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THE PENETRATION OF UREA INTO THE CENTRAL NERVOUS SYSTEM AT HIGH BLOOD LEVELS

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Evidence has been presented during the past few years that intravenous administration of hypertonic solutions of urea has the effect of reducing intracranial pressure and inducing shrinkage of the brain in patients undergoing neurosurgical treatment (Javid & Settlage, 1956; Stubbs & Pennybacker, 1960). In considering mechanisms by which such effects might arise one possibility which comes to mind is that the urea may act in a similar manner to other solutes, such as sodium chloride, which have similar effects when injected in hypertonic solution (Weed & McKibben, 1919) and which are believed to bring about withdrawal of water from the cerebral tissue by virtue of the increased osmotic pressure of the blood plasma which results from their presence in it. However, an obvious difficulty about accepting such an explanation in the case of urea is the fact that this substance is generally held to distribute itself freely throughout the whole of the intracellular as well as the extracellular water of the human body (McCance & Widdowson, 1951) and, in particular, it appears not to behave as an osmotically active solute in relation to the membrane of the receptor organs responsible for controlling the output of antidiuretic hormone which are believed to lie in the hypothalamic area of the brain (Verney, 1954).

The experiments reported in this paper were undertaken in order to determine directly the extent to which intravenously injected urea would penetrate the water of central nervous structures, and hence to study its ability to promote the transfer of water from the brain cells under a simple osmotic gradient. The opportunity was also taken to collect data on changes in water and electrolyte content of brains from animals exposed to constantly elevated concentrations of urea in their blood for periods of several hours. The majority of the experiments were done on cats but isolated observations were also made on a rabbit and a dog.

METHODS

Preparation of animal. Seventeen adult cats, fourteen males and three females, weighing between 2·0 and 4·8 kg, were used for the main investigation. All animals were deprived of food for 16 hr and were initially anaesthetized with intraperitoneal sodium pentobarbitone 49 mg/kg body wt., supplemented by further small doses of the same drug given intravenously as required. Plastic tubing inserted through the left femoral vein into the inferior vena cava was used for infusions, and the right femoral artery was cannulated to provide for the withdrawal of blood samples. The urethra was then catheterized and the abdomen opened in order that the bladder might be compressed to facilitate complete emptying. For the priming dose 'Urevert' (Baxter Laboratories Ltd.), which is a sterile solution containing 30 g/100 g urea and 6·5 % invert sugar, was injected over 5–10 min into the inferior vena cava, 4·0 ml. being given/kg body wt. of cat. Further urea was administered as a 3 or 6 % solution with 5 % glucose by means of an electrically driven syringe; control animals received 10 % invert sugar followed by 5 % glucose but no urea.

Urine was collected half-hourly in short experiments and hourly in long experiments and samples were immediately analysed for urea content by the hypobromite method of Van Slyke (1929). It was thus possible to adjust the rate of infusion in the light of the urea excreted, and of previous experience, and so to establish a constant blood level. Urea levels in plasma, estimated at half-hourly or hourly intervals, rarely varied by more than 6 % from the concentration at the time of removal of the brain, except during the initial 10 min after the loading dose. The mean final concentration in plasma was 273 mg/100 ml. (S.D. $\pm$ 12). In the experiments described as 'open skull' an area of bone measuring roughly 4 cm^2 was removed from the left side of the vault of the skull, without the dura mater being opened, before the urea was injected, the skin being then sutured over the exposed dura mater.

Removal of specimens for analysis. The brain and cervical cord were removed at $\frac{3}{4}$, $1\frac{1}{2}$, 3, 6 or 9 hr after the start of the infusion. Duplicate specimens of grey matter (parietal lobe) and white matter from the cerebral hemispheres (centrum ovale), grey matter and white matter from the cerebellum, and specimens from the pons and cervical cord (each weighing approx. 200 mg) were immediately dissected out and placed in covered tared pots. These specimens were weighed and then freeze-dried to constant weight. The tips of the frontal, temporal and occipital lobes of both sides of the brain (each piece weighing approx. 1 g) were resected and placed in covered pots before oven-drying at 105° C to constant weight.

