J. Physiol. (1961), **158**, *pp.* 366–373
With 4 text-figures
Printed in Great Britain

TRANSPORT OF ^{42}K FROM BLOOD TO CEREBROSPINAL FLUID IN CATS

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(*Received* 6 *March* 1961)

The availability of radioactive elements has provided a tool useful in the study of the blood–brain barrier. As has been mentioned by Bakay (1956), the uptake of radioactive ions by the brain is relatively slow in comparison with that of other tissues in the body. Most of the reports in the past have dealt with the time to reach equilibrium between the blood and the brain. Fishman (1959) performed a series of experiments, using ^{24}Na, in which various procedures were employed to affect the rate of attainment of equilibrium between blood and cerebrospinal fluid (c.s.f.).

The present experiments are concerned with the transfer of ^{42}K from blood into c.s.f., using the method of perfusion of the cerebral ventricular system described by Bhattacharya & Feldberg (1958). This technique makes possible a clearance similar to that commonly used in renal studies. With this technique the effect of physostigmine, of air emboli and of a number of organic cations, on the permeability of the blood–c.s.f. barrier to ^{42}K was investigated.

Physostigmine apparently increases the permeability of the blood–brain barrier to barbiturates and acid fuchsin (Greig & Holland, 1949; Greig & Mayberry, 1951; Greig & Carter, 1954; Beiler, Brendel & Martin, 1956) and decreases it to glucose (Greig & Gibbons, 1959). The effect on transfer of ^{42}K into the c.s.f. has not been investigated. Air embolism temporarily increases the permeability of the cerebral blood vessels to albumin, so that injected albumin enters the brain (Lee & Olszewski, 1959), but it is not known whether this procedure also results in an enhancement of transfer into the c.s.f. Since a number of organic cations have been shown to compete with potassium for secretion by the renal tubules (Kandel & Domer, 1957; Domer, 1960) it was of interest to find whether a similar interrelationship existed in the exchange from blood into the c.s.f.

* Life Insurance Medical Research Fund Fellow.

METHODS

The experiments were performed on cats weighing 2·1–2·2 kg, anaesthetized with intraperitoneal pentobarbitone sodium 35 mg/kg. The method of perfusion of the cerebral ventricles was that described by Bhattacharya & Feldberg (1958). A Collison cannula was placed in the left lateral ventricle. Artificial c.s.f. (Merlis, 1940) was perfused at the rate of 0·1 ml./min from the lateral ventricle, through the third ventricle, into the upper part of the aqueduct, where it entered the outflow cannula inserted through the opened cisterna magna, and was collected in graduated centrifuge tubes.

The ^{42}K was received as 344 mg (4·44 m-equiv) $^{42}K_2CO_3$, having an activity of 5 mc. The salt was neutralized with HCl to form the salt ^{42}KCl. To antagonize the severe cardiac depression caused by the intravenous administration of the potassium, 278 mg (5 m-equiv) $CaCl_2$ was added to the ^{42}KCl solution. The resulting solution was then divided into two portions and used in experiments on two successive days; owing to the short half-life of the ^{42}K (12·4 hr), portions of 1/5 and 4/5 of the total volume were used, so that approximately 1 mc was injected in each experiment. The experiments in which 1/5 or 4/5 of the original ^{42}KCl solution was injected will be referred to as experiments with the small or large quantity of potassium respectively.

The ^{42}K was injected into the cannulated femoral vein and the perfused artificial c.s.f. solution was collected subsequently for eight 15 min periods. At the midpoint of each of these periods a 0·5 ml. blood sample was taken from the femoral artery by means of a siliconed-needle cannula which was in the artery. The blood was immediately transferred to counting tubes containing sufficient heparin to prevent clotting. The blood and c.s.f.-solution samples were counted in a well-type scintillation counter.

