The Influence of the Pituitary on Phosphorus Metabolism of Brain

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A previous investigation (Reiss & Rees, 1947) dealt with the carbohydrate metabolism of rat brain under different endocrine conditions. The hexokinase activity of grey matter was found to be increased after hypophysectomy, as was also the anaerobic glycolysis; the latter function was restored to normal by treatment of the animals with preparations of corticotrophic hormone.

On account of the important part played by phosphate in carbohydrate metabolism, it was considered of interest to follow these experiments with investigations of the phosphorus metabolism, and the present paper describes some investigations which have been made in this field by radioactive tracer methods.

Previous observations of the uptake of radioactive phosphorus by brain after injection have been reported. Borell & Örström (1947) demonstrated by this means that the phosphate turnover in the pineal body is considerably higher than for any other part of the brain. Similar experiments, carried out in animals of different ages, were recorded by Friess & Chaikoff (1941) and by Schachner, Friess & Chaikoff (1942), who also examined the distribution of absorbed phosphorus of fractions of different solubility, especially the lipid fraction.

The experiments described in this communication were designed mainly to reveal differences in behaviour between the brains of normal and hypophysectomized animals.

METHODS

Measurements. Measurements of radioactivity on dry samples were made with G.M. 4 Geiger counter tubes (General Electric Co. Ltd.). All samples were placed in the lead castle at the same distance from the counter. Liquid samples were dealt with by the use of the Alltools M.R.C. 1 liquid counter.

Animals. Rats (both hooded and Wistar types) were used throughout the investigations, and were killed by decapitation. Generally pairs of rats, as nearly as possible equal in weight, were used, one serving as control to the treated animal. Where bigger groups of animals were used, one third to one half of the total number were controls. Hypophysectomy was performed by the paratracheal route, and rats were used at varying intervals after operation.

Preparation of material for measurements. Small amounts of tissue were best dealt with by mounting on filter papers of 2.5 cm. diam. which had been previously weighed on a torsion balance. After spreading the tissue on the paper, the combined weight was read and the material dried in an oven at 100°. Very thin layers were thereby obtained, and the weights of samples thus used could be as low as 1.0–2.5 mg. for a whole pineal. For other organs, such as brain, liver, or spleen, the weights were within the range of 20–60 mg. Blood (20–40 mg.) was soaked into the paper, and, after first drying in air, was dried in the oven as before. When ready the papers were laid flat on a sample tray in the lead castle containing the G.M. 4 counter. Repeat counts for the same sample varied by 1–5%. In some experiments the β -radiation of the brain surface only was measured. The brain (without olfactory lobes) of the decapitated animal was prepared, and, after removal of traces of blood, was placed on a microscope slide in the centre of the lead castle. The counts/min. for upper and lower surface are combined in the tables. For liquid counts the whole brain (excluding olfactory lobes) was first homogenized in the presence of a few ml. 25% trichloroacetic acid with an Elvehjem homogenizer (Potter & Elvehjem, 1936). After centrifuging, the supernatant fluid was made up to 10 ml. with water, and the solution was thoroughly mixed and immediately poured into the liquid counter for measurement. Lipid material was then extracted from the precipitate by suspending in Bloor's mixture. The mixture was placed in a hot water bath until the solvent was brought to the boil, then cooled, the volume made up to 50 ml. with fresh Bloor's solvent, and the mixture centrifuged. Samples of 10 ml. were taken for measurements.

The residue finally remaining from the acid and lipid extractions was ashed by heating in a hard glass tube over a microburner with 2 ml. 60% HClO_4 , until the charred material had clarified and the mixture become homogeneous. After cooling, this was made up to 10 ml. with water and transferred to the liquid counter.

RESULTS

Representative results of a series of twenty one experiments in which the animals were sacrificed 40 min. after intravenous injection with ^{32}P , and the ^{32}P uptake by the various organs measured, are indicated in Table 1. It will be seen that the phosphorus uptake of grey matter in the normal animal is very low, being only about one eighth of that of the liver. The rate of uptake by the pineal is considerably higher than for other organs investigated; the pineal accumulates (per 100 mg. fresh

Table 1. *Comparison between ^{32}P uptake of normal and hypophysectomized animals and hypophysectomized animals injected with corticotrophic hormone*(Intravenous injection of 2 μc . ^{32}P 40 min. before dissection.)

Organ	Counts/min./100 mg. tissue		
	Normal animals	Hypophysectomized animals	Hypophysectomized animals injected with corticotrophic hormone 1 hr. before ^{32}P
Brain, grey matter	35, 48, 60	123, 162, 140	34, 72
Olfactory lobe	118, 93, 88	33, 64, 76	49, 53
Pineal	381, 333, 280, 216	1028, 532, 644, 728	293, 272
Liver	228, 196	259, 320	296, 280
Adrenal	64, 78	85, 96	92, 96
Thymus	109, 145	50, 76	90, 63
Testis	89, 94, 82	49, 56, 62	66, 55, 48
Blood	21, 51, 23, 28, 42	24, 42, 17, 38, 48	18, 12, 16

Table 2. *Comparison between ^{131}I uptake of normal and hypophysectomized animals*(Intravenous injection of 2 μc . ^{131}I 1 hr. before dissection.)

