STUDIES ON THE BRAIN PHOSPHATASES.

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(Received July 23, 1938.)

ACCORDING to the first investigators (Kay (17), King (18), Macfarlane, Patterson and Robison (19), Edlbacher, Goldschmidt and Schläppi (13)), the phosphatase activity of the brain is only one-fifteenth to one-quarter of that of the kidney. Cedrangolo (11) and Giri and Datta (14) have shown that mammalian brain contains two phosphatases, with optima at pH 5 and pH 9 respectively. Yates and Fleischhacker (28) found the alkaline-phosphatase activity of ox-brain cortex to be about five times that of the corpus callosum and to approach within 66% of that of kidney cortex. Intermediate values were found for other parts of the brain; the nucleus caudatus, parts of the corpus quadrigeminum anterius and the nuclei of the medulla oblongata were only slightly less active than the cortex, while the thalamus and the globus pallidus gave lower values, approaching those of the white matter. Different parts of the cortex showed small variations, the precentral area always having the highest activity. The activity of the white matter of the spinal cord was higher than that of the corpus callosum (Yates (29)). As under optimal conditions the variations in phosphatase activity in the same parts of different brains (of the same species) were relatively small, within +33, −20%, it was hoped that standard figures might be obtainable and a still finer differentiation possible. Before such an attempt is made, two other problems should be dealt with and are the objects of the present investigations:

(1) Phosphatase activity obtained with different enzyme preparations from the same parts of the brain.

(2) The activity of various parts of the brain toward different substrates at different pH levels with and without the addition of an activator or an inhibitor.

METHOD OF EXPERIMENT.

Preparation of dried tissue-powder.—The technique of Yates and Fleischhacker (28) for the preliminary treatment of the brain-tissue was followed with some modification. 100–200 mgrm. of fresh tissue were weighed into a slightly tapered centrifuge tube, ground and washed three times with pure acetone, and if the "natural" substrate should be removed, three times with

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70% acetone, again three times with pure acetone, and three times with ether. A quantity of solvent equal to 15–20 times that of the tissue was used for each washing which was of 5 minutes' duration, after which the solvent was removed by centrifugation and decantation. The powder was finally dried in an evacuated desiccator, weighed and stored at 0°; under these conditions its activity was retained for at least 6 weeks. Generally about 1·2 mgrm. of the dried powder from grey matter and about 1·5 mgrm. from white matter were equivalent to 10 mgrm. fresh tissue.

Estimation of phosphatase activity.—Phosphatase activity was estimated at pH 5, 7 and 9 with the aid of the Michaelis veronal-acetate buffer. The substrates used were sodium-α- and β-glycerophosphates, sodium hexosediphosphate (prepared from calcium hexosediphosphate), yeast adenylic acid, and in a few experiments, inosinic acid. In most of the experiments the molar concentration of these substrates were 0·00115 or 0·0023; in other experiments the concentrations were optimal = 0·012 mol. In some tests, magnesium chloride (0·08 mol.) was added as activator or sodium taurocholate (0·75% solution) as inhibitor. The latter solution was made up freshly before use. The hexosediphosphate solution (0·35%) contained 24 mgrm.%, the yeast adenylic acid (0·5%) 6 mgrm.% "free phosphate". The sodium taurocholate was free from inorganic phosphate. Incubation for 1–2 hours in different buffer solutions did not liberate any phosphate from these substances, nor from the inosinic acid used in some experiments. The sodium taurocholate solution forms very often slight opacities with the other reagents, especially with the yeast adenylic acid. It is precipitated by trichloracetic acid; but the precipitation cannot be centrifugalized; such suspensions must therefore be filtered (Whatman filter No. 42) and a double amount of all substances and solutions has to be used. 2 c.c. from the filtrate are taken for the P estimation. The test-tubes containing taurocholate and yeast adenylic acid show very often, 10–15 minutes after the addition of the stannous chloride, an opacity which disturbs the colour. The reading of these samples must be done earlier, and the respective figures can be regarded only as approximate ones.

Inorganic phosphate was estimated by the colorimetric method of Kuttner and Lichtenstein as modified by A. Bodansky (7).

