ABSTRACT

STUDY OF THE AVIAN CEREBROSPINAL FLUID USING BRAIN VENTRICULAR PERFUSION

By

Douglas Kroulik Anderson

Brains of anesthetized chickens were perfused from the left lateral ventricle to the cisterna magna with an artificial chicken cerebrospinal fluid (CSF) containing radio-iodinated human serum albumin (RIHSA) or inulin, ^{22}Na, ^{42}K, ^{45}Ca, ^{14}C-glucose, ^{14}C-creatinine and unlabelled creatinine. Inflow ($\dot{V}_i$) and outflow ($\dot{V}_o$) rates and concentrations of all test molecules were measured. The steady-state clearances of RIHSA (C_{RIHSA}), ^{22}Na (C_{Na}) and creatinine (C_{cr}) from the perfusate were calculated at various intraventricular pressures. C_{RIHSA} increased and outflow-inflow ($\dot{V}_o$-$\dot{V}_i$) decreased linearly (with equal but opposite slopes) with increasing intraventricular pressures, suggesting C_{RIHSA} was a measure of CSF bulk absorption ($\dot{V}_a$). CSF formation rate ($\dot{V}_f$) calculated as the algebraic sum of C_{RIHSA} and $\dot{V}_o$-$\dot{V}_i$, was approximately 1.4 µl/min., and was independent of intraventricular pressure. Resistance to $\dot{V}_a$ (4545 cm · min/ml) was 12-350 times that reported for mammals and 2.5 times

that reported for turtles. This high resistance may indicate either a lack of valve-like channels in the arachnoid villi (as described for mammals) or high resistance pathways in the arachnoid membrane.

Total clearance of sodium and creatinine was divided into a pressure-dependent component related to $\dot{V}_a$ and a pressure-independent component termed an efflux coefficient (K_D). The steady-state efflux coefficients for creatinine ($K_{D_{cr}}$), ^{42}K (K_{D_K}), and ^{45}Ca ($K_{D_{Ca}}$) were not affected by their perfusion inflow concentrations suggesting that the non-bulk removal of all three molecules from chicken CSF is by simple diffusion paralleling, previous studies in various mammalian species. The large K_D for creatinine (relative to $K_{D_{Na}}$, $K_{D_{Ca}}$, and K_{D_K}) suggests active transport may be involved in creatinine efflux.

Ventricular CSF volume was estimated from the distribution volume of RIHSA, ^{22}Na and ^{14}C-glucose (determined by integrating the outflow concentration of these molecules with respect to time) and total CSF volume was estimated from the maximum volume of CSF that could be withdrawn from the cisterna magna. Ventricular volume was 140 µl; total CSF volume was 350 µl. Brain spaces for RIHSA, ^{22}Na, ^{45}Ca and ^{42}K were estimated from brain residual radioactivity after 1-6 hours of perfusion. The smallest brain space was 4 percent of brain weight for RIHSA and the largest was

91 percent for ^{42}K. Magnitude of brain space was dependent on several factors: molecular size; magnitude of brain uptake; and extent of uptake by brain cells.

Perfusion of 0-40 mEq/L of potassium through the chicken cerebral ventricles was without effect on systolic, diastolic or mean blood pressure. Ventricular perfusion with 0-5 mEq/L calcium produced similar results for diastolic and mean blood pressure but systolic blood pressure was significantly depressed at elevated calcium concentrations. With the exception of the systolic response to calcium, these results do not confirm previous studies in mammals. It is suggested that the lack of response in chickens is due to anesthetic interference with the sensitivity of the vasomotor center to ionic changes in CSF.

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INTRODUCTION

The central nervous system (CNS) during development assumes a tubular configuration and retains the characteristic of being a hollow organ throughout life. This neutral tube evolves into the various brain ventricles which contain cerebrospinal fluid (CSF), a low protein secretion derived from blood plasma (Millen and Woollam, 1962; Davson, 1967). CSF is also found in the subarachnoid spaces which cover the entire surface of the CNS.

The circulation of CSF has been well described for mammals (Davson, 1967; Millen and Woollam, 1962; Truex and Carpenter, 1969). CSF flows from the two lateral cerebral ventricles through the foramina of Monro into the third ventricle. From the third ventricle the CSF passes through the Aqueduct of Sylvius into the fourth ventricle. CSF leaves the fourth ventricle by way of 3 foramina, a medial foramen of Magendie and two lateral foramina of Luschka and enters the subarachnoid spaces surrounding the brain and spinal cord. Total CSF volume in humans has been estimated to be 140 ml (Millen and Woollam, 1962).

Since the studies of Frazier and Peet (1914) and Dandy (1919) on dogs, it has been generally accepted that CSF is a secretion from specialized vascular structures

(choroid plexuses) which, along with a lining of ventricular ependyma, project into all four brain ventricles. This has been confirmed by de Rougemont and his co-workers (1960) who collected newly formed CSF directly from the choroid plexus of cats. Recent evidence indicates that in mammals, the ventricular ependyma (Bering and Sato, 1963; Milhorat, 1969; Pollay and Curl, 1967; Welsh, 1963) and the cranial subarachnoid spaces (Bering and Sato, 1963; Sweet and Locksley, 1953; Wallace and Brodie, 1940) may also play a role in the elaboration of CSF.

