RADIOACTIVE ISOTOPE STUDIES OF THE CONNECTION BETWEEN THE LYMPH CIRCULATION OF THE NASAL MUCOSA, THE CRANIAL CAVITY AND CEREBROSPINAL FLUID

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In an earlier work it has been shown by dye techniques (China ink, trypane blue, methylene blue) that a lymphatic communication exists between the mucosa of the nasal septum and the cranial cavity, inasmuch as the lymph flowed from the middle and posterior thirds of the septal mucosa toward the cranial cavity. In that work evidence was obtained showing that the dye reached not only the subarachnoidal space, but also the hypothalamus and in this way contributed as yet undescribed information [1].

The existence of the connection having thus been established, a new quantitative method has been sought for and found in the use of radioactive isotopes.

The results obtained being closely identical, it seems that the chemical properties of the radioactive indicator did not interfere decisively with the reliability and accuracy of the method.

With regard to the above points of view, we have used Thorium B and P^{32}. Hevesy [2] detected substantial amounts of P^{32} in the cerebral cortex after intravenous injection. Tobias, Bertrand and Waine have shown the presence of Au^{198} in the cerebral cortex, in the wall of the lateral ventricle and in cerebral blood vessels after intravenous administration carried out under physiological conditions [3]. Bakay [4] studied the distribution of tracer elements in the central nervous system after intrathecal and intravenous injection. On the other hand, in the reports dealing with the fluid circulation of the nasal mucosa to our best knowledge no mention has been made of investigations involving the use of radioactive isotopes.

Materials and methods

In part of the experiments the lead isotope ThB has been used. A few tenths ml of 0,2 *N* $Pb(NO_3)_2$ added to the radioactive preparation have been used as carrier. The results of the ThB experiments needed no other correction than the deduction of the natural effect. The thin samples absorbed so little of beta rays that autoabsorption could be ignored. The results are given for weight unit.

In the P^{32} (built into H_3PO_4) studies the samples were adjusted to equal inactive material per surface unit and were prepared from the dry residue of single digested test substances.

Thus, autoabsorption was negligible. The amount of radioactive isotope introduced into the organism was 0,1 mC in rats and 0,2 mC in rabbits.

Measurements were made by means of an end-window counter tube, about 5 to 6 hours after injection. To ensure an equal geometrical distribution, the samples were placed into leaden drawers in the leaden cover of the tube.

Twenty adult male rabbits and 60 adult male rats were used in the experiments. The solution containing the radioactive isotope was injected into the middle third of the septal mucosa, as it had been done in the dye series. The pH of the injected solution was 6. The animals were sacrificed after 15, 30, 45, 60, 75, 90 and 120 minutes. The brain was divided into the following parts : olfactory lobe, frontal lobe, temporal lobe, occipital lobe, hypothalamus, hypophysis, cerebellum, pons and oblong medulla. In some cases centres in the basal ganglia, corpora quadrigemina were also examined for activity.

The controls were given 0,5 ml of the solution containing the radioactive isotope subcutaneously, intravenously, or intraperitoneally and were sacrificed at the same time as the experimental animals.

Results

In both the $ThB(NO_3)_2$ and the $H_3P^{32}O_4$ series the labelled ion reached its maximum concentration in the central nervous system 60 minutes following injection, in contrast with the intravenous experiments. This fact offers proof that the radioactive isotope injected into the nasal mucosa reached the central nervous system through another route and in shorter time than after intravenous administration.

As to distribution, it was remarkable that in our experiments the activity of the hypophysis was significantly lower than that of the hypothalamus. This is in sharp contrast with what HEVESY [2] has observed after intravenous administration, when 88 per cent of the injected radioactive isotope were demonstrated in the pituitary and corpus pineale. In this connection reference is made to the studies by BORELL and ÖRSTROM [5] in which considerable amounts of the intraperitoneally injected P^{32} accumulated in the choroid plexus and in the wall of the lateral ventricles. In our studies with ThB it was intended to elucidate eventual differences in total brain activity after administration through different routes. The results have been tabulated (Table I)

Table I

Distribution of $ThB(NO_3)_2$, *30 minutes after injection*

Route of administration	Brain	Liver + muscle	Number of rats
	discharges/min/g		
Intranasal	120,3	27,6	5
Subcutaneous	59,0	223,0	3
Intravenous	67,4	294,0	3
Intraperitoneal	48,0	1500,0	3

It is obvious from the data in Table I that ThB introduced into the middle third of the nasal mucosa reached in the central nervous system a concentration

twice as high as that attained by injection through either the intravenous, or the subcutaneous, or the intraperitoneal route. On the other hand in parenchymal organs and different tissues low levels resulted after intranasal administration and high levels with the latter methods. The results for liver and muscle, two organs richly supplied with blood and thus representative are presented in Table I. It should be added as explanation that the radioactive indicator injected into the nasal mucosa entered directly the CSF space. BAKAY [6] has reported that the radioactive isotope reaches the brain sooner and in higher concentration if injected intracysternally.