The organic cations examined were dimethylaminoethanol, tetraethylammonium bromide, choline chloride, tolazoline hydrochloride (Priscoline) and *N'*-methylnicotinamide. They were dissolved in 0·9 % NaCl solution and infused into the superficial vein of the right foreleg. Infusion was begun 30 min before the injection of the ^{42}K and was continued for 2½ hr. The rate of infusion was 0·4 ml./min. In the experiments with tolazoline and *N'*-methylnicotinamide a priming dose of 4 and 0·25 mg/kg respectively was given in addition, by single injection, into the femoral vein at the start of the infusion.

Unless otherwise stated each value given in the figures is the average obtained in two experiments. The values of each pair of experiments have been compared by *t* test and were found not to differ significantly ($P > 0·05$). When the effects of physostigmine, air embolism and the organic cations were investigated, the results were considered to be significantly different from the controls if $P < 0·001$.

RESULTS

Following the intravenous injection of the small and the large quantities of ^{42}K there was apparently an immediate transfer into the perfused artificial c.s.f. Subsequently the ^{42}K concentration in the artificial c.s.f. decreased in a manner similar to that in the blood. This suggests a rapid equilibration of the potassium between blood and c.s.f. When the large quantity of ^{42}K was injected, the rate of its transfer did not increase proportionally. These findings are illustrated in Fig. 1*A* and *B*; and the decreased rate of transfer in the experiments with the larger quantity of ^{42}K is more clearly seen in Fig. 2, which gives the ratios of ^{42}K in the artificial c.s.f. to that in the blood after injection of the large and the small quantities of ^{42}K.

Figure 3 shows, in experiments in which the small quantity of ^{42}K was injected intravenously, the effects on its transfer into the artificial c.s.f. of physostigmine (at *A*), of the organic cation dimethylaminoethanol (at *C*) and of air embolism (at *B*). The dotted lines represent the control ^{42}K transfer, uninfluenced by these procedures. Neither the intravenous infusion of dimethylaminoethanol (25 mg/kg/hr), nor air embolism produced by injecting 0·4 ml. air through a fine needle into each carotid

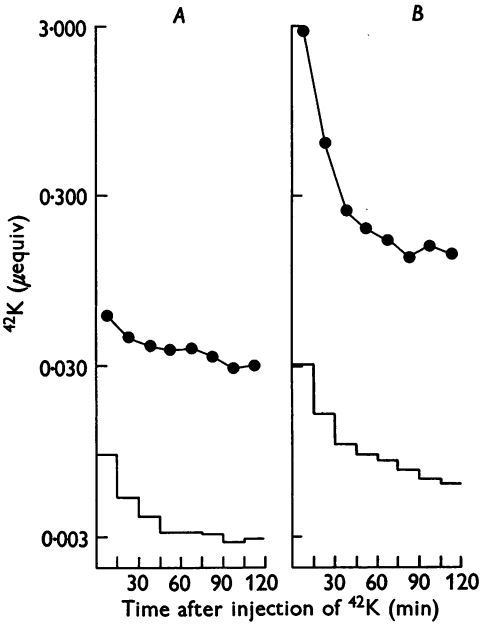

Fig. 1. Concentration of ^{42}K in blood (upper record) and in perfusing artificial c.s.f. (lower record) after an intravenous injection of the small (at *A*) and the large (at *B*) quantities of ^{42}K. Semi-log scale.

artery 10 min before the ^{42}K injection, significantly altered the potassium transfer. On the other hand, the intraperitoneal injection of physostigmine (2 mg/kg) together with atropine sulphate (0·5 mg/kg) 15 min before the ^{42}K injection greatly decreased its transfer. The transfer decreased to such an extent that the ratios became similar to those obtained in the experiments in which the large quantity of ^{42}K was injected without physostigmine.