The studies of Frazier and Peet (1914) and Dandy (1919) indicate that, in dogs, CSF is reabsorbed into venous blood mainly (if not solely) from the subarachnoid space. Weed (1914) proposed that the arachnoid villi (evaginations of the arachnoid membrane into the dural venous sinuses) are the structures responsible for draining CSF into blood. The importance of the arachnoid villi in CSF drainage was recently confirmed by Welsh and Friedman (1960) who described these structures in monkeys as a labyrinth of small tubes which establish an open connection between CSF and venous blood. They showed that these villi function as valves which open when CSF pressure is higher than sagittal sinus pressure and close when the dural sinus blood pressure exceeds CSF pressure.

One function of CSF is mechanical, i.e., it serves as a fluid cushion for the CNS (Millen and Woollam, 1962;

Truex and Carpenter, 1964). In addition CSF may: (1) serve as a nutrient source for the brain and spinal cord and aid in waste removal (Millen and Woollam, 1962; Truex and Carpenter, 1964); (2) carry the hypophyseal hormones (Millen and Woollam, 1962); and (3) play a role in the control of respiration (Pappenheimer et al., 1965).

As for most non-mammalian species, inadequate data are available to describe CSF formation and reabsorption characteristics in birds. From a comparative physiological standpoint, and as an extension of a recent study on the normal composition of chicken CSF (Anderson and Hazelwood, 1969) this information on avian species is important. Further, the effect of changes in CSF concentrations of K^+ and Ca^{++} on blood pressure and heart rate has been studied in several mammalian species (reviewed by Tschirgi, 1960 and Winterstein, 1961), however no studies of this type have been performed with birds.

The control of the avian cardiovascular system differs from that of mammals. Although birds have a carotid sinus homologue, they do not exhibit a carotid sinus reflex (Heymans and Neil, 1958; McGinnis and Ringer, 1966). Sturkie (1965) indicates that baroreceptor mechanisms are present in birds; however, their anatomical locations are unknown. Since birds differ from mammals in the control of their blood pressure and heart rate, it is important to determine if birds and mammals vary in their response to changes in

the ionic composition of CSF. Although direct neutral control of blood pressure in chickens has been postulated (Rodbard and Tolpin, 1947), no evidence is available to determine the presence or to establish the location of a vasomotor center (Sturkie, 1965). In general, perfusing or injecting solutions with a high K^+-Ca^{++} ratio (due either to high levels of K^+ or low levels of Ca^{++}) into the CSF system of mammals results in an elevated arterial pressure concomitant with a slowing of the heart rate (Tschirgi, 1960). These cardiovascular responses are attributed to the direct action of elevated K^+ or reduced Ca^{++} levels on the medullary cardiovascular centers (Tschirgi, 1960; Winterstein, 1961). Changes in blood pressure and heart rate in chickens during ventriculocisternal perfusion with CSF having an altered K^+-Ca^{++} ratio might be taken as presumptive evidence for the location of vasomotor and cardiac centers in the avian brain in proximity to the path of the CSF perfusate.

LITERATURE REVIEW

The purpose of this review is to present studies in several species (mostly mammalian) on: (1) cerebrospinal fluid (CSF) formation and reabsorption rates; (2) molecular exchange between CSF, brain and blood; (3) determination of the brain ventricular and extracellular fluid volumes; and (4) the effect of changes in CSF K^+ and Ca^{++} concentrations on blood pressure, heart rate, electrocardiogram (EKG), electroencephalogram (EEG) and respiration.

Cerebrospinal Fluid Formation and Absorption

Frazier and Peet (1914) were the first workers to quantitatively determine the rate of CSF production. They sutured blunt cannulas (connected to a rubber tube and graduated pipette) into the cisterna magna of anesthetized dogs. The dog was placed on an inclined plane, head down, with the level of the outflow cannula below the head so that all the fluid entering the cisterna magna would be collected. The rate of formation in dogs was found to vary widely under normal conditions, the average being 0.231 ml/min. Frazier and Peet also obtained an estimate of the rate of CSF reabsorption by determining the rate of appearance of phenolsulphonephthalein (PSP) in the urinary bladder after a known

quantity of this dye had been injected into the ventricles. They assumed CSF is reabsorbed at a rate comparable to PSP, clearance by the kidney indicating that 0.4-0.5 percent of the CSF is reabsorbed every minute. In a more recent study using the drainage technique, Greenberg et al. (1943) found the CSF formation rate in anesthetized dogs to average 0.2 ml/hour/kg body weight. For a 20 kilogram dog this would be 0.06 ml/min. These workers indicate that the major objection to the open drainage technique is that the CSF pressure must be maintained at levels far below normal.

Flexner and Winters (1932), using anesthetized adult cats, improved upon the drainage technique by measuring the amount of CSF leaving the aqueduct of Sylvius under normal intra-ventricular pressures. A catheter, surrounded by a balloon, was placed in the caudal portion of the aqueduct of Sylvius and the balloon inflated to provide a seal with the walls of the fourth ventricle. The amount of fluid flowing out of the aqueduct and through the catheter was measured using a bubble-manometer. A fluid reservoir was attached to one end of the manometer so that intraventricular pressure could be held constant. At a ventricular pressure of about 110 mm H_2O approximately 12.1 ml of CSF per day or 0.0084 ml/min was collected from the aqueduct, representing CSF formation by the lateral and the third ventricles. They assumed that CSF was formed only by the choroid plexuses and that the amount of CSF formed by a single plexus is directly

proportional to its weight. Since the choroid plexus of the fourth ventricle weighed about 25 percent of the total weight of the other plexuses, the fourth ventricle would contribute about 3 cc of CSF per day. Thus, total CSF production in the anesthetized cat was 15.1 ml per day or 0.0105 ml/min.