It has been also found (BAKAY [4]) that the isotope administered intracisternally is demonstrable after 30 minutes throughout the brain (except in the frontal lobe and the caudal cord). This shows that the route of the isotope after nasal injection is somewhat different from that administered intracysternally.

Table II

The distribution of intranasally injected thorium B in the brain

Animal, No.	Amount demonstrated				Discharge/gram	Discharge/minute
	1	2	3	4	total	gram
I.	29.80 / 0,3 g	8.00 / 0,259	1.55 / 0,014	58.96 / 0,307	98.31 / 0,880 g	111,7/g
II.	41.64 / 0,261	18.84 / 0,273	11.64 / 0,020	36.84 / 0,240	108.96 / 0,794	137,2/g
III.	8.2 / 0,257	5.4 / 0,200	1.2 / 0,016	6.6 / 0,347	21.4 / 0,820	25,8/g
IV.	28.4 / 0,233	15.4 / 0,261	14.4 / 0,025	22.0 / 0,328	80.2 / 0,837	95,8/g
V.	67.4 / 0.222	58.4 / 0,261	6.6 / 0,025	16.4 / 0,349	148.8 / 0,857	173.6/g
VI.	10.0 / 0,252	56.0 / 0,264	3.0 / 0,028	13.4 / 0,309	82.4 / 0,854	96,6/g
Total:	185.4 / 1,515	162.0 / 1,518	38.19 / 0,128	154.2 / 1,880		
E/min / gram	122.4	106.7	298.3	82.02		—

1. Left frontal lobe.
2. Right frontal lobe.
3. Hypothalamus.
4. Parietal, occipital lobes, cerebellum.

In our experiments the activity of the frontal lobe developed sooner and was higher, because the radioactive isotope reached the CSF space from the anterior scala. This is consistent with the statement made by BAKAY [6] that the parts of the brain adjacent to the CSF space develop a higher activity.

In our studies on the distribution of the tracer in various parts of the brain the following results were obtained. The highest number of discharges per gram weight was found in the hypothalamus. The frontal lobe exhibited about 1/3 of this activity, while the activity in other cerebral areas (as compared to that in the hypothalamus) was insignificant. In this case the animals were killed 20 minutes after injection (Table II).

To eliminate adsorption and radiocolloids, inactive lead was added to the solutions containing the radioactive isotope. In these experiments performed in 10 rats it was found that ThB accumulated in the hypothalamic region, but considerable amounts were detectable also in the basal ganglia. This time the measurements were made 30 minutes after injection (Table III).

Table III

Activity of different parts of the brain

Part of brain tested	Measured value	Discharges/minute/g
Frontal and olfactory lobes	367,5 / 0,472 g	778,7/g
Hypothalamus	57,5 / 0,022 g	2613,6/g
Basal ganglia	39,0 / 0,030 g	1300,0/g
Temporal lobe	124,2 / 0,313 g	396,8/g
Cerebellum, oblong medulla	84,0 / 1,250 g	64,7/g

In the course of the investigations the question arose, what changes occur after injection in the activities of CSF, blood and brain and how are these correlated during the period of observation? To elucidate this point, the above values were followed up over periods of from 15 to 120 minutes after injection (6 rabbits). The results are presented in Fig. 1.

Until 30 minutes after injection the activity of CSF increases steeply, showing no substantial alteration during the next 2 hours. This indicates that ThB passes slowly from the CSF into venous blood. The curve for total brain activity increases for 15 to 30 minutes, then runs parallel (at a lower level)

with CSF activity. The activity of blood showed no substantial changes during the 120-minute observation period.

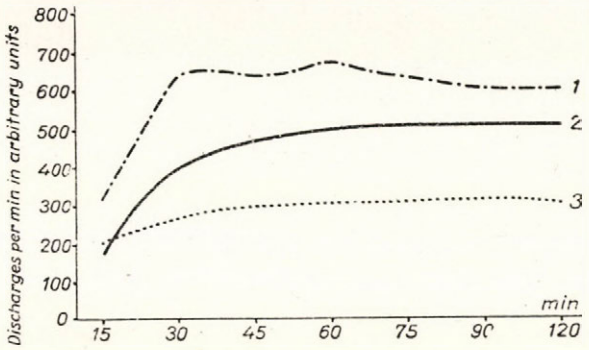

Fig. 1. Appearance of isotope ThB after injection into the nasal mucosa
1: In CSF; 2: in brain; 3: in blood

To make longer observation possible, similar experiments have been carried out with P^{32}, with the difference that in these series the controls, too, were tested for activity in the CSF and the blood. The results are shown in Fig. 2.

