Effects of Intraventricular Osmotic Pressure on the Formation Rate of Cerebrospinal Fluid and Subsequent Distribution of Electrolytes Between the Fluid and Serum in Rabbits

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INTRODUCTION

It is well known that experimentally elevated osmotic pressure in the cerebrospinal fluid (CSF) system causes significant increase in the rate of formation of CSF.[17)27)] With respect to osmosis, two major considerations arise: 1) Water transport across an osmotic gradient between CSF and serum and 2) the concept of water-solute coupling.[12)]

Since there is a paucity of experimental data about both the rate of formation of CSF as well as the nature of electrolytes under altered osmotic conditions, the present study was designed to investigate such relationships.

METHODS

Adult albino rabbits of both sexes, weighing 2.5–3.2 Kg were used throughout the experiment. Anesthesia induced by intravenous sodium pentobarbiturate, at 15 mg/Kg, was maintained by additional doses as necessary. Tracheostomy was performed and an endotracheal tube, 0.5 cm in diameter was inserted and connected to a respirator. The rabbit was placed in a stereotaxic instrument and the head was tightly fixed.

Blood pressure was recorded by means of an electric manometer (Nihon Kohden Co.) via a catheter in the femoral artery. In every animal, blood pressure was stable within the range of 100–110 mm Hg. Arterial blood samples withdrawn from the indwelling femoral arterial catheter were submitted for analysis of blood gases. Blood gas values were maintained within almost normal range.

Surgical Technique: Through a midline scalp incision, right parietal craniostomy was performed with a twist drill 2 mm in diameter, 1 cm caudal to the coronal suture and 0.8 cm lateral to the midline. The nuchal musculature was then incised transversely and the deep ligaments were preserved for obtaining the effluent from the cisterna magna.

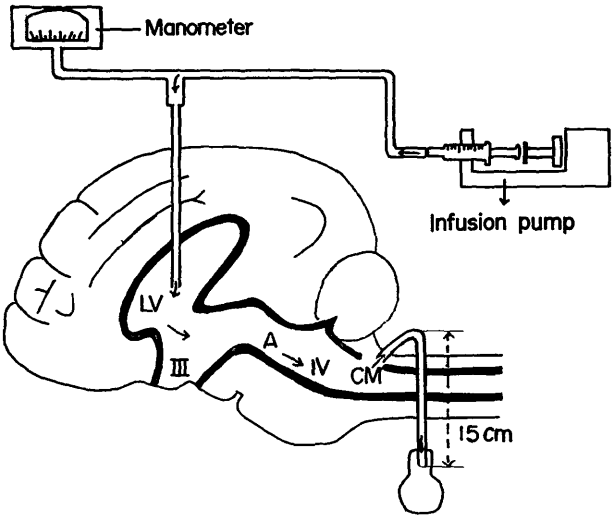

Fig. 1. Diagramatic representation of ventriculo-cisternal perfusion system.
LV; lateral ventricle, III; third ventricle, A; aqueduct
IV; fourth ventricle, CM; cisterna magna

Ventriculo-cisternal perfusion: The inflow device consisted of a 21-gauge blunt-tipped needle with attached fine polyethylene tubing, 0.8 mm in diameter, from the 20-ml syringe, wich was placed in the infusion pump (Nakagawa Seikodo Inc.). The outflow cannula, an 18-gauge needle, was connected to the polyethylene tube and measured approximately 2 mm in diameter.

The inflow cannula was driven into the lateral ventricle as far as 0.8 cm deep from the bone surface with the aid of a micromanipulator. The proper placement of the cannula was assured by the rapid drop of manometric pressure upon entry into the ventricle. While the cannula was advanced through the brain parenchyma, the manometer indicated progressive increases in pressure up to a level of 15–20 cm H_2O. As the cannula tip entered the cavity of the lateral ventricle, an abrupt drop to 4–5 cm H_2O was observed.

After insertion of the inflow cannula, the twist drill hole of the skull was sealed with surgical adhesive. Confirmation of proper placement of the cannula was done by trans-cannular perfusion with dye (Trypan blue) immediately after termination of the experiment. Then the rabbit was sacrificed and the brain was removed. Failure to reach the ventricle was indicated by the absence of ependymal staining.

The standard artificial CSF for perfusion was made of 154 mM NaCl, 2.8 mM KCl, 1.1 mM $CaCl_2$, 0.8 mM $MgSO_4$ and inulin (80mg/dl) according to the method of Bradbury and Davson.[7] The rate of perfusion was 46 μl/min.