Another method used to determine the rate of CSF formation consists of withdrawing a measured volume of CSF and recording the time required to restore CSF pressure to pre-withdrawal levels. Using this technique Masserman (1934) found that in humans CSF was formed at a rate of 0.305 ml/min. CSF pressure measurements and fluid withdrawal were made by lumbar puncture under a local anesthetic. Recently, Katzman and Hussy (1970) repeated Masserman's study and obtained a CSF formation rate in humans of 0.323 ml/min. These workers state a number of objections to this method: (1) withdrawing CSF might alter its rate of formation, (2) CSF absorption might continue (despite the lowered CSF pressure) leading to an underestimation of the rate of formation, and (3) the elasticity of the subarachnoid space would make the measurement imprecise. Thus it remains uncertain why this technique gives values quite similar to those obtained with the more sophisticated steady-state perfusion technique that will be discussed later.

Welch (1963) devised a method for measuring the rate of CSF secretion by the rabbit choroid plexus. Linear velocity of flow in the main choroidal vein was estimated from

cinematographic recordings of the movement of a bubble of 1-octyl alcohol injected into this vessel. Following determination of the cross-sectional area of the main choroidal vein, blood flow in this vessel was calculated as the product of the cross-sectional area of the vein and the linear velocity of the alcohol drop. Plasma volume loss during passage of blood through the plexus was calculated as the difference in arterial (aorta) and venous (main choroidal vein) hematocrits. CSF secretion was then estimated from the choroid plexus blood flow and the fractional plasma volume loss during the transit of blood through the plexus. A CSF production rate of 0.37 µl/min per mg was calculated for the choroid plexus of the lateral ventricle. From this and the total weight of the choroid plexuses of the rabbit, a total choroidal production of CSF was calculated to be 0.0078 ml/min.

Greater quantitation in the study of CSF formation and absorption along with greater versatility in the study of the CSF system was made possible following the adaptation of a ventriculocisternal perfusion technique developed by Leusen (1950) to the unanesthetized goat by Pappenheimer et al. (1962). Guide tubes were implanted above the dura mater over the cisterna magna and above the ependymal linings of the lateral ventricles in anesthetized goats. At a later date, in the unanesthetized state, needles were passed through the guide tubes into one lateral ventricle and

cisterna magna. Synthetic CSF was pumped into the lateral ventricle and collected from the cisternal outflow. Pressure in the system was set by the height of the outflow tubing. Inflow and outflow perfusion rates were determined quantitatively at various intraventricular pressures. They assumed that fluid could enter from perfusion inflow via the lateral ventricular needle and from CSF formed by the animal. Fluid could exit by the cisternal outflow needle and by the subarachnoid space (distal to the cisterna magna) into blood. Then the sum of the two entry rates equaled the sum of the two exit rates (Heisey et al., 1962). By adding to the perfusion fluid a large molecule (inulin) that leaves the CSF system predominantly from the subarachnoid space distal to the cisterna magna, they were able to estimate the rate of bulk CSF absorption from the subarachnoid space into dural venous sinuses by calculating the clearance of inulin from the CSF. Net formation of CSF could then be calculated as the algebraic sum of the difference between outflow and inflow perfusion rate and the clearance of inulin. Using this method, a CSF formation rate of 0.16 ml/min was calculated for the unanesthetized goat. Rate of CSF formation was found to be independent of intraventricular pressure over the range of -10 to +30 cm H_2O relative to the external auditory meatus. However a direct relationship between bulk absorption of CSF and hydrostatic pressure was observed. Bulk absorption varied from zero at an intraventricular

pressure of -18 cm H_2O to approximately 0.4 ml/min at an intraventricular pressure of +30 cm H_2O.

Ventriculocisternal perfusion has been applied to the determination of CSF formation in a variety of species (Table 1). The values are expressed as absolute rates (µl/min), as rate per milligram of choroid plexus tissue (µl/min per mg) and as rate per total volume of CSF (percent per minute; CSF turnover rate). Although there are wide variations in the absolute rates of CSF formation among the various species, the CSF turnover rate or the rate per mg of choroid plexus tissue appears to be fairly costant at least among warm-blooded species. This suggests that the secretion of CSF by choroid plexus tissue is relatively constant among species and that there is an increasing amount of choroid plexus tissue (and total CSF volume) with increasing brain size.

The fact that rate of CSF formation per mg of choroid plexus tissue is fairly constant is an indication of the importance of these structures as the source of CSF. Several other studies have pointed to the choroid plexuses as being the site of CSF secretion (Dandy, 1919; de Rougemont et al., 1960; Welch, 1963; Ames et al., 1965). Recently evidence has been advanced that CSF can also be formed extrachoroidally in the ventricular system and the subarachnoid space. Welch (1963) concluded that there may be two sources of CSF formation which are distinguishable under the influence of

Table 1. Cerebrospinal fluid formation and turnover rates in different vertebrate species.