The entire estimation was carried out in glass-stoppered centrifuge tubes. The reaction mixture, total volume 1·1 ml., consisted of 0·9 or 0·8 ml. enzyme solution, containing 10 mgrm. fresh tissue or its equivalent of dried tissue-powder, in buffer solution of the required pH, 0·1 or 0·2 ml. substrate solution and 0·1 ml. of the activator or the inhibitor solution. For the control tubes the volume was made up to 1·1 ml. with the buffer solution. All tubes were brought to a temperature of 37° prior to mixing. The tubes were kept in a thermostat at 37° for 60 minutes and continuously shaken in the direction of their long axis or placed in a nearly horizontal position so that the sediment was spread evenly on the under wall. Hydrolysis was stopped by addition

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of 0·9 ml. 10% trichloracetic acid, and after 20 minutes the tubes were sharply centrifuged for 10 minutes. The P estimation was made in the same tube by adding 1·6 ml. of the molybdate and 0·4 ml. of the stannous chloride solution and mixing with a glass rod. The small amount of sediment does not influence the colour developed, as was shown by parallel tests in which the protein precipitate was filtered off in the usual way (Table I, p. 950).

In the absence of a microcolorimeter, the colours were compared with a series of standards containing 1–24 mgrm. P. Following Bodansky the activity is given as mgrm. P liberated as inorganic phosphate at 37° by 100 grm. tissue.

The average error for this method of estimation is, for the alkaline phosphatase activity, less than $\pm$ 10%; for the neutral and the acid range it may be higher, even if duplicate estimations show no great differences. Because of some variations of the technique (e. g. lower concentration of substrate, shorter incubation-time, no shaking of test-tubes) most of the figures published in Tables II and III cannot be compared without a correction with our previous figures which were about twice as high for the grey matter (Yates and Fleischhacker (28)). Experiments carried out with different concentrations of substrates showed that the phosphatase activities of the respective brain-parts run parallel in principle, and were in general agreement with the known facts. With a few exceptions the quantities of P liberated by phosphatase activity without activation by magnesium were less than 20% of the P present in the substrate. In the tables, with a few exceptions, no reference is made to the respective concentrations.

Larger and smaller amounts of tissue, from 50 mgrm. to 1 mgrm. were used in some experiments, the amounts of substrate being proportionately varied. For the smallest quantity of tissue, tapered centrifuge tubes of 1 ml. capacity and micro-pipettes (0·2 ml.) were employed and some modifications were made in the technique:

2 mgrm. of brain-tissue were weighed out in a micro-tube and ground and washed by the aid of a tiny glass rod with 0·3–0·4 ml. of acetone and ether respectively in the same manner as described above. From an ice-cold solution, containing in the usual ratio, substrate, activator or inhibitor, and buffer, 0·4 c.c. are added to the dried powder which is stirred up, and from this suspension 0·2 c.c. are transferred to another micro-tube containing 0·18 c.c. of trichloracetic acid for the determination of the free phosphate. The first tube is kept at 37° for 90 minutes. Then, 0·18 c.c. of trichloracetic acid are added and the tubes centrifuged after 20 minutes standing. Next, 0·31 c.c. of the molybdate and 0·07 c.c. of the stannous chloride solution are added and mixed, and read against a series of standards.

The error of this method is less than $\pm$ 15% (for grey matter). Results as published in Table I show that reasonable agreement exists with various amounts of tissue.

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TABLE I.—*Phosphatase Activity, in Bodansky-units, determined on Different Quantities of Brain-tissue.*

Substrate: Sodium-β-glycerophosphate (final concentration: 0·012 mol.). pH: 9·0.

Tissue.	Equivalent of mgrm. fresh tissue.			
	50.*	25.†	10.†	1.†
Ox-brain (9) cortex . . .	240	245	..	..
Human brain (13) (G.P.I.) motor cortex .	70	70	73	..
G.P.I. (14) motor cortex:				
Uppermost layer . . .	..	..	..	110 118
,, and 2nd layer .	..	130	..	140
Deeper layers . .	..	145	..	160
G.P.I. (15) cortex:				
Uppermost layer . . .	..	82	..	68 70 80
Deeper layers . . .	..	98	..	..
Guinea-pig (I):				
Cortex	..	140 130	..	150
Nervus ischiadicus . .	..	51 63	..	45

* P estimated in filtered deproteinized solution.
† ,, without removal of centrifuged tissue.