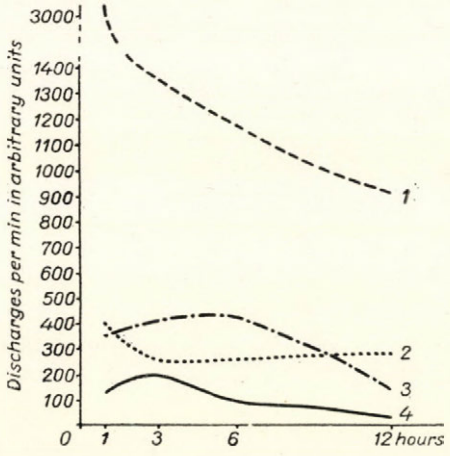

Fig. 2. 1: After intravenous injection, in blood; 2: after intranasal injection, in blood; 3: after intranasal administration, in CSF; 4: after intravenous administration, in CSF

It was found that, after intravenous injection, P^{32} reached a very high initial concentration in blood and after 12 hours, although it was substantially lower than the initial value, the level was about 3 times as high as that attained by using the nasal mucosa route. After rising for about 3 hours, the activity of CSF decreased, fast at first and slowly later.

After injection into the nasal mucosa the blood level remained low, while the activity of the CSF was still about twice as high as that observed after intravenous administration. After 12 hours the CSF activity curve began to decline, but it was still high above the curve of CSF activity in the controls.

Discussion

The radioactive isotope experiments have fully borne out the results obtained by dye techniques. It should, however, be noted that for certain isotopes blood-brain barrier does not exist.

It is known from the literature that only small amounts of parenterally introduced radioactive isotopes (Cu^{64}, I^{131}, Cl^{36}, S^{35} and Zn^{65}) were demonstrable in the brain [7, 8, 9, 10, 11].

In contrast with this, our tabulated data reveal that considerable proportions of the radioactive isotopes ThB and P^{32}, respectively, enter the brain when injected intranasally. The distribution in the brain of the radioactive isotope injected into the nasal mucosa is significantly different from that recovered after intravenous administration. In terms of the arbitrary activity units per gram weight the following differences were found in the brain ; ThB intranasally : 120,3, subcutaneously 59,0, intravenously 67,4 and intraperitoneally 48,0. Thus, the intranasal value was about twice as high. As regards the activities of liver and muscle, a similar comparison will show the intranasal inoculation to produce an activity not amounting to more than a mere one-tenth or one-sixtieth of that produced by administration by other parenteral routes.

Brain activity, as expressed in the above arbitrary units, reaches the peak after 30 to 60 minutes, and begins to decrease after 2 hours. The CSF curve shows an almost parallel course, while the activity in blood increases slowly. From this it follows that during the first 2 hours of examination the radioactive isotope content of the cerebral vessels may be ignored when evaluating the effect in the brain.

In his studies on the distribution in the central nervous system of intracysternally or intravenously injected P^{32}, BAKAY [4] found that on intravenous administration the highest activity was reached in the pituitary, while on intracysternal injection the radioactive isotope attained its highest concentration in tissues adjacent to the aqueduct and choroid plexus, with only very small amounts detectable in different parts of the brain. These results can be explained by the remarkably abundant vascularisation of the pituitary and compare interestingly with our results.

In our intranasal experiments the olfactory lobe, frontal lobe, tissues adjacent to the lateral ventricle and the hypothalamus exhibited a strong

activity. In connection with the hypothalamus it may be suggested that in it the relatively high concentration of radioactive isotope can be explained by the abundant vascular supply of that area and by absense of a blood-brain barrier. The high activity in the basal ganglia might have also been due to their favourable circulation. In earlier injvestigations made by injecting dye into the nasal mucosa we succeeded in following up by gross examination its course on the cerebral base to the hypothalamus.

In the present study we have succeeded again to prove that the blood-brain barrier can be by-passed in the way described earlier and to contribute quantitative data concerning the distribution in the central nervous system of the injected test substances. KROMPECHER et al. [12] have made use of these investigations in therapy, when they instilled insulin and streptomycin on the nasal mucosa.

SUMMARY

The quantitative distribution in the central nervous system of radioactive isotopes (ThB and P^{32}) injected into the nasal mucosa has been studied.

It has been found that:

1. The greatest proportion of the radioactive isotope thus introduced is accumulated in the hypothalamus, with considerable amounts present also in the basal ganglia, olfactory lobe and frontal lobe.

2. The peak of CSF and brain activity is attained 60 minutes following injection.

3. The radioactive isotope injected into the nasal mucosa is absorbed at a higher rate than that injected either subcutaneously or intravenously.

4. The activity reached in the brain after the intranasal administration surpassed in every case the activity measured after intravenous or subcutaneous injection. On the other hand, the parenchymal organs showed a considerably lower activity.

5. Observations continued over prolonged periods revealed that the concentration of radioactive P^{32} did not decrease in the CSF even as late as 6 hours after injection.

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