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The Turnover of Phosphate in the Pineal Body Compared with that in Other Parts of the Brain

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(Received 11 December 1946)

The metabolism of the brain has been investigated by various methods. Chute & Smyth (1939) and Broman & Jacobsohn (1941) have shown that the oxygen consumption of isolated perfused cat brain amounts to about 100–300 ml. O₂/100 g. of brain/hr. An approximate calculation of the Q_{O_2} results in values of between 4 and 12 ($Q_{O_2} = \mu\text{l./hr./mg. dry weight.}$) Another method of determining the metabolism of the brain consists in measuring, in the intact brain *in situ*, the composition of the arterial blood flowing into and the venous blood flowing from the brain. This method is however less suitable for obtaining absolute values, because the velocity of the blood streaming through the brain must be evaluated. This meets with certain difficulties.

In the method most usual at present, metabolism is measured in surviving brain slices by the Warburg procedure. The Q_{O_2} values thus found for cortex slices have usually varied between 5 and 10. This method also shows that the metabolism of the brain is chiefly associated with carbohydrates. Pyruvic acid is one of the most important metabolic products. The oxidation of this substance in the brain has been studied by, for example, Simola & Alapenso (1943) and Long (1943). A number of enzymic reactions takes place in the brain, but no scheme for the carbohydrate metabolism in this tissue has yet been established (Evans, 1944).

Transphosphorylation in the brain has also been studied by this method. Macfarlane & Weil-Malherbe (1941) thought that the breakdown of glucose in the brain takes place with the aid of a cycle of phosphate transfers. This supposition was based on the observation that inorganic phosphate in slices of rat brain undergoes no change during

anaerobic glycolysis, whereas the pyrophosphate is reduced to one-third of its original value. Borell & Örström (1945) showed later that radioactive phosphate chiefly accumulates as hydrolyzable acid-soluble phosphate esters, which supports the above supposition.

The employment of P³² constitutes an advance in technique. This method allows us to measure, in very small parts of the brain, the velocity at which the marked P atom participates in the metabolism. The phosphorus participates, however, not only in the carbohydrate metabolism, but also in that of the phospholipids and nucleic acids. These latter processes in the brain have been studied by several workers (Chargaff, 1939; Chargaff, Olson & Partington, 1940; Hevesy & Hahn, 1940; Hevesy & Ottesen, 1943; Hevesy, 1940).

The investigations of Hevesy & Hahn (1940) show that a strikingly low metabolism is found in the brain, when measured by means of radioactive P; this we are in a position to confirm. Their subsequent work furnished further evidence for the view of Hevesy & Ottesen (1943) that there is only a low metabolism in the brain. These authors isolated deoxyribonucleic acid from the brain and the metabolism proved to be very low even in this quantitatively considerable fraction.

In our investigations we attempted to divide the brain into sections comparatively well-defined anatomically, and to compare their phosphate metabolism by means of radioactive P. A very unexpected result was obtained, in that the pineal body was found to be the most active portion of the brain (Borell & Örström, 1945). These problems will be dealt with further in this work.

MATERIAL AND METHODS

Rats were generally used in the present investigation. Each animal was injected intraperitoneally with 1 ml. of a solution of radioactive P (as sodium phosphate) containing 0.03–0.04 mc./ml. 5% glucose. The concentration employed gave an activity readily measurable in the small parts of the brain that were investigated.

The animals were decapitated, usually 40 min. after the injection. Blood (0.2 ml.) was drawn off immediately after decapitation, after which the portions of the brain were dissected according to the scheme of Borell & Örström (1945). Table 2 gives the parts of the brain investigated.