A histochemical method has been used in order to check the chemical results and to show that the phosphate liberated by the action of the enzyme is found mainly in cellular elements. Good results were obtained, especially with the large cells of the anterior horn of the spinal cord, which show after the incubation an intense blue colour. A finer differentiation of phosphatase activity in different cellular elements has, so far, not been possible.

Fresh brain-tissue is cut in small pieces, one of them is put into Ringer's solution, containing 2·5% of the substrate, another in pure Ringer's solution; both are kept at 0° for 24 hours and the solutions renewed. Sections are cut by a special freezing microtome for brain-tissue, rapidly washed with distilled water and put for 45 minutes into the molybdic acid, which has been diluted by three times its volume of water. The sections are rapidly washed

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again with distilled water and put into the stannous chloride solution (0·25 of the stock solution diluted in 100 c.c. of water) for 2–3 minutes, washed again, and examined under the microscope. The tissue pieces, meanwhile, have been put for 1–2 hours at 37° in small covered vessels containing a few drops of the respective solutions. After that, sections are taken and treated as above.

If it is desired to use " dried " tissue, the pieces may be put into absolute alcohol at 0° for at least one week, after, on the first day, the alcohol had been changed after 2, 4, 8 and 24 hours. For preparing the incubation, the tissue is brought, at 0°, for periods of 12 hours into 90, 80, 60 and 50% alcohol and finally into Ringer's solution until all traces of alcohol have disappeared ; then the tissues are treated as above.

Results.

Influence of Time after Death on Guinea-pig and Human Brain and Brain-tumour Tissue.

In previous experiments it was shown that the activities of the alkaline ox- and rat-brain phosphatase remain stable until 30 hours after death at 0° and 16–18° (Yates and Fleischhacker (28)). This was confirmed in further experiments.

(*a*) The left hemispheres of two guinea-pigs were minced and ground in acetone, within 6 and 10 minutes after death (by bleeding), respectively ; the right hemispheres were kept for 1–1½ hours at 18°, and then similarly treated. The differences in phosphatase activity of the two hemispheres lay within the experimental error of the method.

(*b*) The brain of another guinea-pig was kept in the skull after death, first for 7 hours at room-temperature and then for another 16 hours in the refrigerator. The phosphatase activity was not markedly different from that of the guinea-pig brains removed and treated immediately after death.

By the kindness of Mr. D. W. C. Northfield (of the London Hospital) I was able to obtain brain and brain-tumour tissue during brain operations, immediately after the tissues had been removed. In one case the apparently normal cortex over a subcortical tumour showed a normal activity, and no marked change after 2½ hours. The activity of the tumour (clinical diagnosis : astrocytoma) was about half that of the cortex, and again there was no change after some hours. In the second case a very soft vascular tumour (clinical diagnosis : glioblastoma multiforme) had penetrated the cortex. The activities of different pieces of " cortex " were very low and were considerably reduced after the tissues had been kept at room-temperature for 2½ hours.

The possibility of a rapid loss of phosphatase activity after death, if pathological conditions are present, should be kept in mind.

The results of these experiments and of the experiments previously

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published (Yates and Fleischhacker (28)) agree well with the observations of Kay on the phosphatases of the kidney (16) with the only difference that the phosphatase activity of the brain decreases somewhat earlier than that of the kidney.

The Phosphatase Activities of Different Enzyme Preparations.

As former investigators had used other enzyme preparations, especially extracts, it was thought that the differences of method might explain some of the divergent results. A number of different enzyme preparations from the same brain areas were made up and tried at different pH levels. For the estimation of white matter activity the central white matter was taken.

PREPARATIONS.

(*A*) Fresh tissue, finely ground in distilled water.

(*B*) Crude extract from (*A*), after the whole suspension was kept in the refrigerator, several times stirred up, centrifuged. The decantate was used for the estimation of phosphatase activity.

(*C*) Filtrate from (*A*), after the tissue had been kept in the refrigerator for 2–3 hours and had been stirred up several times (Whatmann filter No. 42).

(*D*) Tissue-powder, treated with acetone and ether as described above.