In the experiments with kittens eight animals from four litters were used. The overall range of weights was from 300 to 810 g but litter-mates differed by less than 30 g. Each animal that received urea was compared with a litter-mate that received no urea, but was otherwise treated similarly. The whole forebrains from the kittens were quickly removed, $\frac{3}{4}$ hr after the start of the infusion. The cerebrum was bisected into its two hemispheres, any cerebrospinal fluid being lightly blotted from its surface and from the ventricles, and was then immediately placed in a covered nickel crucible, before weighing. The same procedure was followed in the experiments with the dog and rabbit as in those with adult cats, except that no specimens were removed for electrolyte analyses.

Chemical procedures. Urea was extracted from the vacuum-dried specimens by pounding them with a glass rod in approximately 2 ml. of water which was added slowly. The mixture was allowed to equilibrate for 1 hr, at the end of which time it was thoroughly stirred with the rod and centrifuged. The pot and extract were weighed immediately before removing the supernatant to determine the exact weight of water added. Urea in plasma and in the tissue extracts was determined in duplicate by Conway's (1947) microdiffusion technique after treatment with urease; the ammonia was liberated with saturated potassium carbonate solution and absorbed into 2 ml. of 1 % boric acid and titrated with 0·05 N or 0·0125 N-HCl. The tissue extracts gave an appreciable blank without urease, generally equivalent to 10–15 mg/100 ml. urea in tissue water; this was estimated for each extract and the appro-

priate correction applied. The solid matter in each extract averaged 1 g/100 ml. It was thus possible to arrive at the urea in 100 ml. of tissue water from a factor relating the volume of water added in making the extract to that evaporated from the brain during drying. The distribution ratio was calculated by dividing the urea concentration in tissue water by that in plasma water.

The sodium and potassium contents of the oven-dried specimens, three from the same side of each cat, were estimated by flame photometry after digestion with 1·0 ml. of concentrated sulphuric acid and 3·0 ml. of concentrated nitric acid, all the latter being boiled off. The digests were diluted in two stages to give approximately 0·2 m-equiv/l. sodium in the first dilution, and 0·2 m-equiv/l. potassium in the second. The emissions from these solutions were recorded on an EEL flame photometer (Evans Electroselenium Ltd.) and compared with those from standards, the mean of the four readings being taken for each solution. The whole brains from the litter-mate kittens were dry-ashed in nickel crucibles for 8 hr at 450° C and extracted with 0·001 N nitric acid before flame photometry.

The chloride estimations were performed on dilute nitric acid extracts of the remaining three oven-dried specimens from each cat. A sample of an extract of the dried brain with 0·75 N nitric acid was digested with a slight excess of silver nitrate in concentrated nitric acid. The excess silver ions were determined by Volhard titration (Lowry & Hastings, 1942).

The blood content of some fragments of brain similar to those analysed for urea was determined by the method of Klein (1945). The average content found was under 2 % and this was considered to be so small as not to warrant any attempt to allow for it in the results of the other analyses quoted.

Statistical methods. The limits (±) given in Table 2 are the standard errors of the means, and in Table 3 the standard error of the difference of the two means. The probability of the difference of the two means being due to chance (P) was estimated by Student's t test.

RESULTS

Distribution ratios for urea

Table 1 gives the tissue: plasma urea ratios from different regions of the cat's nervous system, calculated at different times after the beginning of the infusion, and in Fig. 1 the mean urea ratios for each region have been plotted against time. Entry into nervous tissue is seen to be rather slow by comparison with that into muscle. Equilibrium distribution ratios (r_{eq}), judged from the figures for cats receiving no urea exogenously and from the nature of the entry curves, appear to be near or somewhat above unity for all parts of the nervous system studied, except for pons and cervical cord, where these ratios are 0·96 and 0·89, respectively. The ratios for cerebrospinal fluid and aqueous humour are notably lower, 0·62 and 0·75. In the one rabbit studied the ratios at 6 hr were somewhat below those for the cat at the same time. In the dog, on the other hand, the ratios had reached unity for most parts of the brain by 6 hr.

Table 2 gives the mean values for water and electrolyte concentrations in brain from adult cats, 'closed' and 'open-skull' groups, removed between ¾ and 3 hr after starting the infusion of urea. Values for the brains from the control cats are given, and values for electrolyte concentrations in plasma from all groups are also included for comparison.