The hydrostatic pressure was monitored by a manometer inserted within the perfusion system throughout the experiment. The end of the polyethylene tube from the outflow cannula was maintained at a level 15 cm below the external

auditory meatus. The hydorstatic pressure thus measured was in the range of O ~ minus 5 cm H_2O.

Collection of samples from the outlflow tube was done each 15-minute interval. The quantity of each sample was measured gravimetrically. If blood appeared in the effluent at any time, the experiment was terminated. The entire perfusion system is diagramatically illustrated in Fig. 1.

Adjustment of Osmotic Pressure of Perfusion Fluid: Three different types of the experimental fluid for perfusion were prepared. (1) Control Group: Standard artificial CSF as mentioned avove was used. Osmotic pressure at each experiment averaged 298 ± 9.2 mOsm/L. The chemical composition of the standard artificial CSF, cisternal CSF and plasma of rabbit is indicated in Table 1. (2) Xylose-Glucose Group: In this group, osmotic pressure of the perfusion fluid was reached from 315 to 345 mOsm/L by adding various amounts of xylose and glucose. The details appear in Table 2. Care was taken to prevent the total concentration of glucose from exceeding 300 mg/dl, because beyond such a concentration, the color of the fluid itself interferes with spectrophotometric measurement.[28] (3) NaCl Group: Adjustment by addition of 141 mg/dl of NaCl yielded the osmotic pressure of 335 mOsm/L.

Table 1. Concentrations of electrolytes (mEq/Kg H_2O) and osmolality (mOsm/L) in plasma and cerebrospinal fluid of the rabbit, and artificial CSF

Substance	Plasma*	CSF*	Artificial CSF
Na	148	149	153
K	4.3	2.9	3.0
Ca	5.6	2.47	2.0
Cl	106	130	156
Osmolality	298.5	305.2	298.2

* From Davson

Table 2. Constituents of perfusion fluid used in each experimental group

Experimental Group	Number of Experiment	Quantities of Added Osmotic Agents	Osmolality (mOsm/L)
Control	12	none	298±9.2
Xylose-glucose group	6	5 mM-glucose 10 mM-Xylose	315
	8	5 mM-glucose 40 mM-xylose	345
NaCl group	8	24 mM NaCl	335

Chemical Analysis: Estimation of osmotic pressure was performed by freezing point determination using an osmometer (Model LS, Advanced Co.). Inulin concentration was determined by the resorcinol method of Hubbard and Loomis.[19] A spectrophotometer (Hitachi-Parkin Elmer, type 139) was used for the analysis at the wave length of 450 mμ. Concentration of Na^+ and K^+ was determined by flame photometer (Model k43, IL Inc.). For the estimation of CL^-, an auto-analyser was used, employing the N-21, b/II method.

RESULTS

Intraventricular pressure was estimated as low as -2.5 to $+1.9$ mm H_2O in seven normal rabbits. **Hydrostatic pressure** during ventriculo-cisternal perfusion was measured in the range of 0 to -5 cm H_2O with the orifice of the collecting tube 15 cm below the auditory meatus in the control group. In the xylose-glucose group and the NaCl group, a tendency toward 0 to -10 cm was seen in most cases at the same height of the outlet orifice and such a tendency was more marked in the NaCl group. Those data indicating hydorstatic pressure below -10 cm H_2O were discarded because of evidence that the formation rate of CSF is affected by hydrostatic pressure outside the ragne of -10 cm to $+28$ cm H_2O, as reported by Pappenheimer, et al.[25]

Calculation of CSF formation was performed according to the method of Heisey, et al,[17] namely that inulin, a molecule of high molecular weight, is barely diffusible across the ependymal linings, and is lost from CSF only by bulk absorption distal to the fourth ventricle. Therefore, the clearance of inulin represents virtually the rate of absorption of inulin. On the other hand, in the steady state perfusion system, the rate of bulk formation of CSF is expressed as outflow-infolw difference plus bulk absorption. Thus,

$$(1)\quad Vf = Cin + Vo - Vi$$

$$(2)\quad Cin = \frac{Vici - Voco}{co}$$

where Vf is the formation rate of CSF; Cin, inulin clearance; Vo, rate of effluent flow; Vi, rate of perfusion; and ci and co represent the concentration of inulin in influent and effluent fluid.