(*E*) Extracts, prepared by 24–40 hours " autolysis " in distilled water from
- (*a*) tissue-powder, prepared according to Giri and Datta (14),
- (*b*) preparation (*D*).

(*F*) Residua from (*B*) and (*E*).

The experiments were carried out on the cortex and white matter of five ox and one calf brains with different substrates at varying pH levels. The results, some of which have been published in Table II, show :

(1) Extracts of grey matter (*C* and *E* (*b*) exhibit a low phosphatase activity with an acid and an alkaline optimum.

(2) Grey matter preparations which contain either the whole tissue (*A*, *D*, *F*) or great amounts of active tissue in the suspension of the crude, unfiltered extract (*B*) have an activity which is very high at pH 9.

(3) Fresh central white matter and its crude extracts may sometimes exhibit a weak acid optimum. All the other preparations (whole tissue-powder or extracts) belong to the alkaline type. If magnesium is added, extracts from acetone-ether treated white matter show an optimum activity at pH 7.

Some of these findings not only confirm generally the results of previous investigators, especially of Giri and Datta (14) concerning the acid optimum of certain extracts, but seem also to prove the existence of significant differences between the grey and central white matter. The evidence, unfortunately, is not quite satisfying.

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TABLE II.—*Phosphatase Activity of Different Enzyme Preparations.*

Phosphatase activity in mgrm. P liberated by 100 grm. tissue. Incubation-time : 50 min.
Final substrate concentration : 0·0023 mol. or in ( ) 0·012 mol.

Ox-brain XII.

	Substrate	Fresh tissue. pH 5.	7.	9.	Giri-Datta extract. 5.	7.	9.	Residue from Giri-Datta extract. 5.	7.	9.	Tissue-powder (D). 5.	7.	9.
Cortex	β-glycerophosphate	—	—	—	2	1·3?	8·8	5	7	66	4	22	68
	Ditto + magnesium	13·5	43	122	5·3	4·0	13·7	7	10	75	10	34	104
	Hexosediphosphate	—	—	—	13·3	—	—	2·3	6	78	—	—	100
	Ditto + magnesium	29	56	140	16·0	12·2	21·3	5	9·3	91	—	28	116
	Yeast-adenylic acid	—	—	—	0	1·0	2·0	..	..	..	..	..	..
	Ditto + magnesium	1·5	32·5	50	2	8·6	16·7	..	..	..	..	..	..
Central white matter	β-glycerophosphate	—	—	—	2·7	3·3	5·3	1·5	4·5	27	5	6	38
	Ditto + magnesium	26	22	54	5·3	10·0	8·7	4·5	8·3	44	6	14	64
	Hexosediphosphate	—	—	—	2·0	—	12·7	4·5	4·5	40	—	—	70
	Ditto + magnesium	26	15?	72	3·3	4·7	14·0	9	10·3	46	—	—	82
	Yeast-adenylic acid	—	—	—	—	4	3·3	..	..	..	..	..	..
	Ditto + magnesium	12	17	35	2	8	14	..	..	..	..	..	..

Ox-brain XIV.

	Substrate.	Fresh tissue. pH 5.	7.	9.	Crude extract. 5.	7.	9.	Filtrate from crude extract. 5.	7.	9.	Extract E (a). 5.	7.	9.
Cortex	β-glycerophosphate	12	22	104	10	12	74	10	4	36	4 (8·8)	1 (2·7)	6 (10)
	Ditto + magnesium	—	—	—	—	—	—	—	—	—	(14)	(15·3)	(21)
	Hexosediphosphate	..	..	..	..	..	..	..	..	..	..	..	..
	Ditto + magnesium	..	..	..	..	..	..	..	..	..	..	..	..
	Yeast-adenylic acid	8	7	82	—	8	52	4	10	24	—	—	—
	Ditto + magnesium	—	—	—	—	—	—	—	—	—	—	—	—
Central white matter	β-glycerophosphate	12	20	48	2	6	16	4	8	12	(3·5)	(3·5)	?
	Ditto + magnesium	—	—	—	—	—	—	..	..	..	(5·5)	(9)	?
	Hexosediphosphate	..	..	..	..	..	..	..	..	..	..	..	..
	Ditto + magnesium	..	..	..	..	..	..	..	..	..	..	..	..
	Yeast-adenylic acid	18	14	26	—	—	—	4	6	8	—	—	—
	Ditto + magnesium	—	—	—	—	—	—	—	—	—	—	—	—

The Phosphatase Activity of Different Parts of the Brain on Different Substrates at Different pH.