Species	CSF Formation µl/min	CSF Formation µl/min per mg choroid plexus	CSF Turnover rate	Reference
spiny dogfish	4	0.05		Oppelt et al., 1963
nurse shark	5			" " "
lemon shark	4			" " "
turtle	1.4	0.06	0.19	Heisey & Michael, 1971
rat	2.2	0.55		Cserr, 1965
rabbit	13	0.56		Pollay & Curl, 1967
cat	20	0.50	0.45	Davson et al., 1961
cat	22	0.55	0.50	Graziani et al., 1965
cat	20	0.50	0.45	Hockwald & Wallenstein, 1967
dog	47	0.59	0.53	Bering & Sato, 1963
dog	44	0.56	0.50	Sahar et al., 1971
goat	164	0.37	0.66	Heisey et al., 1962
human (child)	350		0.38	Culter et al., 1968
human (adult)	429	0.22	0.31	Rubin et al., 1966

acetazolamide. He indicated that CSF secretion from the choroid plexus is almost completely stopped under the influence of intravenously administered or topically applied acetazolamide. Welch cites other studies which show that total CSF production (as measured by inulin dilution) is decreased by only about 50-60 percent under the influence of this drug. Since acetazolamide essentially stopped choroidal production of CSF *in vitro* while only cutting CSF production in half *in vivo*, he concluded there was a dual source of CSF formation, from the choroid plexuses and from the surrounding nervous tissue. Cerebral ventricular perfusion can also be used to demonstrate extra-choroidal CSF formation. Pollay and Curl (1967) using an aqueductal-anterior fourth ventricle perfusion system in the rabbit calculated a mean rate of CSF formation (determined by inulin dilution) originating from the ventricular ependyma of 0.33 µl/min per cm^2 of ependymal surface. The total area of ventricular ependyma in the rabbit was 12.8 cm^2 giving a CSF formation rate attributed to the ventricular ependyma of 4.23 µl/min which is approximately 33 percent of the total intraventricular CSF formation rate in the rabbit. Milhorat (1969) found that CSF production was decreased by only 26 percent in bilaterally plexectomized rhesus monkeys (studied by ventriculo-aqueductal perfusion) as compared to normal nonplexectomized controls. He concluded that in the monkey the choroid plexus is not the sole or even major source of CSF, this being either a secretion

of the ventricular ependyma or a product of cerebral metabolism which enters the ventricular system across the ependymal lining. Bering and Sato (1963) using ventriculocisternal and subarachnoid-cisternal perfusion, found in the anesthetized dog that about 43 percent (0.020 ml/min) of total CSF production was by the cranial subarachnoid space. However, Hammerstad et al. (1969) using a cisternal-lumbar perfusion in anesthetized cats could not detect any CSF formation from the spinal subarachnoid space.

The ventriculocisternal perfusion technique does not only allow the determination of the sources and rates of CSF secretion but also possesses capabilities for studying the relationship between the formation of CSF and intraventricular pressure. As mentioned previously, (Heisey et al. (1962) demonstrated in the goat the independence of CSF formation and acute changes in intraventricular pressure. This relationship between CSF formation and intraventricular pressure has been well established in a variety of species, notably the turtle (Heisey and Michael, 1971), cat (Katzman and Hussey, 1970), human (Cutler, et al., 1968), and dog (Bering and Sato, 1963). Bering and Sato further state that the rate of CSF formation is unaffected by the development of hydrocephalus. Recently Sahar (1970) demonstrated, in individual anesthetized cats with kaolin-induced obstructive hydrocephalus, that there was a decrease in the CSF formation rate of 3.5 percent/cm H_2O as perfusion pressure was elevated.

Their stated purpose for looking at individual animals was that in studies previous to theirs, conclusions concerning the influence of perfusion pressure on CSF formation rate have been based only on statistical evaluation of whole groups. Thus, studying individual animals would allow paired comparisons. They propose that the reduction in CSF production was due to an adverse effect of increased intraventricular pressure on the structure of the chorid plexuses and ventricular ependyma. In a later study Sahar et al. (1971) found a 30 percent reduction in CSF production in individual anesthetized kaolin-induced hydrocephalic dogs compared with normal anesthetized dogs. They found that this reduction in CSF production is not as great as that seen in their previous study on cats because the choroid plexuses in the hydrocephalic dogs were not damaged. It appears that although the increased intraventricular pressure had a deleterious effect on the structure of the ventricular ependyma in dogs, it did not reduce CSF production by the choroid plexuses of these animals.

The technique of cerebral ventricular perfusion has been instrumental in the greater quantitation of studies on CSF formation and absorption rates in a variety of animal species. The versatility of this technique also allows the study of CSF secretion at various intraventricular pressures in either acute or chronic pathological (hydrocephalus) situations. Measurements of the ventricular and brain

extracellular fluid volumes, studies of the movement of various substances into and out of the CSF system, and investigations of peripheral autonomic function by ventriculocisternal perfusion (all to be discussed later) further emphasize the adaptability of this technique.

Molecular Exchange Between Blood, Brain and Cerebrospinal Fluid

Determination of the CSF formation and absorption rates is integral to the study of molecular flux between CSF, brain and blood. With knowledge of these parameters appropriate permeability coefficients can be devised that take into account material influx into the ventricular system with freshly formed CSF or subtract the material outflux from the ventricles with CSF absorbed in bulk. These permeability coefficients have been used to compare the relative rates of movement of a variety of materials across the ventricular wall and have resulted in greater quantitation in the study of the blood-brain and blood-CSF barriers.

Movement from blood to CSF and brain

Early studies with intravenously injected dyes showed that the brain was spared from staining while most of the other body tissues were stained. It was this restricted passage of dissolved dyes out of the blood into brain tissue and CSF that gave rise to the concepts of

blood-brain and blood-CSF barriers. However, if dyes were injected directly into CSF, the whole brain became heavily stained (Tschirgi, 1960; Davson, 1967). Thus, while there appears to be a barrier between blood and nervous tissue and CSF, this barrier can be circumvented by direct injection into CSF as there does not appear to be a barrier between CSF and brain.