The steady state condition was reached an hour after the beginning of the perfusion. This condition was realized by the constant value of inulin dilution and the constancy of effluent flow rate (Fig. 2). Since before steady state was achieved, these figures were extraordinarily high and variable, the first three successive samples were discarded. The period requiring emptying of the already accumulated fluid was somewhat longer than we had expected.

The mean values, with standard errors, for inflow rate (Vi), outflow rate (Vo),

outflow-inflow difference (Vo-Vi), and inulin clearance (Cin) are given in table 3.

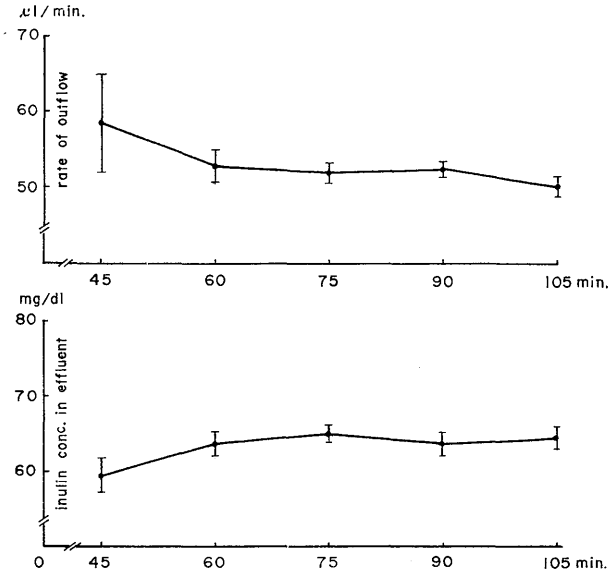

Fig. 2. Rate of outflow and inulin concentration of effluent in each time interval of the control experiment

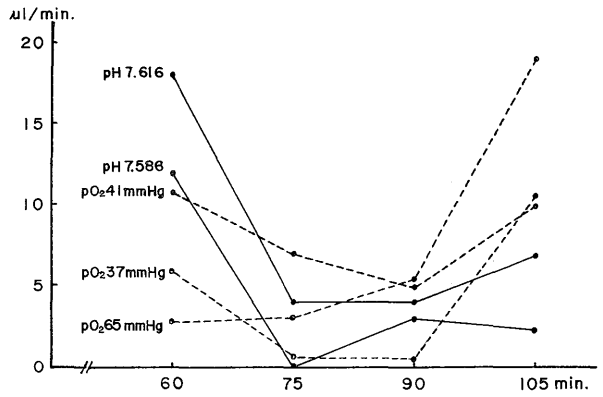

Fig. 3. Rate of CSF formation in rabbits with poorly regulated respiration.
—— Alkalotic animals ····· Hypoxemic animals

Correlation of the formation rate with blood gases: Two cases in the control group were rendered alkalotic because of poorly regulated respiration (Fig 3). In these cases, the rate of formation was greatly reduced. Three animals in the xylose-glucose group were also maintained by poorly regulated respiration during the experiment, and, in these cases, hypoxemia developed which subsequently

resulted in a marked reduction of the formation rate in almost the same fashion as in the alkalotic cases. Such inadequate cases were eliminated from the experimental groups.

The formation rate of CSF in 3 groups : The mean formation rate of CSF in the control group (12 animals) was 10.9 ± 0.5 μl/min. This value compares favorably with that reported by Bradbury and Davson.[7]

In the xylose-glucose group, no significant increase of the formation rate occurred at the osmolality of 315 mOsm/L of the perfusate. At 345 mOsm/L in the same group, however, there occurred a 66 % increase from the control level in 60 minutes and a 53.6 % increase in 75 minutes following the beginning of the perfusion (p less than 0.01). Thereafter, the values returned to the control level. The mean formation rate in the 345 mOsm/L level was 14.5 ± 2.0 μl, which represented a 31 % increase from the control group (8 animals). This phenomenon is illustrated in Fig. 4.