The next subject of inquiry, therefore, is to determine whether any significant "qualitative" differences for the phosphatase activity of different parts of nervous tissue can be found.

For this purpose the phosphatase activities of physiologically and structurally different regions of the brain on different substrates under varying conditions of pH, addition of magnesium as an activator and sodium taurocholate as an inhibitor have been investigated. As the structures and functions of different brain-parts vary very much, only small quantities of each region can be used. The method described above allows about 50 phosphatase estimations to be done on 0·5 grm. of fresh brain-tissue. Two normal ox-brains and two pathological human (G.P.I.) brains have been investigated. Some of

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the results obtained are published in Table III. Some of the results detailed in Table II will be found to correlate with these data. Besides the substrates of those experiments α-glycerophosphate and in a number of experiments yeast-adenylic acid (adenosin-3-phosphoric acid) and inosinic acid (adenosin-5-phosphoric acid) have been used. Reis (23) claims that only substances like the last-named are the specific substrate of the phosphatase of nervous tissue, with an optimum at the neutral pH. An activator and inhibitor were used, as it was thought that a differentiation might be possible, similar to that in experiments carried out by O. Bodansky (8) on phosphatases of other tissues.

The results obtained from four parts of central nervous tissue (motor cortex, occipital cortex, corpus quadrigeminum anterius, central white matter) exercising different physiological functions, show that there is no great divergence between them.

Thus :

(1) All phosphatases have their highest optimum at about pH 9.

(2) Magnesium always activates. It activates the hydrolysis of β-glycerophosphate the most, especially at the neutral pH when the activity of the enzyme is low.

(3) Sodium taurocholate inhibits generally in the alkaline range. Apparently it also influences the hydrolysis of the β-salt the most. Its action in pathological cases is possibly less regular.

(4) α-glycerophosphate is hydrolysed the least, β-glycerophosphate more and together with magnesium it very often reaches the hydrolysis rate of hexosediphosphate, which is always hydrolysed the most.

(5) The activity of the motor cortex is always the highest, a result which confirms our previous figures (Yates and Fleischhacker (28), Yates (29)).

A few experiments were carried out with inosinic acid, kindly sent by Dr. Reis. The results obtained with crude grey and white matter extracts at different concentrations of substrate and dilutions of the reaction mixture after deproteinization, seem to indicate that the activity on inosinic acid is quite different from the activity on yeast-adenylic acid ; it is very high, especially at the neutral pH. After the addition of the stannous chloride solution a precipitate develops when both inorganic phosphate and inosinic acid are present, and disturbs the readings. The precipitate is dissolved after the addition of NH_4OH.

So far no " qualitative " differences in the phosphatase activity of three parts of the cerebral grey matter could be observed. As regards the central white matter, it may be remarkable that at the alkaline, and perhaps at the neutral pH the activity on the hexosediphosphate is relatively the highest of all tissues investigated. This can be seen easily if the respective ratios of hydrolysis $\frac{\text{grey matter}}{\text{white matter}}$ for the different substrates are compared. When various enzyme concentrations are incubated in qualitative constant substrate

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TABLE III.—*Effect of Different Cortex and White Matter Phosphatase on Different Substrates.*

Dry powder preparation. Phosphatase activity in mgrm. P liberated by 100 mgrm. fresh tissue. Incubation-time: 50 minutes.
Final substrate concentrations: Ox-brain 10 and 11, 0·0023 mol.; G.P.I. 17, 0·0012 mol.; in ( ) 0·012 mol.