Davson (1967) has investigated the penetration from blood of various non-electrolytes into the brain and CSF of rabbits. The rate of penetration decreased in the following order: ethyl alcohol > propyl thiourea > ethyl thiourea > methyl thiourea > thiourea > creatinine. Davson states that the decreases in the rates of penetration are related to the decreasing lipid-solubility of the substances.

One way of explaining the permeability characteristics exhibited by the blood-CSF and blood-brain barriers to certain lipid-insoluble molecules is by carrier mediated transport which includes both active transport and facilitated transfer. When a solute diffuses down an electrochemical gradient, the process is described as passive diffusion. When the material moves against an electrochemical gradient, exhibits saturation kinetics, requires metabolic energy and can be competitively inhibited, the process is termed active transport. If the transport system exhibits saturation kinetics and competitive inhibition but the transport is not against an electrochemical gradient and

is not energy dependent, the process is termed facilitative transfer. Fishman (1964) reported on the movement of sugars between blood and CSF in anesthetized dogs. He found that CSF glucose concentrations approached a maximum despite increasing intravenous glucose loads, suggesting saturation kinetics. Competitive inhibition between glucose and 2-deoxyglucose was also demonstrated. Fishman concluded there was a carrier transport system for glucose in the membranes separating blood and CSF. Bradbury and Davson (1964) also found evidence for a saturable carrier transport system for D-glucose and D-xylose into and out of the CSF of anesthetized rabbits. These workers found no evidence for transport against a concentration gradient or energy requirement suggesting a facilitative rather than an active transport system for these monosaccharides.

One of the first quantitative studies of the movement of ions across the blood-brain and blood-CSF barriers was by Wallace and Brodie (1940). These workers administered bromide, iodide and thiocyanate by iv injection in anesthetized dogs. They found that the uptake of these ions by the CNS and CSF was slow and restricted in comparison to other tissues and concluded they must cross a barrier "which offers some selective hindrance to their course" in passing from plasma to the brain extracellular fluid (ECF) and from there into CSF. Greenburg et al. (1943) studied the permeability of the blood-CSF barrier to a number of radioactively

labelled ions in anesthetized dogs. They found that the rate of increase in concentration of the labelled ion in CSF, following intravascular administration was in the order potassium > sodium > bromide > rubidium > strontium > phosphate > iodide. These workers indicated that the rate of accumulation in CSF of the injected ions (both positively and negatively charged) is selective and is a slow process in comparison to other tissues. They concluded that CSF formation must be a secretory process rather than passive diffusion. Katzman and Leiderman (1953) found that the rate of equilibration of ^{42}K between the plasma and brain of rats was slow. In addition they found that despite variations in the plasma potassium levels, the potassium flux into brain from plasma is approximately the same, suggesting that potassium movement into brain is carrier mediated; a finding they confirmed in cats several years later (Katzman et al., 1965). Graziani et al. (1967) studied the calcium flux from blood to brain and CSF (as measured by ^{45}Ca flux) in anesthetized cats during ventriculocisternal perfusion. These workers found that when the serum calcium levels were varied, a component of the calculated permeability coefficient for ^{45}Ca from blood to CSF and brain was reciprocally related to serum calcium concentrations indicating an active or carrier-mediated process. Another smaller component of the coefficient was constant which is consistent with passive diffusion. When ouabain was added to the perfusate both CSF formation

rate and calcium influx declined suggesting a calcium influx component related to CSF formation. However, when acetazolamide was added to the perfusate, CSF formation declined but a component of calcium influx continued independent of the reduced CSF formation suggesting that calcium can enter CSF by ways other than with freshly formed fluid. Bito (1969) presented evidence that the dog cerebral cortex ECF has a low potassium and high magnesium concentration even when compared with the concentration of these cations in cisternal CSF. He concludes that these brain ECF concentrations of potassium and magnesium can not be maintained by the secretory activity of the choroid plexuses and a passive diffusional barrier between blood and brain but necessitate the existence of an active transport function across the blood-brain barrier.

Any interpretation of ionic distributions and fluxes between CSF, brain and blood requires knowledge of any electrical potential differences existing between these compartments. Held et al. (1964) determined the steady-state electrical potential difference between cisternal fluid and jugular venous blood averaged +6.5 mV in unanesthetized goats and varied from -2 to +7 mV in anesthetized dogs. They found there was an inverse linear relationship between the pH of arterial blood and the CSF potential changing from +15 mV at pH 7.1 to -3 mV at pH 7.6. They also observed an inverse relationship (slope = 1 mV per mEq/L) at constant

arterial pH between the potassium concentration of fluid perfusing the cerebral ventricles and the CSF potential. In addition, they found that the rate of CSF secretion was unaffected during large changes in CSF potential. These workers concluded that the CSF potential must contribute to the exchanges of all charged particles between CSF, brain and blood and must play a role in determining the steady-state ionic composition of CSF. They further suggest that the CSF potential is derived from ion transport across the ventricular ependyma and is not associated with CSF formation.

Movement from CSF to blood and brain

The removal of substances from CSF depends upon several mechanisms: bulk flow of fluid via the arachnoid villi into blood, active transport of substances into nervous tissue or blood, and diffusion of substances into nervous tissue or blood. As discussed previously, bulk absorption refers to the pressure-dependent removal of CSF with its total contents through the one-way valve system of the arachnoid villi. This is mainly how large, non-diffusible molecules (e.g., proteins, inulin, dextran) exit the CSF system. Since there is no filtration across the arachnoid villi, one component of the total removal of all substances from the CSF compartment will be with the bulk absorption of fluid through the arachnoid villi into blood.