The mean water content of oven-dried brain from treated cats was lower than that from the control animals, but this difference was not significant ($P > 0{\cdot}05$). The water content of brains from treated cats with open skulls was lower still, but again the difference from the control animals was

TABLE 1. The concentration of urea in the tissue water expressed as a fraction of that in plasma-water (the distribution ratio) at various intervals after raising the plasma concentration

Sex cats	Body wt. (kg)	Duration of infusion (hr)	Grey cortex	Grey cerebellum	White centrum ovale	White cerebellum	Pons	Cervical cord	Muscle	c.s.f.	aq.h.
F	2·8	¾	0·53	0·42	0·40	0·38	0·36	0·31	0·87	0·37	0·48
*M	3·2	¾	0·47	0·45	0·41	0·44	0·42	0·40	0·86	—	0·51
M	4·8	1½	0·72	0·65	0·50	0·52	0·51	0·34	1·03	0·42	0·59
M	2·5	1½	0·66	0·60	0·51	0·54	0·53	0·44	0·96	0·44	0·60
*F	1·9	1½	0·52	0·52	0·41	0·44	0·49	0·46	0·97	—	0·65
M	2·3	3	0·62	—	0·56	—	0·57	0·56	—	—	0·64
M	4·2	3	0·69	0·68	0·56	0·53	0·56	0·43	0·98	0·46	0·60
*M	3·7	3	0·67	0·61	0·55	0·53	0·54	0·47	0·98	0·45	—
*M	2·5	3	0·61	0·55	0·50	0·47	0·51	0·46	1·00	0·43	0·57
M	2·6	6	0·85	0·80	0·72	0·72	—	0·63	—	—	—
M	3·0	6	0·82	0·72	0·72	0·67	0·68	0·62	—	—	—
M	4·0	6	0·75	0·78	0·60	0·83	0·71	0·66	—	—	0·69
M	4·0	9	0·91	0·86	0·83	0·78	0·77	0·68	1·01	0·61	0·78
M	3·3	0	1·08	1·12	1·09	1·02	0·91	0·80	1·08	—	0·76
M	3·7	0	1·10	1·13	1·19	1·13	0·99	0·99	1·22	0·61	0·76
M	2·6	0	1·15	1·04	1·06	1·05	0·94	0·86	1·16	0·63	0·68
F	2·2	0	1·14	1·05	0·86	1·03	—	0·88	1·11	—	0·76
Dog											
M	8·6	6	1·01	0·96	0·98	0·94	0·93	0·89	0·94	0·80	0·69
Rabbit											
M	2·3	6	0·63	0·66	0·63	—	0·60	0·57	0·94	0·56	0·74

aq.h. = aqueous humour; c.s.f. = cerebrospinal fluid; * = open-skull experiments.

TABLE 2. The concentration of water and of some electrolytes in the brains of adult cats following intravenous infusion of urea. Some plasma values are also given for comparison

Treatment	No. of animals	Mean water content of brain (%)	Mean sodium concentration (m-equiv/l.)		Mean potassium concentration (m-equiv/l.)		Mean chloride concentration (m-equiv/l.)	
			In plasma water	In brain water	In plasma water	In brain water	In plasma water	In brain water
No urea	4	77·68±0·57	158·8±0·8	62·6±0·3	4·0±0·2	119·6±0·8	120·0±2·5	47·2±1·2
Intravenous urea, closed skull	5	77·22±0·46	159·5±1·3	69·1±0·5	3·8±0·1	126·4±2·1	123·3±2·2	51·1±0·9
P_1		> 0·05	> 0·05	0·001	> 0·05	0·05 > P_1 > 0·02	> 0·05	0·05 > P_1 > 0·02
Intravenous urea, open skull	4	76·68±0·75	158·3±1·4	70·3±0·8	3·7±0·4	124·1±0·3	123·8±2·6	53·1±0·8
P_2		> 0·05	> 0·05	0·001	> 0·05	0·01 > P_2 > 0·001	> 0·05	0·01 > P_2 > 0·001

Brains removed at ¾, 1½ or 3 hr after starting the infusion of urea.

P_1 and P_2 are the probabilities of the difference between the 'no-urea' group and the 'closed-skull' and 'open-skull' groups respectively, arising by chance.

not statistically significant. The vacuum-dried specimens gave similar results.