Table 3. Rate of formation of CSF and related values in ventriculocisternal perfusion

Exeprimental Group	Osmolality mOsm/L	Vi μl,/min.	Vo μl,/min.	Vo-Vi μl,/min	Cin μl,/min.	Vf μl,/min.
Control (12)	298±9.2	46.4±0.5	52.8±0.9	6.0±0.6	5.1±0.8	10.9±0.5
Xylose-glucose group(8)	345	46.3±0.2	55.2±0.7	8.2±0.7	7.8±1.5	14.5±2.0
NaCl group (8)	335	46.5±0.2	59.0±1.2	12.2±1.1	11.7±1.1	23.6±0.9

Numerals are expressed as mean±S.E., Vi ; Rate of perfusion
Vo ; Rate of effluent flow, Cin ; Inulin Clearance=Absorption rate
Vf ; Formation rate,
Numerals in parenthesis indicate the number of experiments

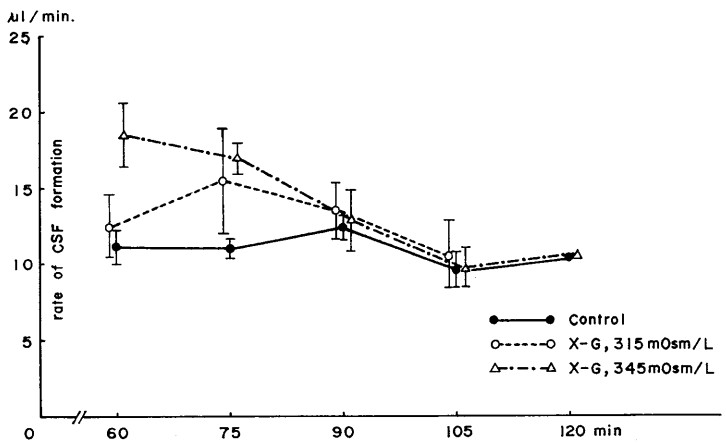

Fig 4. Rate of CSF formation in xylose-glucose group.

In contrast to the xylose-glucose group, NaCl group revealed different results. The formation rate of CSF markedly increased and remained high throughout the entire experiment. Increases of 116.9 %, 140 %, 77.4 %, 122.9% and 133.9% from the control level (p less than 0.01) were observed at 60, 75, 90, 105, and 120 minutes, respectively, after the beginning of perfusion; the mean value was 23.6 ± 0.9 μl/min in the eight animals (Figs. 5 and 6).

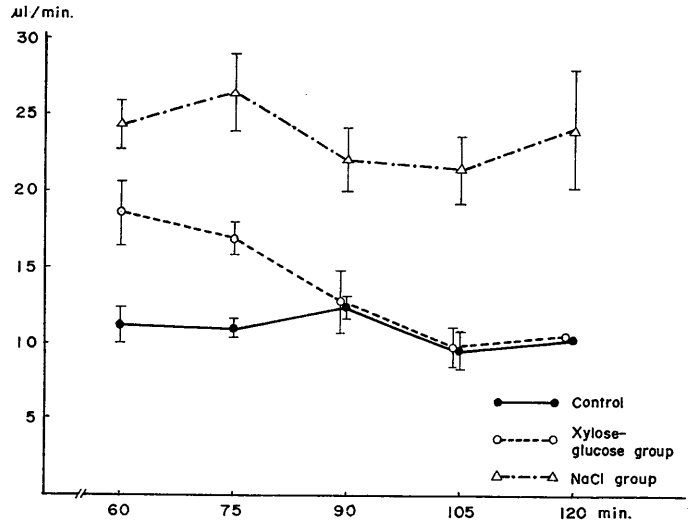

Fig. 5. Rate of CSF formation of ventriculo-cisternal perfusion in 3 experimental groups.

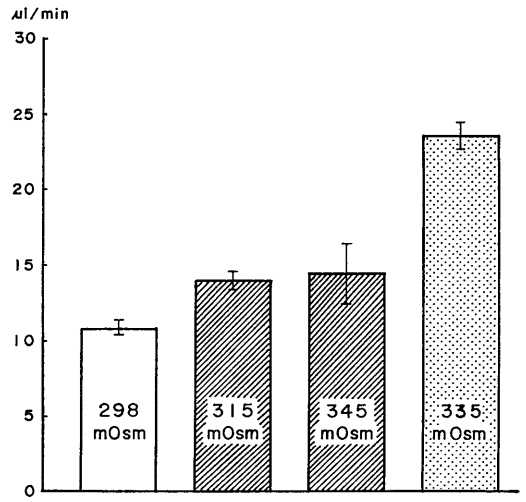

Fig. 6. Comparison of mean rate of CSF formation in 3 experimental groups.
□ Control group, ▨ Xylose-glucose group, ⁘ NaCl group

It is of interest that substantial difference exists in the value of formation rate of CSF between two hyperosmotic groups, i. e., the high osmolality perfusate, 345 mOsm/L, induced by non-electrolytes, brought about minor changes in the formation rate of CSF in comparison with that of the 335 mOsm/L level, induced by NaCl. Furthermore, the effect in increasing CSF formation with the xylose-glucose group was of short duration (Fig. 5).