Organ	Counts/min./100 mg. tissue	
	Normal animals	Hypophysectomized animals
Brain, grey matter	129, 172, 145, 110, 160	138, 123, 170, 165, 142
Olfactory lobe	185, 275, 298, 242, 152	236, 270, 210, 190, 196
Pineal	480, 606, 820, 704, 634	946, 1260, 1285, 1122, 1420
Liver	328, 382, 422	349, 304, 318
Adrenal	264, 325, 364, 411, 321	450, 443, 360, 520, 288
Thymus	344, 343, 480	483, 354, 512
Testis	342, 556, 193, 664	336, 321, 282, 312
Thyroid	4850, 6148, 7261, 5224, 5368	620, 678, 1500, 996, 352, 826
Blood	892, 680, 630, 721, 866	531, 842, 1240, 1260, 482, 636

tissue) about ten times as much phosphorus as brain, six times as much as the adrenal and 50 % more than the liver.

Hypophysectomy appears to bring about a general increase in the absorption. In different experiments the increase in the case of brain amounted to 50–500 %. The pineal shows an increase in uptake of 70–900 %, while the liver showed increases of 0–100 %.

The effect of corticotrophic hormone is clearly demonstrated. Administration to the hypophysectomized animal of about 3 sudanophobic units 1 hr. before injection of ^{32}P reduced the phosphorus uptake of the brain and of the pineal to a normal level.

That the high activity of the pineal is not restricted to phosphorus only is shown in Table 2, which gives the results of parallel experiments carried out with ^{131}I (the only other radioactive indicator available at these laboratories). After intravenous injection, more ^{131}I is taken up by the pineal than by any other tissue with the exception of the thyroid. The iodine uptake by the pineal after hypophysectomy is increased in the same

manner as the phosphorus uptake, but no corresponding behaviour was shown by brain.

Table 3 illustrates experiments where radiation of the brain surface was measured at different intervals after injection of various doses of ^{32}P . There is, without exception, a considerable increase in phosphorus uptake ranging between 53 and 588 %, and this increase is borne out by the results of measurements on the trichloroacetic acid extract of the whole brain. At the same time, the content of ^{32}P in the lipid phosphorus fraction showed little change when measured 1 hr. after injection of the phosphorus. There was, however, a considerable increase, compared with normal control animals, 17.5 hr. after injection.

DISCUSSION

The increase of phosphorus uptake of the brain after hypophysectomy should be considered in combination with the previous finding of Reiss & Rees (1947) that hypophysectomy results in increased hexokinase activity and anaerobic glycolysis.

The increased activity of the brain with regard to

Table 3. Investigation of the whole brain after injection of ³²P

Dose of ³² P injected (μc.)	Time interval between injection and dissection (hr.)	Normal animals (counts/min.)			Hypophysectomized animals (counts/min.)			Percentage increase in ³² P uptake after hypophysectomy		
		Brain surface	10 ml. trichloro-acetic acid extract of total brain	10 ml. Blood extract of total brain	Brain surface	10 ml. trichloro-acetic acid extract of total brain	10 ml. Blood extract of total brain	Brain surface	Trichloro-acetic acid extract	Blood extract
7	72	347	—	—	657	—	—	89	—	—
7	96	264	—	—	470	—	—	78	—	—
5	4	208	—	—	318	—	—	53	—	—
3	4	140	—	—	232	—	—	66	—	—
2	2	24	18	—	128	191	—	430	960	—
2	2	22	27	—	151	224	—	588	730	—
2	2	89	98	—	263	425	—	191	338	—
2	1	40	66	—	93	152	—	132	283	—
2	0.5	67	39	—	132	145	—	97	273	—
6	16	77	86	—	142	155	—	91	80	—
2	18	—	96	90	—	127	145	—	32	61
2	17.5	—	53	45	—	126	150	—	137	233
2	17.5	—	61	40	—	148	135	—	142	234
2	1	—	67	45	—	147	65	—	119	44
2	1	—	89	0	—	95	5	—	7	—

phosphorus may possibly be due to increased carbohydrate utilization, and this supposition would appear to be justified by the large increase of phosphorus in the acid-soluble fraction, which contains the various carbohydrate phosphoric esters. These observations are interesting in the light of certain long-established therapeutic applications of phosphates: the administration of sodium dihydrogen phosphate or phosphorus-containing syrups in cases of fatigue may be justified on the basis that freshly administered inorganic phosphate can be rapidly mobilized for phosphorylation processes in the brain, thus providing a stimulus to carbohydrate metabolism.