In view of the inconstancy in the water content of adult cats, it was decided to carry out some studies on litter-mate young animals in an attempt to reduce this source of variation; pairs of matched kittens were accordingly treated in the following way. For each kitten that received urea, a litter-mate which had received only 10% invert sugar or else no intravenous fluid was also studied and the brains of the two animals were then analysed. The results of experiments with four such pairs of animals are shown in Table 3. In each case the water content of the whole forebrain

TABLE 3. Changes in the water content of the brains of kittens and in the concentration of sodium and potassium in the plasma and brains of the same animals following intravenous urea

Treatment	No. of animals	Mean water content of brain (%)	Mean sodium concentration (m-equiv/l.)		Mean potassium concentration (m-equiv/l.)	
			In plasma water	In brain water	In plasma water	In brain water
No urea	4	82·68	160·3	63·7	4·2	109·2
Intravenous urea	4	81·80	158·8	68·7	4·3	116·1
Mean difference		−0·88	−1·5	+5·0	+0·1	±6·9
S.E. of difference		±0·2	±2·4	±1·0	±0·7	±2·6
P		0·05 > *P* > 0·02	>0·05	0·02 > *P* > 0·01	>0·05	>0·05

All brains removed ¾ hr after onset of infusion. The 'no-urea' group includes two animals which received intravenous hexose solution and two which received no intravenous fluid, but there was no systematic difference between these.

was lower in the animal that had received urea than in its fellow which had not. The mean difference in water content (expressed as a percentage of wet weight) is 0·88. This difference is significant at the 0·05–0·02 probability level. It should be noted, however, that this small difference in percentage of wet weight represents a change of 5·0 g/100 g of water, assuming the solid matter to remain constant, and the resulting mean change in brain bulk would be some 6%.

The sodium, potassium and chloride concentrations/kg tissue water, as shown in Table 2, were significantly higher in the two groups of adult animals that had received urea than in the control animals, but there were no differences between the 'closed' and 'open-skull' groups. Similar changes were noted in the litter-mate kittens, but the difference in potassium concentration in this instance did not reach the 0·05 probability level of significance. No substantial divergences in plasma electrolyte concentrations were noted between any of the groups of animals.

DISCUSSION

The results recorded in the preceding section are in general agreement with those of Conway & Fitzgerald (1942) in so far as they reveal an unequal distribution of exogenous urea between brain water and plasma water and with those of Schoolar, Barlow & Roth (1960), who demonstrated differing rates of entry of [^{14}C] urea from the blood stream into different regions of the central nervous system (C.N.S.).

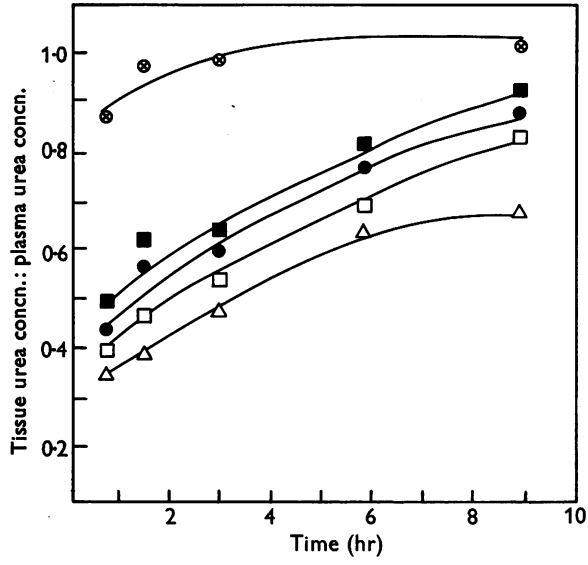

Fig. 1. Graph of ratio of concentration of urea in brain water to that in plasma water at various intervals of time after starting intravenous infusion. ■, grey matter of cerebral cortex; □, white centrum ovale; ●, grey matter of cerebellum; △, cervical cord; ⊗, skeletal muscle.

In the present experiments, as is clear from Fig. 1, each of the areas of the C.N.S. which were sampled appeared to exhibit a distinct pattern of behaviour as regards the concentration of urea in its tissue water relative to that in plasma water at various time intervals following the artificial elevation of the latter. These apparently systematic differences between the several regions have prompted us to enquire whether the course of events in each region might be summarized in some over-all quantitative index, which in turn might assist in the elucidation of the origin of these inter-regional differences. A possible index of this kind would be a transfer constant derivable by the kinetic treatment of permeation employed by Davson (1956) in connexion with his experiments on the aqueous humour of the eye. This treatment is developed from a consideration of the rate of

penetration of a solute into a single cell immersed in a relatively large volume of liquid containing that solute at constant concentration. For such a system an equation of the following form has been shown to apply.

$$\ln\left(1-\frac{C_t}{r_{eq}\times C_{out}}\right) = Kt, \tag{1}$$

where C_t is the concentration in the cell at time t, r_{eq} is the ratio of internal to external concentration at equilibrium (i.e. at infinite time) and K is a rate constant, called the 'transfer constant'.