Critical value of osmotic gradient for increase in the formation rate of CSF: In the xylose-glucose group, we investigated the correlation of the formation rate of CSF with osmotic gradient between perfusion fluid and serum. Perfusion fluid was prepared in each experiment so as to keep a graded osmotic gradient against serum osmolality previously measured. Thus, four grades of osmotic differences were established between perfusate and serum: 10, 20, 30 and 40 mOsm/L. In the results, a significant increase in the formation rate resulted at the level greater the 30 mOsm/L difference between perfusion fluid and serum (Fig. 7). This phenomenon led us to believe that a critical magnitude of osmotic pressure would be required to cause a significant increase in osmotic flow. However, allowance must be made for variation in estimated values of osmotic pressure. In order to confirm this phenomenon, more extensive investigation would be required.

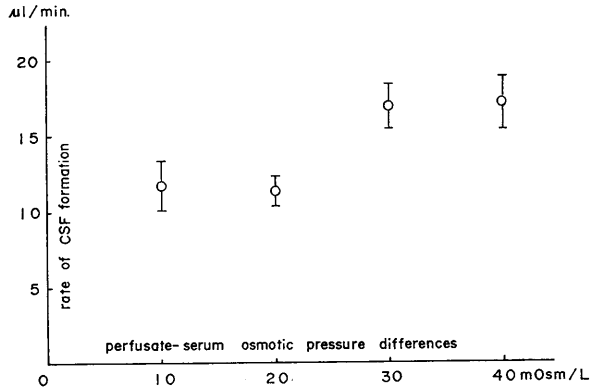

Fig. 7. Relation between rate of CSF formation and osmotic gradient across the choroidal epithelium.

Table 4 (A). Concentrations of electrolytes in perfusion fluid, effluent and serum (mEq/Kg H_2O)

Substance	Perfusion Fluid			Effluent Fluid		
	Control Group	Xylose-Glucose Group	NaCl Group	Control Group	Xylose-Glucose Group	NaCl Group
Na	153.8±1.01	154.8±0.6	177.8±2.8	152.3±1.6	144.1±1.7	169.2±4.5
K	2.8±0.05	2.9±0.1	3.0±0.1	2.8±0.07	2.8±0.1	2.8±0.2
Cl	158.7±4.2	150.6±2.3	173.7±7.1	147.1±6.0	137.4±2.3	176.9±5.5

Values are expressed as mean ± S. E.

Serum Before Perfusion			Serum in Steady State		
Control Group	Xylose-Glucose Group	NaCl Group	Control Group	Xylose-Glucose Group	NaCl Group
154.3±1.4	151.7±1.3	157.0±2.9	151.0±2.2	148.8±2.0	153.2±3.3
3.9±0.2	3.7±0.3	3.8±0.1	3.5±0.3	3·8±0.2	3.8±0.3
114.6±2.3	112.8±0.2	118.6±.21	114.6±2.3	116.4±1.4	118.6±2.2

Table 4 (B). Distribution ratio (Rcsf) of electrolytes

Substance	Control Group	Xylose-Glucose Group	NaCl Group
Na	1.01	0.97	1.10
K	0.8	0.74	0.74
Cl	1.28	1.19	1.49

Rcsf: Concentration in effluent / Concentration in serum

Distribution of electrolytes in effluent fluid (Tables 4A and B): In the control group, concentrations of Na^+, K^+ and Cl^- in the effluent were estimated as 152.3 ±0.2, 2.8±0.07 and 147.1±6.0 mEq/Kg H_2O respectively. The distribution ratio (Rcsf = concentration in effluent/ concentration in serum) was calculated 1.0 for Na^+, 0.8 for K^+ and 1.28 for Cl^-.

In the xylose-glucose group, Na^+ concentration in the effluent (144.1±1.7 mEq/Kg H_2O) decreased slightly from the control value, while Rcsf (0.97) was near unity, thus suggesting that Na^+ concentration in CSF was not markedly affected by elevated osmotic pressure. The concentration of Cl^- was 137.4±2.3 mEq/Kg H_2O, slightly less than the control, with an Rcsf of 1.19. The differences in values were not statistically significant. The concentration of K^+ in this group was exactly the same as the control: 2.8±0.1 mEq/Kg H_2O, with an Rcsf of 0.74.