A number of substances have been shown to be actively transported from the CSF system. Pappenheimer et al. (1961) presented evidence for the active transport of Diodrast and phenolsulphonephthalein (PSP) from CSF to blood in anesthetized goats perfused from one lateral ventricle to the cisterna magna. These workers found that Diodrast moved from CSF to blood against a concentration gradient, was competitively inhibited by para-aminohippuric acid (PAH) and PSP and exhibited saturation kinetics. They presented evidence implicating the choroid plexus of the fourth ventricle as the site of the active transport. Snodgrass et al. (1969) perfused the ventriculocisternal system in anesthetized cats to study the transport of neutral amino acids out of the CSF system. They found that the exit of L-leucine and cycloleucine from CSF exhibited saturation kinetics. In contrast, the movement of α-aminoisobutyric acid (AIB) and L-alanine showed no saturation kinetics. These workers concluded that neutral amino acids were not cleared from CSF by bulk absorption or diffusion alone, but for some amino acids there was a saturable transport out of the CSF. Murry and Cutler (1970) studied the rate of clearance of glycine from the CSF of anesthetized cats during ventriculocisternal and ventriculolumbar perfusion. They found that glycine was cleared from the ventricular system and spinal subarachnoid spaces by a saturable mechanism, suggesting carrier-mediated transport, and also demonstrated that the choroid plexuses and

periventricular thalamic tissue could concentrate glycine to about 10 times that of the surrounding medium.

Several workers have found that various anions are transported out of the CSF. Pollay (1966) has indicated that active transport of thiocyanate from CSF is responsible for maintaining the observed concentration gradient of thiocyanate between brain and CSF. Destroying this transport process results in a more accurate estimate of the brain thiocyanate space (see page 32 of this review). Cutler et al. (1968), using ventriculocisternal perfusion in anesthetized cats, showed that iodide and sulfate were cleared from CSF against a concentration gradient, exhibited saturation kinetics and could be competitively inhibited. In addition, they found that sulfate was cleared from the CSF of adult cats at a rate 3 times greater than that from kittens and concluded that transport from CSF was more efficient in adult cats than in kittens. Hammerstad (1969) used a technique of cisternal-lumbar perfusion in anesthetized cats to study the transport of iodide and sulfate out of the spinal subarachnoid space. They found that iodide was cleared from the spinal subarachnoid spaces by a saturable process, against a concentration gradient, which could be competitively inhibited by thiocyanate. In contrast, sulfate removal from CSF was unaltered by changing the perfusate sulfate concentration. These workers concluded that iodide was transported

out of the spinal subarachnoid fluid by a carrier-mediated process whereas sulfate exited by passive diffusion.

Many substances other than sulfate have been shown to leave the CSF by a process of simple or passive diffusion. Davson et al. (1962) found a "relatively high rate of escape" from the CSF of lipid-soluble substances such as ethylthiourea as compared with ^{24}Na. The rate of inulin loss was found to be small and these workers indicated that inulin loss was probably almost exclusively by way of the arachnoid villi. Pappenheimer et al. (1961) reported that after inhibition of active Diodrast transport a passive component of transfer of this molecule was revealed. Studies with creatinine, fructose and inulin showed that passive movement of these molecules from CSF is by both bulk absorption and diffusion at rates comparable with diffusion rates from the capillaries of 1 gm of skeletal muscle. Heisey et al. (1962) introduced increased quantitation in the study of molecular movement out of the CSF by deriving a diffusional permeability coefficient, K_D, that accounts for movement of substances out of the CSF by means other than by bulk absorption. These workers compared the steady-state rates of exit of several molecules from the CSF of unanesthetized goats during ventriculocisternal perfusion. They found that the permeability of the ventricles in goats (as measured by the K_D for the various solutes) decreased in the following order: labelled water (TOH) > urea > creatinine > fructose. This appears to

indicate that the flux is inversely related to molecular size. They also observed that the K_D for ^{42}K was greater than that for ^{24}Na. Heisey et al. concluded that the passive permeability of the ventricular system in the goat is at least as great as that for the vasopressin-treated toad bladder. Bradbury and Davson (1964) found no evidence for a saturable transport system for either urea or creatinine from the CSF of anesthetized rabbits studied by ventriculocisternal perfusion. They found that the flux of urea out of CSF exceeded creatinine efflux,indicating diffusion rate for these two substances is related to molecular size. These workers concluded that there was no carrier-mediated transfer of urea or creatinine, these two molecules exiting the CSF by simple diffusion.