Table 1. *Calculation of relative specific activity*

Organ	Radio-activity (impulses/min.)	Phosphorus content (μ g.)	Sp. activity	Rel. sp. activity
Pineal	137	71.5	1.92	33
Cerebellum	130	2280.0	0.058	1
Blood	800	149.0	5.35	92

The values are expressed in terms of relative specific activity. The way in which this is calculated is seen from Table 1. First the specific activity is calculated, this being the number of impulses/ μ g. P. To obtain values which are comparable with one another and not so highly dependent on the biological variations of the material, the specific activity in the different organs was referred to a standard. We have chosen the specific activity of cerebellum as such a standard and the values for all other parts of the brain in the same experiment have been referred to it. Values thus obtained are called relative specific activities.

Table 2. *Specific activity and relative specific activity in different parts of the brain of the rat*

(The animals were killed 40 min. after injection of P^{32} .)

Part	No. of animals	Sp. activity	Rel. sp. activity
Pineal body	36	8.2 $\pm$ 1.8	26.5 $\pm$ 5.5
Pituitary: ant. lobe	36	2.14 $\pm$ 0.42	8.6 $\pm$ 1.2
Pituitary: post. lobe	30	2.61 $\pm$ 0.53	7.9 $\pm$ 1.4
Choroid plexus	24	3.27 $\pm$ 0.71	11.2 $\pm$ 2.8
Olfactory lobe	18	0.41 $\pm$ 0.08	1.30 $\pm$ 0.17
Grey matter of brain	9	1.05 $\pm$ 0.17	1.74 $\pm$ 0.30
White matter of brain	9	1.19 $\pm$ 0.54	1.98 $\pm$ 2.50
Telencephalon	21	0.31 $\pm$ 0.06	0.76 $\pm$ 0.13
Tuber cin.: Ant. part	24	0.39 $\pm$ 0.05	1.12 $\pm$ 0.15
Post. part	24	0.48 $\pm$ 0.11	1.20 $\pm$ 0.14
Cent. part	21	0.62 $\pm$ 0.12	2.72 $\pm$ 0.46
Habenula	27	1.10 $\pm$ 0.27	2.68 $\pm$ 0.48
Thalamus	24	0.31 $\pm$ 0.07	0.78 $\pm$ 0.12
Mammillary body	24	0.58 $\pm$ 0.14	1.49 $\pm$ 0.95
Perforated substance	24	0.42 $\pm$ 0.09	1.16 $\pm$ 0.80
Corpora quadrigemina	24	0.27 $\pm$ 0.01	0.69 $\pm$ 0.07
Pons	24	0.28 $\pm$ 0.02	0.72 $\pm$ 0.17
Medulla oblongata	24	0.46 $\pm$ 0.10	1.02 $\pm$ 0.23
Cerebellum	36	0.33 $\pm$ 0.05	1.00
Blood	36	19.9 $\pm$ 4.35	40.5 $\pm$ 6.6

RESULTS

As shown in Table 2 the different parts of the brain have been divided according to their activities into two distinct groups. The first group contains very

active parts of the brain. It consists of the pineal, the anterior and posterior lobes of the pituitary and the choroid plexus. The other group comprises the fifteen other parts tested. Finally the relative specific activity of the blood also figures in the table (cf. also Borell & Örström, 1945).

The very powerful activity of the pineal seems to be the most remarkable phenomenon. The value of the relative specific activity exceeds that of the pituitary in all cases. The mean value is about 3–4 times the average value of the anterior and posterior lobes.

The values in Table 2 are for animals killed 40 min. after injection of P^{32} . For the purpose of determining the importance of the time elapsing between the injection and the killing of the animals, a series of experiments was carried out at different times after the injection (Figs. 1 and 2). The specific activities of all parts are high 40 min. after the injection. In parts of brain such as the pineal, choroid plexus and the anterior and posterior lobes of the pituitary body, on the other hand, the specific activity shows a strong diminution as early as about 2 hr. after the injection (Fig. 1). In parts with low metabolism, however, the decrease is insignificant (Fig. 2). No appreciable change from the two-hour values was observed 24 hr. after the injection.

The specific activity decreases most rapidly in the blood. This reduction is due to the fact that free phosphate disappears very rapidly (Table 3).