In our experiments, if we consider the nervous system as equivalent to the inside compartment and the blood to the outside compartment of a two-chambered system, an equation of the same type as equation 1 could be applied to measurements of the kind which we have made. However, the equation requires some modification, in that, although the external (plasma) concentration was kept constant during any one of our experiments, it was not necessarily the same from animal to animal. Nevertheless, for any given observed figure for the concentration of urea in brain water the corresponding plasma concentration is known. It therefore seems permissible, as a first approximation, to substitute r_{eq} for $r_{eq}\times C_{out}$ and r_t for C_t respectively in equation (1), where r_t is the ratio of internal (brain or spinal cord) to external (plasma) concentration at time t. Algebraically this corresponds to dividing the terms in equation (1) by the appropriate C_{out}, and leads to the following relationship:

$$\log\left(1-\frac{r_t}{r_{eq}}\right) = K't, \tag{2}$$

in which K' is a new rate constant with similar dimensions to K in equation 1 but with a different numerical value.

Graphs of the function $\log(1-r_t/r_{eq})$ against time, based on the mean values of r_t and r_{eq} for cortical grey matter and cervical cord, are shown in Fig. 2, the ratios found in the absence of infused urea being used as estimates of r_{eq}. These regions were chosen as representing the extremes of the range of behaviour of the distribution ratios, and it will be seen that in both cases the points lie reasonably close to straight lines. Moreover, these lines are not widely separated over the time interval covered by the measurements. Similar graphs for the other regions studied revealed a similar degree of linearity, and this was taken as justification for estimating the K' values for each region by the conventional formula for linear regressions. Such calculations disclosed no significant differences between the transfer constants of the regions for which distribution ratios appear in Table 1.

Although this result is not altogether surprising in view of the resem-

blance between the contours of the four curves plotted in Fig. 1, it is at first sight not readily reconciled with the fact that the actual mean ratios show at each time interval a regular downward gradation from grey cortex to cervical cord, and that, with two minor exceptions, a similar trend through the regions holds for the individual animals in the injected group, as may be seen from Table 1. The lack of a statistically significant difference between the calculated rate constants does not of course exclude the possibility of there being a real difference which is too slight to be demonstrable from our data; but, leaving aside this particular possibility,

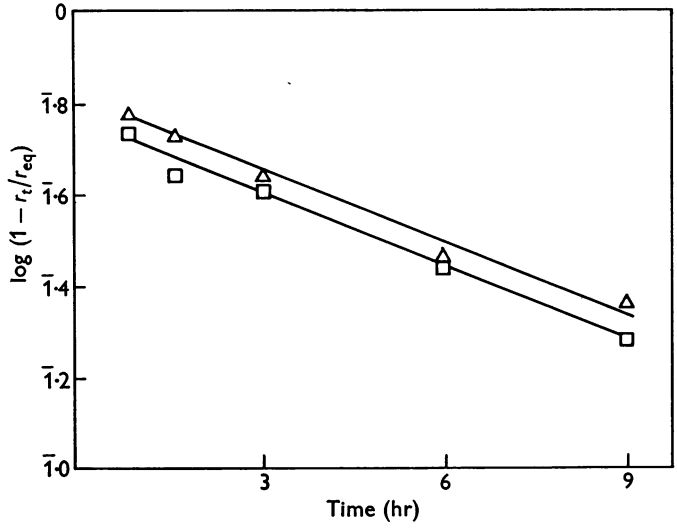

Fig. 2. Graph of function log $(1-r_t/r_{eq})$ against time. □, grey matter of cerebral cortex; △, cervical cord; r_t = (tissue urea concn.)/(plasma urea concn.) at time t; r_{eq} = (tissue urea concn.)/(plasma urea concn.) at equilibrium.