Substrate.	Ox 10. Corpus quadrigeminum anterius.			Ox 11. Motor cortex.			Ox 11. Occipital cortex.			Ox 11. Central white matter.*			G.P.I. 17. Broca.			G.P.I. 17. Frontal cortex.		
	pH 5.	7.	9.	5.	7.	9.	5.	7.	9.	5.	7.	9.	5.	7.	9.	5.	7.	9.
α-glycerophosphate	1	4	46	3	7	64	4	13	39	3	9	27	0	3	25	0	5	26
Ditto + magnesium	2	9	50	5	9	70	9	19	47	5	12	34	1	5	30	0	13	35
,, + taurocholate	—	—	47	5	2	50	9	13	35	12	12	15	3	4	23	5	12	32
β-glycerophosphate	2	7	54	3	7	74	4	15	63	3	17	33	1	5	44	0	5	45
Ditto + magnesium	2	22	96	7	17	?	9	27	81	5	27	57	1 (5)	8 (12)	64 (86)	0 (9)	13 (17)	55 (85)
,, + taurocholate	3	6	42	3	11	62	9	13	39	8	9	18	3	4	30	2	4	31
Hexosediphosphate	0	13	64	5	9	90	3	23	81	3	29	73	0	3	46	1	2	45
Ditto + magnesium	9	13?	82	11	15	110	10	29	95	6	34	91	0	5	60	5	7	53
,, + taurocholate	5	8	60	5	9	82	7	23	69	3	27	59	4	2	46	3	3	45
Yeast-adenylic acid	—	0	52	0	?	88	0	9	57	0	3	27	..	..	..	..	..	..
Ditto + magnesium	—	18	48 ?	0	5	100	0	29	85	0	21	63	..	..	..	..	..	..
,, + taurocholate	—	—	—	0	0	48	0	0	24	0	0	12	..	..	..	..	..	..

* High "natural" activity.

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concentrations, it is found that the quantities of P liberated are in direct proportion to the amounts of enzyme ($\frac{1}{2}$–$2\frac{1}{2}$ mgrm. dried powder from the white matter), whether β-glycerophosphate or hexosediphosphate be used.

Discussion.

The alkaline phosphatase appears to be closely connected with the nervous tissue, even with so soft a tissue as the grey matter of the cerebral cortex, and it is not possible to extract considerable quantities either within a few hours from fresh tissue or within 24–40 hours from acetone-ether treated tissue-powder. If a crude extract is made up from brain-cortex, the phosphatase remains connected, at least for the first few hours, mainly to the particles which do not pass a Whatman filter No. 42, and the activity of the filtrate is low. These facts, in connection with those concerning the distribution, may explain the low activities of brain phosphatases found by some investigators who used extraction methods (Edlbacher *et al.* (13), Giri and Datta (14), Reis (23)). On the other hand, Kay (17) and King (18), who autolysed the fresh-ground tissue for 24–48 hours at room-temperature, and Macfarlane, Patterson and Robison (19) who used very small pieces of dried tissue for a long lasting extraction and incubation period, at pH 8·4–8·6, obtained activity figures more of the order of those obtained in our experiments with whole tissue preparations, or crude extracts. The close connection of the alkaline phosphatase with the tissues and the limited possibility of extraction have been generally discussed by Bamann, Riedel and Diederichs (2).

The evaluation of the activity of the acid phosphatase is difficult. It is evident that its optimum in crude fresh brain extracts and in whole tissue preparations of grey matter appears low and even obscured by the high activity of the alkaline tissue phosphatase. The acid optimum will be demonstrated the best if the main quantity of alkaline phosphatase be removed; thus in extracts from acetone-ether treated grey matter or the whole brain (Giri and Datta (14)), or when the activity of the alkaline enzyme is not too high, thus in white matter. The question why the activity of dried powder extracts from white matter is lower at pH 5 than at pH 7 cannot be decided from our experiments. Another question is, whether the acid phosphatase which belongs to the easily extracted "lyoenzymes" (Bamann, Riedel and Diederichs (3)) is a true tissue phosphatase at all. A survey of the literature affords no striking evidence on this point. Robison and Soames (24) had pointed to the possible existence of an acid phosphatase in pancreas, spleen and liver. The figures of Martland and Robison (21) show a slight acid optimum for bone phosphatase. The existence of an acid phosphatase has been confirmed in several papers by Bamann (3), Belfanti (6) and Busse (9). Roche (25), in several papers, Davies (12), Schoonover (27), Hori (15), Munemura (22) showed that an acid phosphatase is present in the red corpuscles. The Japanese authors demonstrated