Cserr (1965) investigated the potassium exchange between CSF, brain and plasma using ^{42}K and the technique of ventriculocisternal perfusion in anesthetized dogs and rats. She found that two-thirds of the ^{42}K outflux could be recovered from brain tissue indicating that the ^{42}K in the perfusate was exchanging primarily with brain intracellular potassium pools. Addition of $10^{-5}M$ ouabain to the CSF perfusion fluid reduced the ^{42}K outflux to 25 percent of control values, indicating that 75 percent of ^{42}K outflux is dependent on active ion transport. Cserr ascribed the active step of ^{42}K outflux to cellular components in the brain (and not to the ependyma) for two reasons: (1) large molecules like creatinine or even

inulin can diffuse across the ependyma making it unnecessary to assume an active role for ependymal cells; and (2) ouabain does not inhibit transependymal ^{42}K influx. This fact cannot be explained if the active process for ^{42}K outflux is placed at the ventricular ependyma. She thus concluded that transependymal potassium exchange is passive. Two other studies on the exchange of potassium between CSF, brain and blood appeared in 1965. Both investigations utilized ^{42}K and the technique of ventriculocisternal perfusion to measure the flux of potassium between CSF, brain and blood. Bradbury and Davson working with anesthetized rabbits and Katzman et al. using anesthetized cats both reported that ^{42}K flux from the CSF perfusate did not exhibit saturation kinetics, a finding that is consistent with simple diffusion. Like Cserr, both Bradbury and Davson and Katzman et al. found that ouabain in the perfusate depressed ^{42}K outflux and ascribed this phenomena to a poisoning of the Na-K pump in cells of the brain parenchyma resulting in an inhibition of potassium influx into cells. Inhibition of potassium flux into brain cells would result in a reduction in the flux of ^{42}K from CSF. Thus both groups of workers concluded that ^{42}K outflux was by a process of simple diffusion.

Oppelt et al. (1963) injected solutions into the cisterna magna of anesthetized dogs containing ^{45}Ca and either normal or 3-5 times normal concentrations of calcium. They found the ^{45}Ca removal rate was independent of the

injected calcium concentration. These workers concluded there was no active transport of calcium out of the CSF; diffusion and bulk flow accounted for the complete removal of calcium. These results were confirmed by Graziani et al. (1965) who found that ^{45}Ca efflux from the CSF perfusate during ventriculocisternal perfusion in anesthetized cats does not demonstrate saturation kinetics when increasing quantities of unlabelled calcium were added to the perfusate. These workers concluded that calcium efflux from the CSF was due to passive diffusion with one-third of the ^{45}Ca entering brain tissue, the other two-thirds presumably diffusing into blood.

Cerebrospinal and Brain Extracellular Fluid Volumes

As with other tissues, an extracellular fluid (ECS) compartment exists within the central nervous system (CNS). In addition, nervous tissue contains and is surrounded by CSF which, if not the same as brain interstitial fluid, is in communication with it (Tschirgi, 1960). Determination of the volume of these two compartments is helpful for a basic understanding of the chemical environment of the CNS, in the clinical assessment of CSF and CNS abnormalities, in determining intrathecal drug dosages, and in calculating the flux of materials between CSF and brain ECS.

Table 2 compares the CSF volumes of various species as determined by several methods; most experiments employ the technique of perfusing the CSF system. Values for total CSF and ventricular volumes are shown, and, so that comparisons can be made among species, ventricular volume is also expressed as a function of brain weight. Although, among species, there is wide variation in the absolute values for both ventricular CSF and total CSF volumes, the ventricular volume expressed per gm of brain is (excepting the goat) relatively constant. This indicates that, in general, there appears to be a proportionate increase in ventricular volume with increasing brain weight. The data in Table 2 also reveal good agreement between the volumes obtained using the perfusion technique and those determined either by open drainage or ventricular casts. Thus, while similar ventricular volumes have been reported for the same species using different techniques (cat and goat, Table 2) the use of different methods has caused considerable confusion in the determination of the actual volume of brain extracellular fluid. Over the years a number of methods have been employed, many giving conflicting results.

Estimates of the size of the brain ECS have been obtained from electron microscope studies of nervous tissue. These studies have indicated a virtual absence of extracellular space in brain tissue ranging between 0 and 5 percent of the total brain volume (Maynard et al., 1957). These

Table 2. Cerebrospinal fluid volumes in different vertebrate species.

Animal	Method of Measurement	Ventricular volume (ml)	Ventricular volume (µl/gm brain)	Total CSF Volume (ml)	Reference
cat	free drainage from cerebral aqueduct	0.9	33	4.4	Flexner, 1933
cat	ventriculocisternal perfusion	0.8	30		Lorenzo et al., 1970
	ventriculo-lumbar perfusion			4.0	
cat	ventriculocisternal perfusion	1.4	52		Sahar et al., 1970
	ventricular casts	1.5			
dog	ventriculocisternal perfusion	3.0	39		Bering & Sato, 1963
	Sum of values from ventriculocisternal & subarachnoid-cisternal perfusions			8.8	
goat	free drainage from cisternal magna			25	Pappenheimer et al., 1962
	ventriculo-subarachnoid perfusion			22.7	
	ventriculocisternal perfusion	11.0	107		
monkey	CSF dilution	3		9	Wellman et al., 1968
human (adult)	ventricular casts	22	16		Last & Tompsett, 1953
human (adult)		20	15	140	Millen & Woollam, 1962

workers indicated that brain cellular elements were separated by fairly constant gaps, 150-200°A wide. Maynard et al. argued that it is unnecessary to postulate the presence of a special blood-brain barrier and suggested that this barrier is a lack of ECS in the brain. However, the credibility of these electron microscope studies has declined since the recognition that neural tissue swells considerably and rapidly during the process of fixation (Van Harreveld, 1961; Davson, 1967). Van Harreveld et al. (1965) published electron micrographs of rapidly frozen brain tissue, showing enlarged extracellular spaces and yielding values for brain ECS of 18-25 percent of brain weight as compared with approximately 6 percent of tissues that were asphyxiated for 8 minutes before fixation.