it must be concluded that the observed differences between the behaviour of the regions displayed in Fig. 1 are dependent upon some process other than that measured by K'. Now it is important to note in this connexion that the derivation of K' is based on the assumption of a single membrane dividing plasma from brain, through which permeation of urea can take place in both directions. This clearly is a greatly over-simplified conception of the blood–brain barrier, and it may well be that the earliest of our observed ratios (i.e. the 45 min ratio) in the cats injected with urea may arise from the existence of multiple pathways for solute exchange between blood and the tissue water. If the ratios were different for the various regions at 45 min but thereafter increased at the same rate in all regions, the results which we have obtained would be expected. Thus distribution ratios at 45 min may well depend upon movement of urea into pools of tissue water

other than the main intracellular space of the mass of neural tissue under consideration. Such subsidiary pools might be placed in parallel with the vascular bed and the bulk of the intracellular fluid, or they could simply represent areas of the tissue with a poor blood supply. The latter type of non-homogeneity has been postulated by Renkin (1959) to account for his findings with regard to the penetration of radioactive potassium into muscle. Moreover, in practice, multiple rather than single exponential terms are commonplace in the equations describing the time course of mixing of tracer substances introduced into the blood stream with pre-existing body pools (Robertson, 1956); and in the case of labelled carbon dioxide there is some evidence that the rate of exchange of molecules between tissue and blood can be correlated with the ratio of the size of the tissue pool to the regional blood flow (Coxon & Robinson, 1959). It is important to recall in this connexion that Conway & Fitzgerald (1942) inferred from a consideration of data obtained under different experimental conditions from ours the existence of four compartments in the water of the C.N.S. of cats and rabbits each exhibiting differing degrees of accessibility to urea.

While the unequal distribution ratios for urea in the injected cats of our series might be explicable as arising from differences in the rate of mixing of subsidiary tissue pools during the early stages of the experiments, such an explanation could not account for the differences (if they are real) between the distribution ratios for cortical grey matter and spinal cord in the untreated cats. Now Davson (1956) in his derivation of the transfer constants (of which K' in this paper is an example) emphasizes that the greatest caution must be exercised in using these in relation to systems when the geometry is not as simple as in the case of the single cell exposed to a constant environment, and clearly our system is very much more complicated than this. There is, in fact, no certainty that urea enters and leaves cerebral tissue by the same route, while vascular anatomy and glial populations undoubtedly vary in different areas in the C.N.S. It is, therefore, perhaps more reasonable to lay stress upon the finding that many of our results are consistent with the occurrence of one process with a single time constant in several areas of the C.N.S., rather than upon the fact that some of our results appear to depend on other processes.

The results which we obtained in the single rabbit studied show a general similarity to those from our cats, but differ from those found in the dog. The brain of the latter animal seemed much more permeable to urea than those of the cat and rabbit, a finding which is in conformity with the more extensive data of Riser, Valdiguié & Giraud (1938) and seems to indicate a marked species difference.

The relatively slow rate of entry of urea into the nervous system in the

living cats and rabbits contrasts with the rapid passage into isolated tissue slices incubated with urea *in vitro*. This was demonstrated for cat and rabbit by Conway & Fitzgerald (1942). It has also been shown (Coxon, 1957) that virtually complete penetration of brain slices from guinea-pigs has taken place after 2 hr incubation in a urea-containing medium, but the permeability of the brain in these animals *in vivo* has not been exhaustively studied. Davson & Spaziani (1959) have demonstrated inconsistencies between the rates of entry of substances other than urea into the brain water in the whole animal on the one hand and isolated fragments of tissue on the other. In the case of water itself it appears that while isotopically labelled molecules attain a uniform distribution in all parts of the brain which corresponds with that in plasma, equilibration takes place more rapidly in grey than in white matter (Bering, 1952).