In the NaCl group, the concentration of Na^+ and Cl^- in the effluent was naturally high because of the addition of these ions to the perfusate. Na^+ was 169.2±4.5 mEq/Kg H_2O, with an Rcsf of 1.10, and Cl^- was 176.9±5.5 mEq/Kg H_2O, with an Rcsf of 1.49, which appeared to be moderately high in comparison with that of the control group. When these values were compared with those of perfusion fluid, Na^+ concentration decreased following passage through the ventricles, probably due to dilution by newly formed CSF. Cl^- concentration was not changed from the perfusate, the reason for which is not clear. Concentration of K^+ was not affected by adding NaCl to the perfusate (2.8±0.2 mEq/Kg H_2O, with an Rcsf of 0.74).

Concentration of electrolytes in serum: There was no appreciable alteration of serum Na^+, K^+, and Cl^- concentration within the conditions of the experiments. Even the high concentrations of Na^+ and Cl^- in the perfusate of the NaCl group could not affect the corresponding concentration of serum electrolytes (Table 4A).

DISCUSSION

The estimated osmotic pressure of artificial CSF for perfusion in the control group was 298 mOsm/L, while the corresponding value for serum indicated 299 mOsm/L. Therefore, isosmolaltiy was maintained on both sides of the choroidal epithelium in the control condition, in which state, CSF was still continuously produced.

According to Davson,[11] the osmolality of the CSF is significantly greater than that of plasma, although the difference is considered to be too small to originate flow of the fluid. In addition, the predicted total osmolality of the brain extracellular fluid is hypertonic with respect to CSF, according to Stern and Coxon.[26] The osmotic influence upon the formation of CSF may be, therefore, negligible, under normal circumstances. In such a situation, it is conceivable that the CSF is produced by secretion.

Because of the composition of CSF, Davson[11] suggested that CSF was produced by secretion. The mechanism for such an isosmolal secretion has been explained by postulating an active transport of ions across the choroidal epithelium. Water would be transferred rapidly into the ventricle by diffusion with the ions so that an approximately isosmolal flow would be achieved in the steady state. Curran and McIntosh,[10] and Diamond[12] supported such a concept, that is, water-to-salt coupling.

Heisey, Held and Pappenheimer[17] investigated the correlation of the rate of formation of CSF with osmotic pressure gradients between plasma and CSF, using the technique of ventriculocisternal perfusion in the unanesthetized goat. The osmolality of the perfusion fluid was adjusted by altering NaCl concentration without changing other ions, as in one of the present experiments. Consequently, they observed a linear relation between net rate of formation and osmotic gradient of the two fluids. For instance, when the osmotic gradient was 340 mOsm/L, a condition similar to ours, the rate of net formation increased twice that of the isosmolal condition. Our data compared favorably with their results.

On the other hand, Welch, Sadler, and Gold[27] found remarkable increase in the rate of CSF formation when the fluid bathing the choroid plexus was replaced with non-electrolyte such that the concentration of Na^+ and Cl^- was reduced but the osmolality was kept the same, as determined by comparing the hematocrit of arterial blood to draining venous blood of the choroid plexus. The results suggest

that low concentration of salt on the ventricular side stimulates transport of water which is followed by movement of ions to establish Donnan equilibrium.

From these observations, it is clear that the rate of formation of CSF is sensitively influenced by an osmotic millieu outside the choroid plexus. However, the concept of a secretory property of the choroid plexus would be incompatible with the fact that the rate of CSF formation depends upon osmotic gradient. Therefore, osmotic flow through the ependymal walls of the ventricles might be considered a contributing source of fluid, as Heisey, et. al.[17] suggested, although the choroid plexus still has the major role in CSF production.

In the presnt study, intraventricular osmotic pressure was enhanced by adding different osmotic agents, NaCl and monosaccharides, into the perfusion fluid. Consequently, the rate of CSF formation increased to twice that of the control level in the NaCl group, while increases in the rate were less marked and of shorter duration in the xylose-glucose group, despite the fact that similar osmolality was achieved in both groups.

Such discrepancy is impossible to explain from the aspect of altered vascular resistance as Harvey[16] demonstrated in the isolated dog kidney, because the change of vascular resistance seems to be secondary to the shift of water by osmotic agents such as mannitol or urea.

Accounting for the different results from the two series of experiments, a different osmotic response of these two osmotic agents, ionic and non-ionic, to the CSF production system should be considered.