In this connexion there may be some grounds for the belief that a more sensitive β-counter could furnish information of diagnostic importance, by revealing something of the state of carbohydrate metabolism in the human brain, through observations of rates of radioactive phosphorus uptake. It would be wise, however, to suspend judgement on this point, until much more work of a fundamental character is accomplished.

The finding of Borell & Örström (1947) that the pineal has the largest phosphorus uptake/100 mg. is confirmed. It may be doubted, however, whether their deduction that the pineal plays an important role in phosphorus metabolism is justified, in view of the equally high activity of this gland with regard to ¹³¹I, demonstrated in the present research. No explanation can at this stage be offered for this behaviour, but it may be permissible to offer the suggestion that the pituitary gland can exert some inhibitory function, controlling the activities of the pineal.

SUMMARY

1. Previous observations by other workers on the uptake of radioactive phosphorus by the brain after injection into normal rats are confirmed; comparative measurements on other organs have been made.
2. The effect of hypophysectomy is to bring about a general increase in the absorption of phosphorus, particularly by the brain and the pineal.
3. Administration of corticotrophic hormone to the hypophysectomized animals, before injection of phosphorus, restores the absorption to a normal level.
4. It is suggested that the increased rate of ³²P uptake of the brain in the hypophysectomized animals is connected with increased carbohydrate utilization.

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REFERENCES

- Borell, U. & Örstöm, A. (1947). *Biochem. J.* **41**, 398.
 Friess, B. A. & Chaikoff, I. L. (1941). *J. biol. Chem.* **141**, 479.
 Potter, V. R. & Elvehjem, C. A. (1936). *J. biol. Chem.* **114**, 495.
 Reiss, M. & Rees, D. S. (1947). *Endocrinology*, **41**, 437.
 Schachner, H., Friess, B. A. & Chaikoff, I. L. (1942). *J. biol. Chem.* **146**, 95.

Studies on Cholinesterase

6. THE SELECTIVE INHIBITION OF TRUE CHOLINESTERASE *IN VIVO*

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When the distinction was made between two types of cholinesterase (ChE) in the animal body, these enzymes were called true cholinesterase and pseudo-cholinesterase (Mendel & Rudney, 1943*a*). The name pseudo-cholinesterase was chosen because it was deemed unlikely that this enzyme could deal effectively with acetylcholine *in vivo* in view of its relative inactivity towards low concentrations of that substrate. To determine the validity of this assumption Hawkins & Gunter (1946) employed a selective inhibitor of pseudo-cholinesterase, the prostigmine analogue Nu-683. *In vivo* experiments with this compound showed that a prolonged and almost complete inhibition of pseudo-cholinesterase in plasma and tissues does not evoke any reaction indicative of the accumulation of acetylcholine. Thus it appeared that in conditions in which the activity of true cholinesterase is relatively unimpaired pseudo-cholinesterase plays no essential role in the removal of acetylcholine *in vivo*.

The possibility remained, however, as these workers pointed out, that an auxiliary role might be assumed by pseudo-cholinesterase, namely, that of serving as a substitute for true cholinesterase whenever the activity of the latter enzyme is impaired. Proof of this hypothesis has hitherto not been put forward. It has awaited the finding of a compound which not only possesses the property of acting as a selective inhibitor of true cholinesterase, but which can also be administered *in vivo* without eliciting any effects other than those attributable to its cholinesterase-inhibiting action.

Selective inhibitors of true cholinesterase have been described previously. Zeller & Bissegger (1943)

have shown that high concentrations of caffeine inhibit the cholinesterases of erythrocytes and brain, two examples of true cholinesterase (Mendel & Rudney, 1943*a, b*), without significantly affecting the cholinesterase activity of human plasma which contains mainly pseudo-cholinesterase (Mendel, Mundell & Rudney, 1943). Recently, Adams & Thompson (1948) have demonstrated that certain 'nitrogen mustards', particularly di-(2-chloroethyl) methylamine (DDM), act as selective inhibitors of true cholinesterase.

The present communication deals with a cholinesterase inhibitor which, apart from possessing a selective inhibitory action on true cholinesterase, produces no effects *in vivo* that could not be attributed to the accumulation of acetylcholine. This compound, a prostigmine analogue, the *N*-*p*-chlorophenyl-*N*-methylcarbamate of *m*-hydroxyphenyltrimethylammonium bromide (Nu-1250; Aeschlimann & Stempel, 1946), has the following formula:

The aforementioned properties of Nu-1250 suggested its use as a tool in determining whether pseudo-cholinesterase can assume an auxiliary role in the hydrolysis of acetylcholine when the activity of true cholinesterase is impaired. The results of experiments dealing with this question are reported here.