Table 3. *The turnover of different phosphate fractions in the pineal body and blood at different intervals after the injection of radioactive P*

Phosphate fraction	Pineal impulses		Blood impulses	
	No.	%	No.	%
	40 min.			
Free phosphate	357	35	3450	90
Total organically bound P	667	65	398	10
10 min. hydrolysis	463	45	110	3
Residual fraction	167	17	240	6
Precipitable by trichloro-acetic acid	37	3	48	1
	2 hr. 10 min.			
Free phosphate	40	28	2320	43
Total organically bound P	103	72	3080	57
10 min. hydrolysis	14	10	1330	25
Residual fraction	62	43	1550	29
Precipitable by trichloro-acetic acid	27	19	198	3

The question arises as to whether the high phosphate turnover in the pineal is connected with a lively turnover of nucleic acid and phospholipids or with a vigorous metabolism of the phosphorylated intermediate compounds appearing

in the carbohydrate cycle, or whether it is just a diffusion of free phosphate from the blood.

By means of hydrolysis we have attempted to determine the percentages of phosphate in different fractions. The results are given in Table 3.

and 72 % respectively after these two periods. If, however, the radioactivity is determined in the individual organic phosphate ester fractions, a distinct change is observed. The fraction obtained after 10 min. hydrolysis shows a strong diminution,

Fig. 1. The specific activity in different parts of the brain from rats at different times after the injection of radioactive phosphorus. In the figure are shown parts of the brain with a specific activity greater than 1.0, with the exception of cerebellum which was chosen as a basis for comparison.

This table gives only the numbers of impulses from the different P fractions and their percentage distribution. Blood has been included as a standard for comparison. The radioactivity in the inorganic phosphate fraction from the pineal is 35 % after 40 min. and 28 % after somewhat over 2 hr. The sum of the organic phosphate esters constitutes 65

while the residual fraction and that precipitable by trichloroacetic acid increase after 2 hr. 10 min. Each phosphate fraction is characterized by the rapidity with which it turns over radioactivity. The maximum in the curve appears first for the rapid intermediate links and later for the slow links (Örström, 1947).

In blood the radioactivity in the free phosphate fraction falls from 90 to 43 % between 40 min. and 2 hr. 10 min. The activity of the pineal is not changed in the corresponding fraction to any appreciable extent. The pineal is thus independent of the activity of the blood in this fraction. We have arrived at the same result by another method. A rabbit, injected with P^{32} , was killed 40 min. afterwards by compression of the thorax. Distilled water (200 ml.) was

the fraction precipitable with trichloroacetic acid (phosphatides, nucleic acids) (Table 3). Two hr. after the injection this value has risen to 19 %. This confirms the findings of Fries & Chaikoff.

Obviously at least two different types of phosphate reaction take place in the brain, namely one rapid, which results in the turnover of carbohydrate esters, and one slow, which results in that of phospholipids and nucleic acids (Table 3).

Fig. 2. Specific activity in parts of the brain with specific activity less than 1.0 at different times after the injection.

then injected slowly into both carotid arteries and was allowed to run out through the cut jugular veins. The pineal still possessed a high specific activity, even exceeding that of the control animals.

Fries & Chaikoff (1941 *a, b*) determined the uptake of phosphate in rats of different ages with P^{32} . They divided the nervous system into four parts, namely, the forebrain, cerebellum, medulla oblongata and spinal cord. They found that the relatively highest uptake of P^{32} was that of young animals. In young animals, the radioactivity of the spinal cord exceeded that of the other parts, whereas in adults the activity of the parts of the brain predominated. They analyzed particularly the phospholipid fractions, which constituted 20–30 % of the total P^{32} in the brain after 24 hr.

