyramidal centres' could mean one of two things: (*a*) More than one neurone of an inhibitory pathway transmits by releasing Factor I; in this case, Factor I can be inhibitory only at the last synapse but must be excitatory at the preceding synapses. (*b*) Factor I is not involved in transmission by neurones belonging to what may be called polysynaptic inhibitory pathways, but rather it would belong to inhibitory tracts involved in direct inhibition of the activity of one centre by the action of inhibitory fibres originating in another centre; these would be single neurone pathways.

Although excitatory actions of Factor I have been demonstrated by Florey & McLennan (1955*b*) and Florey (1956), we have to await final identification of Factor I until it can be decided whether these actions are indeed due to the same substance that causes inhibition of synaptic transmission (Florey & McLennan, 1955*a*, *b*; Florey, 1956; McLennan, 1957).

It is interesting to compare the distribution of Factor I in the central nervous system with that of acetylcholine, substance P, sympathin (noradrenaline) and 5-hydroxytryptamine. No specific data are available on the distribution of gamma-aminobutyric acid in mammalian brain apart from reports that it does occur largely in the central nervous system, is absent in peripheral nerves, and that grey matter contains more than white matter (Udenfriend, 1950; Roberts, Harman & Frankel, 1951; Tallan, Moore & Stein, 1954). The hypothalamus contains large amounts of all the substances under discussion (MacIntosh, 1941; Zetler & Schlosser, 1953, 1954; Vogt, 1954; Amin, Crawford & Gaddum, 1954). The substantia grisea centralis is another structure which contains large quantities of several 'transmitters', noradrenaline (Vogt, 1954), 5-hydroxytryptamine (Amin, Crawford & Gaddum, 1954), substance P (Zetler & Schlosser, 1953, 1954; Amin *et al.* 1954) and Factor I. There are no data concerning the acetylcholine content of this structure. The substantia nigra contains large amounts of Factor I and substance P (Zetler & Schlosser, 1953, 1954), and judging from the high activity of choline acetylase found

in this structure (Feldberg & Vogt, 1948) its acetylcholine content is likely to be high also. Nothing is known, however, about the noradrenaline and 5-hydroxytryptamine content. It can thus be seen that some brain structures contain large amounts of several of these substances. This means that a high content of one 'transmitter' does not exclude the presence of a large amount of another transmitter, and its predominance is not indicated unless it can be shown that the others are indeed present in much smaller quantities.

From the foregoing discussion it must be concluded that, with the data at hand, it is not possible to establish a definite correlation between location and function of Factor I. There is, nevertheless, a characteristic pattern of distribution and our results indicate that there are tremendous differences in the Factor I content of the different brain structures. Since extracts containing Factor I are highly potent in affecting synaptic transmission (Florey & McLennan, 1955*a*, *b*), we assume that the differential distribution of Factor I is actually correlated with a functional differentiation of the brain and that different amounts of Factor I reflect the incidence of a type of neurone which is chemically characterized by its Factor I content. These neurones are definitely not sensory (functionally as well as chemically), they differ also, as shown by their distribution, from cholinergic and adrenergic neurones (for information concerning the distribution of cholinergic and 'adrenergic' tracts, consult the papers by Feldberg & Vogt (1948) and Vogt (1954)). We therefore postulate the existence of a further category of neurones which, because of their Factor I content, we should like to name I-neurones.

SUMMARY

1. With the aid of the crayfish stretch receptor preparation, the Factor I content of a large number of brain structures was determined. Factor I was found to be concentrated within the grey matter of the extra-pyramidal centres. A high content of Factor I was also found in the superior cerebellar peduncle, optic tract and crus cerebri. In general, however, white matter was found to contain only little Factor I.

2. Two alternative explanations for the distribution of Factor I in mammalian brain are offered, both based on the assumption that this factor is a transmitter substance: (*a*) Factor I is contained within neurones concerned with direct inhibition of other nerve centres. (*b*) Factor I is contained within the first and last neurones of polysynaptic extra-pyramidal pathways, in which case it has to fulfil the function of an excitatory transmitter in the first synapses of these pathways.