In contradistinction to electrolytes, glucose is rapidly metabolized by the brain, while xylose is not metabolized in vitro.[24] Although xylose is supposed to affect the transport system, monosaccharides employed as osmotic agents in the present experiment consist of xylose in their major part, so that metabolic influence upon either ependymal cell or choroidal epithelial cell may be ignored.

It is interesting to note that two alkalotic animals and three hypoxemic ones (sustaining poorly regulated respiration) showed subsequent marked reduction in the formation of CSF. Though our cases are few, it might be inferred that the formation of CSF has a close relationship to cerebral metabolism, as Bering[5] stated. Reduced CSF formation with hyperventilation has also been observed in the earlier works of Oppelt et. al.[23] and Ames et. al.[3] Accordingly, under such deranged metabolism, CSF production may not be affected by osmolality. In order to avoid confusion, in the present study, respiration of animals was regulated by a respirator and gas metabolism was kept within normal range during the experiment.

Since molecules of salt and monosaccharide seem to be easily permeable to the ependyma, diffusion of these molecules into brain extracellular fluid across the ventricular wall might have some effect upon the change of formation of CSF.

In order to consider the different attitudes of the changes in CSF formation

between the two series of experiments, differences in permeability of the ependymal wall to NaCl and monosaccharides must be known. Thus, according to Heisey, et. al.,[17] the computed permeability coefficient for ^{24}Na for goat is approximately the same as that for urea, while the permeability coefficient for d-glucose and d-xylose for the rabbit (calculated by Bradbury and Davson[7]), is greater, by four and five times, respectively, than that for urea. Since the value for urea in the goat and rabbit appear to be of the same order of magnitude, monosaccharide may presumably pass across the ependymal epithelium more easily than Na^+, when the ventricle is perfused with a hypertonic solution of these solutes. If so, then, in the case of hypertonic perfusion with monsaccharide, osmotic equilibrium between both sides of the ependyma may be attained more rapidly than in the case with NaCl, so that the osmotic influence upon water movement would disappear earlier in monosaccharide perfusion than in NaCl perfusion.

Yet glucose transport is a carrier-mediated mechanism. Since these carriers are finite in number, carrier saturation could occur, such that transportation would become inhibited.[11] Moreover, in a solution of different sugars, a competition mechanism could account for altered membrane transport. D-glucose and d-xylose have similar structures and approximately the same molecular weight. When infused separately, they penetrate brain tissue rapidly, but when infused together, the rate of penetration diminishes. Therefore, glucose and xylose may compete with each other and affect the transport system.

Experimental evidence supporting the above hypothesis exists both in vivo and in vitro.[1][4][6][7][8][13][14][15][21] Considering the selectivity involved in the transport mechanisms of sugars, it is difficult to reach a single conclusion based on a simple concept of osmotic equilibrium. Therefore, further investigation, including analysis of tissue fluid of the brain, is necessary.

Some solute added to the perfusate may escape from the ventricle not only through the ependyma but probably through the choroidal epithelium. Although there is evidence that the brain cells can accumulate sugars at a higher concentration than that of the surrounding medium,[15] the majority of escaped solutes is supposed to enter the blood stream via either the cerebral capillary wall or the epithelium of the choroid plexus. If so, hyperosmolal perfusion should cause an elevation in osmotic pressure in the circulating blood.

When we measured serum osmolality during hypertonic xylose-glucose perfusion in six animals, we obtained the following results. In three of the animals, the osmolality of the perfusate was 315 mOsm and in the remaining three it was 345 mOsm. All specimens were taken 105 minutes after the beginning of the perfusion. For those animals perfused with 315 mOsm, the serum osmolalities were 320, 321 and 340 mOsm/L, whereas for those perfused with 345 mOsm, the serum osmolalities were 330, 290 and 320 mOsm/L.

Since the total serum osmolality in 12 control animals prior to perfusion was

Table 5. Osmolality of perfusion fluid and serum in experimental groups and that of CSF and serum in normal rabbit (mOsm/L)

Experimental Groups	Perfusion Fluid	CSF	Serum
Cotrol Group	298.2±9.2		299.5±4.6
Xylose-Glucose Group	345		298~313
NaCl Group	335		270~290
Normal Rabbit*		305	298

* From Davson

299.5±4.6 mOsm/L (Table 5), serum osmolality increased 20–40 mOsm/L during hypertonic perfusion with monosaccharides, although we could demonstrate no linear relationship between the osmotic level of the perfusate and the changes in osmolality of the serum before and after perfusion. In addition, those cases in which the osmotic pressure of the perfusate was relatively mild, that is, 315 mOsm, the osmotic gradient between perfusate and serum reversed during a period of 105 minutes, so that water would move in the direction opposite to osmotic flow. In those cases with an osmotic load of 345 mOsm, the osmotic gradient between perfusate and serum still existed. However, in both series of the xylose-glucose group, the rate of formation of CSF decreased and returned to the control level within 90 minutes following the beginning of perfusion.