Figure 4 shows the effect produced on potassium transfer by intravenous infusion of four different organic cations in experiments in which the large quantity of ^{42}K was injected. The infusion of choline chloride (5 mg/kg/hr) greatly increased the transfer (at *A*). The transfer ratios

became even greater than those obtained in the experiments in which the small quantity of ^{42}K was injected without choline. The infusion of tolazoline hydrochloride (9·25 mg/kg/hr) and of tetraethylammonium bromide (5 mg/kg/hr) also significantly increased the transfer ratios (at *B* and *C*),

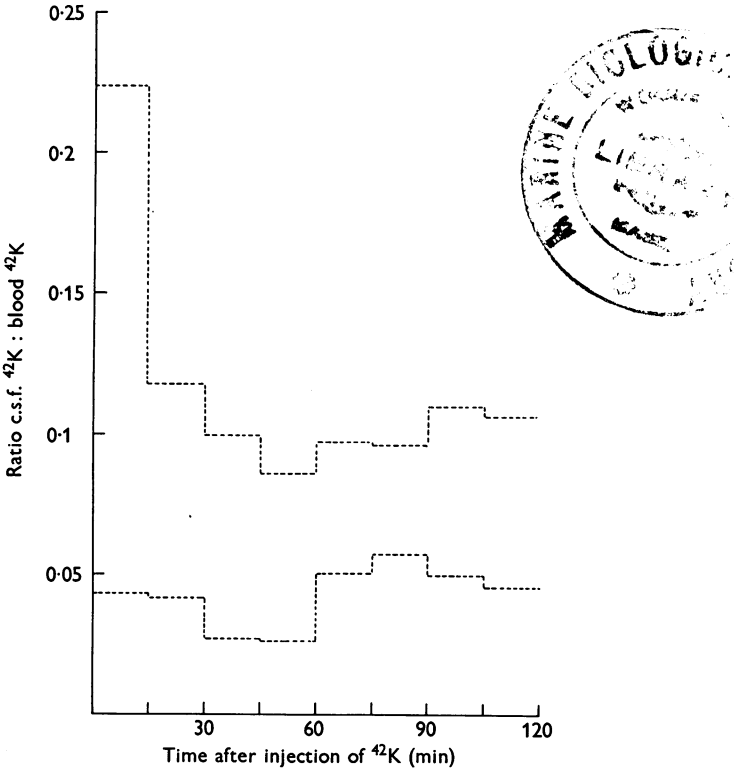

Fig. 2. Ratios of ^{42}K in the artificial c.s.f. to blood ^{42}K after an intravenous injection of the small (upper record) and the large (lower record) quantities of ^{42}K.

but the facilitation of the transfer was small compared to that produced by choline. When *N'*-methylnicotinamide (15 mg/kg/hr) was infused in one experiment, the transfer ratios did not differ significantly from the control ratios (at *D*).

DISCUSSION

Rudolph & Olsen (1956) showed that in dogs the turnover time of ^{42}K from blood to c.s.f. is much shorter than that of ^{24}Na; for ^{42}K the turnover time was 9 min, and for ^{24}Na it was 143 min. From the experiments of Selverstone (1958) we know that the equilibration in the c.s.f. of potassium as well as other ions, takes place most rapidly in the ventricular cavities,