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REFERENCES

AMIN, A. H., CRAWFORD, T. B. B. & GADDUM, J. H. (1954). The distribution of substance P and 5-hydroxytryptamine in the central nervous system of the dog. *J. Physiol.* **126**, 596–618.

BAZEMORE, A., ELLIOTT, K. A. C. & FLOREY, E. (1956). Factor I and γ-aminobutyric acid. *Nature, Lond.*, **178**, 1052–1053.

BAZEMORE, A. W., ELIOTT, K. A. C. & FLOREY, E. (1957). Isolation of Factor I. *J. Neurochem.* **1**, 334–339.

EDWARDS, C. & KUFFLER, S. W. (1957). Inhibitory mechanisms of gamma-aminobutyric acid on an isolated nerve cell. *Fed. Proc.* **16**, 34.

ELLIOTT, K. A. C. & FLOREY, E. (1956). Factor I—Inhibitory factor from brain. Assay. Conditions in brain. Simulating and antagonizing substances. *J. Neurochem.* **1**, 181–192.

FELDBERG, W. & VOGT, M. (1948). Acetylcholine synthesis in different regions of the central nervous system. *J. Physiol.* **107**, 372–381.

FLOREY, E. (1954). An inhibitory and an excitatory factor of mammalian central nervous system, and their action on a single sensory neuron. *Arch. int. Physiol.* **62**, 33–53.

FLOREY, E. (1956). The action of Factor I on certain invertebrate organs. *Canad. J. biochem. Physiol.* **34**, 669–681.

FLOREY, E. (1957). Further evidence for the transmitter-function of Factor I. *Naturwissenschaften*, **44**, 424–425.

FLOREY, E. & MCLENNAN, H. (1955*a*). The release of an inhibitory substance from mammalian brain, and its effect on peripheral synaptic transmission. *J. Physiol.* **129**, 384–392.

FLOREY, E. & MCLENNAN, H. (1955*b*). Effects of an inhibitory factor (Factor I) from brain on central synaptic transmission. *J. Physiol.* **130**, 446–455.

LEMBECK, F. (1953). Vorkommen und Bedeutung der Substanz P in dorsalen Wurzeln. *Arch. exp. Path. Pharmak.* **219**, 197–213.

MACINTOSH, F. C. (1941). The distribution of acetylcholine in the peripheral and the central nervous system. *J. Physiol.* **99**, 436–442.

MCLENNAN, H. (1957). A comparison of some physiological properties of an inhibitory factor (Factor I) and of γ-aminobutyric acid from brain. *J. Physiol.* **139**, 79–86.

PAPEZ, J. W. (1929). *Comparative Neurology.* New York: T. Y. Crowell.

ROBERTS, E., HARMAN, P. J. & FRANKEL, S. (1951). γ-aminobutyric acid content and glutamic decarboxylase activity in developing mouse brain. *Proc. Soc. exp. Biol., N.Y.*, **78**, 799–803.

STADIE, W. C. & RIGGS, B. C. (1944). Microtome for the preparation of tissue slices for metabolic studies of surviving tissues in vitro. *J. biol. Chem.* **154**, 687–690.

TALLAN, H. H., MOORE, S. & STEIN, W. H. (1954). Studies on the free amino acids and related compounds in the tissues of the cat. *J. biol. Chem.* **211**, 927–939.

UDENFRIEND, S. (1950). Identification of γ-aminobutyric acid in brain by the isotope derivative method. *J. biol. Chem.* **187**, 65–69.

VOGT, M. (1954). The concentration of sympathin in different parts of the central nervous system under normal conditions and after the administration of drugs. *J. Physiol.* **123**, 451–481.

ZETLER, G. & SCHLOSSER, L. (1953). Substanz P im Zentralnervensystem des Rindes. *Naturwissenschaften*, **40**, 559–569.

ZETLER, G. & SCHLOSSER, L. (1954). Substanz P im Gehirn des Menschen. *Naturwissenschaften*, **41**, 46.