Our experiments show that 3 % of the radioactivity in the pineal is recoverable after 40 min. in

In order to show that the high activity of the pineal body is not characteristic of rats only, the glands from rabbits, pigs, cats and guinea-pigs have also been studied. Table 4 shows that in all the animals investigated the activity of the pineal was at

Table 4. *Relative specific activity in the pineal body in animal species*

Species	No. of animals	Pineal body	Relative specific activity		
			Pituitary		Blood
			Ant. lobe	Post. lobe	
Rat	36	26.5	8.6	7.9	40.5
Rabbit	15	49.5	47.0	35.5	81.0
Guinea-pig	3	8.7	—	—	147.0
Pig	1	3.0	1.5	4.9	—
Cat	5	40.5	—	—	134.0

least on a level with that of the anterior and posterior lobes of the pituitary gland.

Many attempts have been made to determine the function of the pineal. Most of them yielded no valuable results and in fact disproved one another. An observation verified by several authors (MacCord & Allen, 1917; Huxley & Hogben, 1922; Addair & Chidester, 1928) is the change in colour of tadpoles fed before metamorphosis with an extract of oxypineal. The tadpoles grew white and transparent owing to the contraction of the chromatophores. Attempts have also been made to investigate the function of the organ by extirpation, implantation and extraction experiments, and, moreover, to study possible histological changes in the pineal following the removal of other endocrine organs (cf. Bargmann 1943). Certain conditions of pubertas praecox in man have been explained as a result of a reduction or cessation in the activity of the pineal (Berblinger, 1932).

In connexion with certain surgical experiments carried out for the purpose of determining the significance of the pineal, the following observation was made which may well be quoted in this account of the introduction of P^{32} into the brain. In this experiment we studied the consequences of exoculation. It has been shown that the functional condition of the pituitary is influenced by such an operation (Bissonnette, 1937). Furthermore, Scharrer (1937) showed that the glandular cells observed by him in the diencephalon altered after the eyes had been exposed to light. A week after the removal of the eyes the experiment was performed with P^{32} . Of the different parts of the brain investigated, only the values for the medulla oblongata from the blind animals (six in number) differ from those of the normal material (48 animals) (Table 5). Statistical examination of the figures shows that there is a significant difference only between the relative specific activity of the medulla oblongata of animals with eyes removed and that of the medulla oblongata of control animals. The respective mean values are 2.48 and 1.02. The difference, 1.46, is statistically significant with $p < 0.001$.

No accumulation of radioactive P could be observed in the pineal, the diencephalon or the pituitary. Nor did the metabolism of the corpora quadrigemina deviate from the normal value. A lower metabolism had been expected, because the

Table 5. *Relative specific activity in different parts of the brain of control and blind animals*

Part of brain	Control animals	Blind animals
Pineal body	26.5	18.1
Ant. lobe of pituitary	8.6	4.6
Post. lobe of pituitary	7.9	5.0
Choroid plexus	11.2	14.5
Olfactory lobe	1.30	1.55
Telencephalon	0.76	0.94
Thalamus	0.78	0.67
Ant. part of tuber cinereum	1.12	1.75
Post. part of tuber cinereum	1.20	1.17
Mammillary body	1.49	1.45
Perforated substance	1.16	2.41
Corpora quadrigemina	0.69	1.25
Cerebellum	1.0	1.00
Pons	0.72	0.57
Medulla oblongata	1.02	2.48

anterior corpora quadrigemina contain centres which transfer impulses from the optic tract to the oculomotor nerve and thus play a part in the pupil and accommodation reflexes. These reflexes disappear after removal of the eyes, which, however, obviously entails no measurable decrease in the P^{32} content.

SUMMARY

1. The phosphate metabolism in nineteen different parts of the brain of rats has been measured with radioactive phosphorus. The pineal body has the highest specific activity. It is 3–4 times higher than that of the pituitary gland and choroid plexus. The remaining parts of the brain have a low activity. The high specific activity of the pineal body has also been found in a number of other animals.

2. All parts of the brain have a higher specific activity 40 min. after injection with radioactive P than 145 min. and 24 hr. after.