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that the enzyme has two optima at pH 3·5 and 5·5 and that it is easily removed by good washing from the tissues, while the other authors claim that the only optimum is at pH 6. Davies (12) and Schoonover (27) confirmed the observations of Roche (25), that the acid phosphatase of the red cells hydrolyses much more α- than β-glycerophosphate. According to Cedrangolo (11) his brain extracts hydrolysed more of the α- than the β-salt, but he claims to have removed the red cells. The confusion that exists on the subject of the acid phosphatase has been commented on by Bamann and Salzer (5). It is not intended in this paper to add to the discussion. The ultimate solution of the problem of the origin and localization of the acid phosphatase in brain will only be possible when more is known of the general properties of these phosphatases.

Two other discrepancies which seem to occur between the results of Giri and Datta (14) and those recorded here can be explained. The first of them concerns the higher activity of extracts at the acid pH found by them. In many of our experiments the substrate concentrations were under optimal, and it is well known that the acid enzyme is much more dependent on optimal conditions than the alkaline (see also Tabes II and III). The other point is the activation by magnesium, which according to Giri and Datta (14) and other authors, does not activate the acid tissue phosphatase. This result depends very much on the liberation of the enzyme preparation from alkaline impurities. As many of our extracts were obtained by centrifugation, such impurities may have been present. In two filtered extracts the activation of the acid phosphatase by magnesium was practically *nil*. The influence of the presence of different enzymes has been discussed above, it has been emphasized by Bamann and Riedel (2), and Albers and Albers (1).

The relatively high activity at the neutral pH of white matter extracts is conditioned only by the strong influence of magnesium in this range. This was observed also by Davies (12). The observation of Cattaneo (10) that this activation is especially high when the phosphatase concentration is low is confirmed by the present experiments.

Reis's (23) findings that the hydrolysis of the so-called 5-nucleotidase is very high at the neutral pH and also that a precipitate is formed in magnesium containing filtered fresh extracts is apparently confirmed by some experiments. On the other hand, his results on the different activities of white and grey matter and of the higher hydrolysis of hexosediphosphate, confirm in principle our findings although his figures are low (extracts !). It may be mentioned that Edlbacher, *et al* (13) found a higher hydrolysis by the cortex in those experiments in which magnesium hexosediphosphate was used.

The ratio of $\frac{\text{P liberated from hexose diphosphate}}{\text{P liberated from } \beta\text{-glycerophosphate}}$ by the alkaline enzyme was found to be for extracts from the whole brain by Kay (17) 1·25, by Giri and Datta (14) 1·38. The respective average ratios calculated from my experiments are : for cortex 1·22, and for the white matter 1·75. The average

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ratios $\frac{\text{grey matter}}{\text{central white matter}}$ are for P liberated from β-glycerophosphate about 2·5, and for P liberated from hexosediphosphate about 1·75, in consequence of the higher activity of the white matter on this substrate.

Summary.

(1) Methods for the estimation of phosphatase in small amounts of nervous tissue and a histochemical method have been described, and different enzyme preparations have been used.

(2) The alkaline phosphatase of the brain is firmly united with the tissue. Its maximum activity cannot be demonstrated in filtered extracts from fresh or acetone-ether treated tissue.

(3) When the phosphatase activity is estimated at pH 5, pH 7 and pH 9, an acid optimum is usually only demonstrable in filtered extracts of the grey matter.

(4) The actions of three different regions of the grey matter on α- and β-glycerophosphate, hexosediphosphate and yeast adenylic acid varied directly with the variation of pH.

Incubation with inosinic acid as substrate showed a very high activity at the neutral pH.

The hydrolysis of hexosediphosphate by white matter was found to be relatively higher than that by grey matter.

(5) The influence of time after death, of pathological processes, of magnesium as an activator, of sodium taurocholate as an inhibitor are described.

(6) Previous findings concerning the distribution of the alkaline phosphatase in different parts of the brain have been confirmed.

I wish to thank Prof. F. L. Golla and Dr. S. A. Mann for their helpful criticism and steady encouragement, Prof. H. D. Kay and Dr. J. S. Folley for discussing with me some of the problems, and Mr. D. W. C. Northfield (the London Hospital) for his kindly providing me with operation material.

I am indebted to the Professional Committee, Woburn House, for a grant.

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