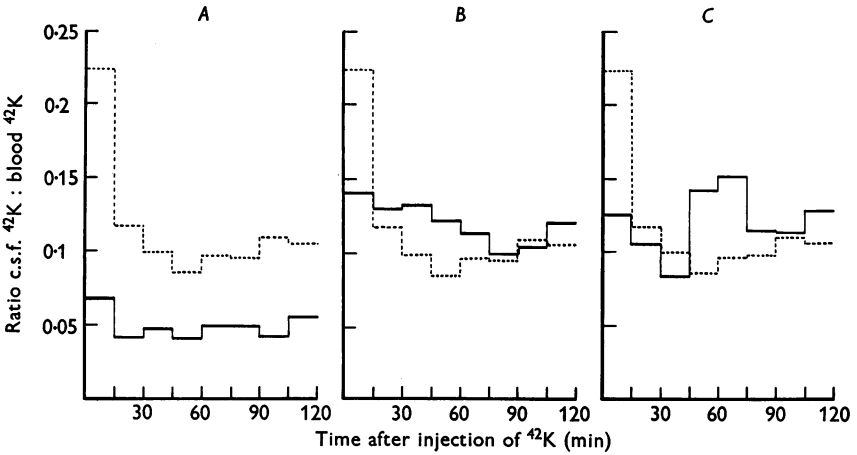

Fig. 3. Ratios of ^{42}K in the artificial c.s.f. to blood ^{42}K after an intravenous injection of the small quantity of ^{42}K. The dotted lines are taken from Fig. 2. The continuous lines are the ratios influenced by intraperitoneal injection of physostigmine (2 mg/kg) plus atropine sulphate (0·5 mg/kg) at *A*; by injection of 0·4 ml. air into each carotid artery at *B*; and by intravenous infusion of dimethylaminoethanol (25 mg/kg/hr) at *C*. For further details see text.

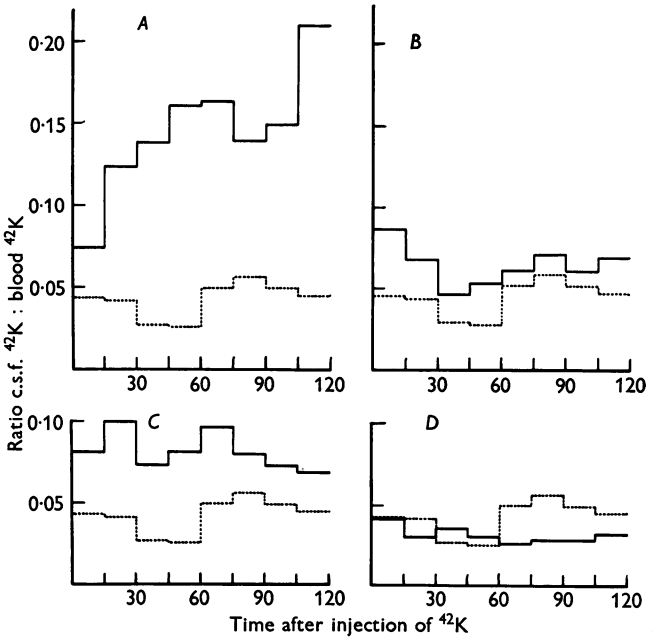

Fig. 4. Ratios of ^{42}K in the artificial c.s.f. to blood ^{42}K after an intravenous injection of the large quantity of ^{42}K. The dotted lines are taken from Fig. 2. The continuous lines are the ratios influenced by intravenous infusion of choline chloride (5 mg/kg/hr) at *A*; of tolazoline hydrochloride (9·25 mg/kg/hr) at *B*; of tetraethylammonium bromide (5 mg/kg/hr) at *C*; and of *N'*-methylnicotinamide (15 mg/kg/hr) at *D*. For details see text.

especially near the chorid plexuses. The method of perfusing the ventricles to the aqueduct thus allows a study of the transfer of substances in these regions of optimal transfer. That this transfer of ^{42}K takes place rapidly is reflected in the high c.s.f. level found in the first 15 min period following the intravenous injection of ^{42}K, and in the decrease of the concentration in the perfused c.s.f. solution during the following 15 min periods, parallel with the decreased ^{42}K level in the blood.

The injection of a large quantity of potassium decreased the transfer ratio from blood to c.s.f. A similar finding was observed with the transfer of compounds in the tubules of the kidneys (Peters, 1960). This so-called self-depression of transport in the kidney is taken as evidence that the system involved is an 'active' one requiring an energy source. The same conclusion may apply to the transfer of ^{42}K into the c.s.f., a view also held by Quadbeck (1959), who found a decrease in the rate of ^{42}K transfer from blood to c.s.f. during hypoxaemia, and in a condition of physical exhaustion. In these conditions the passage of bromide and phosphate ions was unaffected and their movement was thought to occur by passive diffusion.