Further study of the osmotic influences under graded osmotic differences between perfusate and serum revealed the probable existence of a critical magnitude of osmotic gradient which is required to initiate osmotic flow. We estimated that value to be 30 mOsm/L. Stern and Coxon,[26] observing the osmolality of brain tissue fluid in relation to the elevated osmotic pressure in serum with hypertonic saline solution, found that the brain began to respond to an osmotic gradient in excess of 35 mOsm. This implies that water transport occurs from the brain to the serum beyond 35 mOsm/L. It is noteworthy to consider their finding in light of the present experiment, because both situations would be analogous regarding the essential mechanism of water transport involved. However, we are unable to elucidate entirely the mechanism of this phenomenon due to the lack of estimation of brain osmolality.

In the present results, concentration of Na^+ and Cl^- in the effluent deviated not far from the control level except for the NaCl group even under osmotic influences, contrary to our expectation. An active transport mechanism involved in the formation of CSF is the most likely explanation of our findings. In this connection, interesting results were obtained in the recent study of the chemical analysis of CSF from neurosurgical patients in our laboratory[18]. The concentration of Na^+ in the CSF was surprisingly constant even in the presence of excessive

amounts of protein, whereas a good correlation was demonstrated between the concentration of Na^+ and protein in CSF in the condition within the normal range of protein concentration, for example, below 62 mg/dl. Therefore, it is possible that transport of electrolytes may be dependent upon different mechanisms than those of protein accumulation within the CSF, provided the blood-CSF barrier is intact.

The concentration of K^+ remained surprisingly constant in the present experiment. Even an outstandingly high increase in the formation rate of CSF failed to alter the K^+ concentration. Thus, our study corroborated the work of others.[2)9)20)] Although the Na^+, K^+ and Cl^- concentration in the effluent could not reflect the regulation from the separate parts of the CSF system, as Ames, Higashi and Nesbett[2)] suggested, the observed constancy of these ions in bulk flow from the cisterna magna under experimental conditions seems to be due to the same mechanism as that involved in the formation of CSF. Such observations permit the conclusion that transport of Na^+ and Cl^- and especially K^+, is regulated by a high degree of selectivity in the membranes lining the CSF cavity and water cannot, therefore, move to the ventricular side in an unrestricted fashion, responding only to the osmotic gradient, but must move under the control of energy-dependent electrolytes, that is, the concept of water-to-solute coupling.

The probable existence of a critical magnitude of osmotic gradient required to initiate osmotic flow also lends indirect support to such a concept of homeostatic control.

SUMMARY

Ventriculocisternal perfusions with artificial CSF were carried out in rabbits. The rate of formation of CSF was estimated by means of inulin dilution techniques. The rate of formation of CSF in the control animals was found to be $10.9 \pm 0.5 \mu l$/min.

Hypertonic perfusion was performed by adding NaCl or monosaccharides into the perfusion fluid and the osmotic influence these had upon CSF formation was investigated.

A remarkable increase in the rate of formation of CSF was observed in the NaCl group, after a steady state was achieved in the perfusion system, while in the xylose-glucose group, the increase in the rate of formation of CSF was not only less marked but also of shorter duration, despite increased osmolality of the perfusate.

In the xylose-glucose group, when the osmotic gradient between perfusate and serum was produced by the addition of sugars to the perfusate, we found that there probably existed a critical magnitude of osmotic gradient required in order to initiate osmotic flow through the ependymal linings and/or choroidal epithelium.

We estimated this critical value to be approximately 30 mOsm/L.

Analysis of electrolytes in the effluent revealed no remarkable alteration during perfusion with hyperosmotic solutions when compared with those of the controls, except for concentrations of Na^+ and Cl^- in the NaCl group.

The mechanism responsible for the discrepancy found in the results of the two groups of experiments was discussed.

The active transport of electrolytes followed by diffusion of water, saturation of a sugar-transport mechanism, and regulation of electrolyte concentration in the ventricular system are all thought to be factors contributing to the homeostatic mechanism which regulates the production of CSF.

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