Before passing on to a consideration of the osmotic consequences of the delayed entry of urea into brain water there is a further point regarding the equilibrium ratios which calls for some comment. This is the fact that in the case of three of the regions studied all our observed ratios exceed unity and in one instance (white centrum ovale) three of the four do likewise. A comparable distribution has been reported by Ralls (1943) to obtain between red cells and plasma (where it is possibly attributable to binding of urea to intracellular protein) and between isolated frog muscle and medium in which this is incubated by Bozler (1961). Davson & Kleeman (1960) are also on record as having noted a ratio of distribution for urea between brain and plasma in favour of the former, but no details are available at the time of writing. In the brain of normal cats it may be that the slight excess concentration of endogenous urea in brain water as compared with plasma water may be a result of synthesis *in situ* for which evidence, based on tracer experiments, has been advanced by Sporn, Dingman, Defalco & Davies (1959). The possibility of urea synthesis in the brain, if it occurs on any appreciable scale, must introduce a further complication into the kinetic analysis of urea permeation from blood to brain and would need to be taken into account in any scheme which aimed at anything like a complete description of the process. As has been mentioned earlier, it is recognized that the mathematical treatment used in this paper is greatly over-simplified and the possibility of urea synthesis is just one additional factor distinguishing the real situation from the idealized model. Binding of urea to protein, if this occurs, might provide a possible basis for toxic effects (cf. Grollman & Grollman, 1959).

Regardless of the detailed mechanisms underlying it, the persistence over some hours of a substantial concentration gradient for urea between plasma and brain water could certainly bring about the transfer of water from the brain to the plasma by osmotic means. The changes in water

content which were observed in the kittens which we studied were sufficient to account for a change of some 5 % in the bulk of the brain, if we make the reasonable assumption that the total solids of the brain remained unchanged in quantity during the 45 min when urea was being infused. We examined the effect of this on the appearance of the brain through a craniotomy opening in the skull by inserting a balloon into a human skull the interior of which had been converted to an approximately spherical shape by the insertion of Plasticine moulds. It was then found that a change of 5 % in the volume of the balloon produced a change in the visible distension of the portion of it visible through the craniotomy opening which corresponded well with that seen at operation in brains shrunk by the infusion of urea. The fact that most of the body water is readily and rapidly penetrated by urea (McCance & Widdowson, 1951; Bradbury, 1961), although the water of the C.N.S. is entered only relatively slowly, should confer upon urea some degree of selectivity as compared with other agents used to promote osmotic shrinkage of tissues, since many such agents when introduced into the circulation would withdraw water indiscriminately from most tissues and so dissipate their effects.

The measurements on cerebrospinal fluid and aqueous humour which are listed in Table 1 do no more than amplify previous evidence of the slow penetration of urea into these fluids (Davson, 1956). The pressure of the cerebrospinal fluid is known to fall following the infusion of urea, and measurement of volume changes in the several subdivisions of the intracranial contents by methods such as those of White, Verlot, Selverstone & Beecher (1942) or of Rosomoff (1961) might be expected to reveal alterations in the intracranial blood content and in the total content of cerebrospinal fluid in addition to the change in brain bulk which we have inferred. It should be noted, too, that visible shrinkage of the convexity of the brain may be in part a consequence of reduction in ventricular volume.

The measurements of brain electrolytes recorded in Tables 2 and 3 reveal a rise in the concentration of all three ions which were studied as an accompaniment of the infusion of urea. As regards the absolute figures, our estimate of the chloride space, which amounts to 300 ml./kg brain, shows very satisfactory agreement with the figure of 31·4 % quoted by Davson (1956). As regards the variations in electrolytes seen during infusion, the data for the kittens (Table 3) are preferable as a basis for interpretation, since in them the changes in water content were more definite than in the adult cats. Calculations based on these changes reveal that the increase in concentration of both sodium and potassium can be explained as consequent upon the withdrawal of water from the brain. These calculations rest on the assumption that the total solids of the organ remained unaltered in total quantity throughout the infusion.

SUMMARY

1. The distribution of urea between plasma water and brain water was studied at intervals up to 9 hr following the artificial elevation of the plasma concentration to levels about ten times normal.

2. The rapidity with which the concentration of urea in brain water approached that in plasma varied in different regions of the brain and exceeded that found in the spinal cord.

3. The water content of the brains of kittens was found to be reduced following sustained elevation of the plasma urea for 45 min and the changes in water content were sufficient to account for perceptible shrinkage of the brain.

4. Changes in the concentrations of sodium, potassium and chloride in brain water after prolonged elevation of the blood urea were observed and could be interpreted as secondary to the movement of water.

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Note added in proof

A paper published in 1927 by F. Fremont-Smith and H. S. Forbes (*Arch. Neurol. Psychiat. Chicago*, **18**, 550) has recently come to our notice; in it a fall in intracranial pressure is reported to follow the intraperitoneal administration to cats of urea in hypertonic solution.