Although the transport by the renal tubules and the transfer from blood to brain or c.s.f. may have many common characteristics, the systems involved in potassium transfer must be different, since tetraethylammonium and tolazoline facilitated transfer into the c.s.f., whereas these organic cations are known to compete with potassium for renal tubular transport (Kandel & Domer, 1957; Domer, 1960). The system involved in the blood–c.s.f. barrier resembles that concerned with the transfer of sodium by the frog skin, in that this transport is facilitated by a number of organic cations (Skou & Zerahn, 1959). Finally, the facilitation of ^{42}K transport from blood to c.s.f. is scarcely a non-specific property of organic cations, since dimethylaminoethanol and *N'*methylnicotinamide were both found to lack this action.

Physostigmine and other inhibitors of cholinesterase are known to block the active transport of sodium in various tissues (Kirschner, 1953; Holland & Creig, 1950; Koch, 1954; van der Kloot, 1956). The finding that physostigmine greatly decreased the transfer of ^{42}K into the c.s.f. may be another aspect of this action. This conclusion is in accord with the view that energy is required in this transfer. Various authors have investigated the effect of physostigmine on the uptake of substances into the brain. The uptake of glucose, a non-ionized substance, was decreased (Greig & Gibbons, 1959), whereas that of barbiturates, acid fuchsin and sulphanilamide, substances which when ionized form anions, was facilitated (Greig & Holland, 1949; Greig & Mayberry, 1951; Greig & Carter, 1954; Beiler *et al.* 1956; Paulet, Marsol & Coq, 1957). The difference is understandable, since the rate of transfer of substances across the blood–brain

barrier is dependent on the electrical charge of the molecule, the barrier generally being more permeable to positively charged compounds (Friedmann, 1942). It is not certain whether inhibition of cholinesterase, thought to be also the cause of the blockage of the active sodium transport, is the sole explanation for the effect of physostigmine in decreasing the transfer of ^{42}K into the c.s.f. and for the various effects on the uptake of substances into the brain. Paulet *et al.* (1957), for instance, found no parallelism between enzyme inhibiting potency of several anticholinesterases and their action on the uptake of sulphanilamide by rat's brain.

It is unlikely that the depression by physostigmine of the transfer of ^{42}K into the c.s.f. is due to accumulation of undestroyed acetylcholine caused by cholinesterase inhibition since choline infusion had the opposite effect. In this connexion it is interesting that pilocarpine, which shares many of the actions of choline and acetylcholine on smooth muscles and gland cells, apparently does not affect the permeability of the blood–brain barrier (Stern, Slatoweirow & Belkina, 1927).

In the present experiments injection of air into the carotid arteries did not increase the passage of ^{42}K into the c.s.f. This result is different from that of Lee & Olszewski (1959), who found that air embolism caused a great transient increase in cerebral vascular permeability to albumin, but only in those vessels reached by the air embolus. It is possible, therefore, that the failure to observe an increase in the transfer of ^{42}K was due to the fact that the air embolus did not reach the vessels involved.

SUMMARY

1. The transfer of intravenously injected ^{42}K into the perfused cerebral ventricles has been studied in anaesthetized cats.

2. When the same amount of radioactivity was injected in either a large or a small amount of potassium, the transfer of the ^{42}K from the blood into the c.s.f. was decreased by the large amount.

3. An intraperitoneal injection of physostigmine greatly decreased the transfer rate of the intravenously injected ^{42}K.

4. Intravenous infusion of choline, tetraethylammonium or tolazoline significantly increased the ^{42}K transfer, whereas intravenous infusion of dimethylaminoethanol or *N'*-methylnicotinamide did not alter it.

5. Injection of air into the carotid arteries did not alter the ^{42}K transfer.

I wish to thank Sir Charles Harington for hospitality, Professor W. Feldberg for his interest, and Miss S. Fyson for her help with statistics.

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