3. The high phosphate turnover in the pineal body is caused by the pineal body itself and not by blood in the organ.

4. The radioactive phosphate in the pineal changes principally in two ways: (a) a rapid turnover in which P^{32} enters chiefly into the carbohydrate phosphate esters; (b) a slow one during which P^{32} enters the phospholipids and nucleic acids.

5. After extirpation of the eyes a marked increase takes place in the phosphate turnover of the medulla oblongata.

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Antibacterial Action of Some Aromatic Amines, Amidines, Amidoximes, Guanidines and Diguanydes

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(Received 18 December 1946)

The discovery of therapeutic activity in a new type of compound is a noteworthy development in chemotherapy. Such activity may be of a minor order but it can be developed by the art of the organic chemist. Discoveries of this kind often depend upon some accidental observation; it is a laborious task to search systematically, using experimental infections in animals, for specific therapeutic activity in new types of compounds. As however the vital processes of all organisms are fundamentally similar, it seems possible that by choice of a suitable organism which could be easily handled one might be able to uncover unsuspected biological activity in new types of compounds without excessive labour. For this purpose a carefully chosen group of bacterial species seems worthy of consideration.

During the war a long series of aromatic bases was prepared in connexion with investigations into the chemotherapy of virus diseases (Andrewes, King & van den Ende, 1943) and of typhus (Andrewes, King, van den Ende & Walker, 1944; Andrewes, King & Walker, 1946). As these substances were still available it was deemed worth investigating whether any possessed pronounced biological activity as revealed by bacteriostatic action on a range of bacteria *in vitro*. Such indeed proved to be the case. *p*-Methylsulphonylbenzamidinium and *p*-methylsulphonylbenzylamine were found to be outstandingly active against certain obligate anaerobic bacteria. These observations were followed up and the results have been fully reported (Fuller, Tonkin & Walker, 1945; Evans, Fuller & Walker, 1944, 1945). The present communication records the results of bacteriostatic tests on a number of aromatic amines, amidines, amidoximes, guanidines and diguanides which have not hitherto been recorded.

METHODS

The methods used were similar to those previously described (Fuller, 1942). Inhibitory concentrations are recorded as mg./100 ml. (parts/100,000). The sign > means that the drug was inactive at the highest concentration tested. The tests were carried out in 200 μ l. of medium contained in tubes 40 $\times$ 5 mm. (internal) which were afterwards sealed with sealing wax. For the tests with *Clostridium welchii* the broth was first boiled, reduced with 0.1% of thiolacetic acid and neutralized; 4 mm. tubes were used and these were loosely covered with glass caps and incubated in the anaerobe jar. The implant was 10 μ l. of a 1:1000 dilution of an overnight culture of *Streptococcus pyogenes* or *Cl. welchii* and of a 1:10,000 dilution of the other organisms.

The effect of proteins on the activity was determined by testing the drugs against *Strep. pyogenes* in broth and in horse blood instead of in 50% serum broth as previously.

Cultures. The main tests were carried out with the following cultures: *Strep. pyogenes*, group A, type 3, 'Richards' (mouse virulent); *Staphylococcus aureus*, 'Paler'; *Cl. welchii*, 'Roshier'; *Escherichia coli*, '88'; *Proteus vulgaris*, 'Hunt'.

RESULTS

Table 1 summarizes the results obtained on a series of aromatic amidines.

It will be noticed that the aromatic amidines with few exceptions are not highly bacteriostatic. The relatively high activity of the nitro-compounds is probably due to a specific effect of the nitro-group. Of the compounds recorded in Table 1 it seems that *p*-ethoxybenzamidinium (12), and *p*-sulphonamido-phenylacetamidinium (21), might be worth while examining on other organisms including the Protozoa. The *o*-aminophenol grouping in (18) on which Ehrlich laid considerable stress has no eutherapeutic effect in this series.