Allen (1955) incubated brain slices in a medium containing inulin or ferrocyanide as extracellular markers (foreign substances that equilibrate rapidly between blood and interstitial fluid but do not penetrate cells). He found that the brain spaces occupied by these molecules increased with time presumably as a result of the markers entering cells. By extrapolating back to zero time he estimated a normal brain extracellular space of 14-17 percent. Davson and Spaziani (1959) incubated brain slices with sucrose and chloride. They found that the sucrose space was not affected by incubation time, remaining steady at 13 percent of brain weight. They concluded, however, that their tissue slices

were not normal as the chloride-space gradually increased with incubation time,presumably due to the penetration of chloride into cells. Davson (1967) states there are two major sources of error with this in vitro technique of estimating brain ECS. First, unless the tissue slice is very thin, oxygenation of the tissue will be poor, causing cerebral edema,thereby decreasing the size of the ECS. However, cutting the brain tissue slices too thin will cause so many cut ends of cells on the surfaces of the tissue slice that significant penetration of these cut cells by the marker will occur. In addition, any estimate of the brain extracellular space obtained by plotting the brain space of the marker against incubation time and extrapolating back to zero time can result in a falsely low value for the ECS size. Davson (1967) indicates that these in vitro experiments suggest a CNS extracellular space of 10-15 percent but, because the tissue is not normal, they are not convincing.

It has been assumed that the CNS extracellular fluid resembles the extracellular fluid of other tissues and that it is the primary container of the two "extracellular ions," Na^+ and Cl^- (Tschirgi, 1960). In many tissues the extracellular space closely approximates the Na^+ and Cl^- space. Since the mammalian brain has high concentrations of Na^+ and Cl^-, the size of the Na^+ and Cl^- spaces in brain have been accepted as the size of the CNS extracellular space (Katzman, 1966). Elliot (1949) indicated the rabbit brain had a Cl^-

space of 40 percent of the total brain volume while Bourke et al. (1965) found a Cl^- space ranging between 20-40 percent of brain weight in the guinea pig, rabbit, cat, monkey and sheep. The difficulty with using this method of estimating the size of the CNS interstitial space is knowing the extent to which Na^+ and Cl^- are, indeed, extracellular.

Another method of estimating the ECS of brain is to present the extracellular markers via blood (or plasma) to the CNS. Generally the markers used have been inulin, ferrocyanide, sulfate, iodide, thiocyanate, radio-iodinated human serum albumin (RIHSA) and sucrose introduced into the blood by either single intraperitoneal (ip) or intravenous (iv) injection or by constant infusion into blood (Davson and Spaziani, 1959; Reed and Woodbury, 1963; Pollay, 1966; Davson, 1967; Davson and Segal, 1969; Pollay and Kaplan, 1970). This technique has generally given small values for brain space due to the combined effects of their restricted penetration across the blood-brain barrier and the "sink effect" of CSF (Davson and Segal, 1969; Pollay and Kaplan, 1970). Reed and Woodbury (1963) obtained brain spaces in rats of 2 percent for inulin, 6 percent for iodide, and 4 percent for sucrose after giving these markers iv or ip. Davson and Spaziani (1959) found iodide and sucrose spaces in rabbit brains of 3-4 percent following two hours of continuous infusion. Davson and Segal (1969) found a brain sucrose space in the rabbit of about 3 percent following

constant iv infusion of this marker for 1.5 hours. However, if the CSF was replaced by silicone (given via a bilateral ventriculocisternal perfusion) during the iv infusion of sucrose, the sucrose brain space was greater than 6 percent. These workers concluded that CSF acts as a sink and prevents the extracellular fluid from coming into equilibrium with plasma. Removal of the sink action (in this case by perfusing the ventricular system with silicone) resulted in a larger measured brain space. Pollay (1966) infused thiocyanate into the ear vein of rabbits and found (at low plasma thiocyanate concentrations) a brain thiocyanate space of 10 percent of brain weight. If plasma thiocyanate levels were increased (from 1.0 to 3.0 mM/ml) the brain thiocyanate space increased to 20 percent. If sodium iodide was given iv or 2,4-dinitrophenol was injected into the CSF, the rabbit brain thiocyanate space was calculated to be 14-15 percent at low plasma thiocyanate levels. Pollay concluded that thiocyanate was actively transported from CSF to blood and this transport was a major component of the CSF sink action for brain thiocyanate. Altering the effectiveness of this transport process (by saturation, competitive inhibition or inhibiting cellular respiration) reduced the effectiveness of the CSF sink action and gave a more accurate estimate of the brain thiocyanate space.

Presentation of the extracellular marker to neutral tissue by way of the CSF has the advantage of circumventing the blood-brain barrier and maintaining the tissue normal.

The markers can be introduced either by a single intracisternal (IC) injection (Reed and Woodbury, 1963; Bourke et al., 1965; Van Harreveld et al., 1966) or by ventriculocisternal perfusion (Rall et al., 1962; Woodward et al., 1967; Baethman et al., 1970; Heisey, 1971). Reed and Woodbury (1963) found a sucrose brain space in rats of 15-38 percent. Bourke et al. (1965) calculated thiocyanate, sucrose and inulin brain spaces for 11 mammalian species. They found the thiocyanate brain space ranged from 17 percent in the mouse to 56 percent in the whale. Inulin and sucrose spaces ranged from 8.5 percent to 53 percent in the mouse and whale, respectively. These workers found that the sizes of the indicator spaces vary among species as a function of the logarithm of the brain weight. Van Harreveld et al. (1966) computed a sulfate space for the rabbit brain of 15-30 percent of the total tissue volume. Rall et al. (1962) perfused dog brains and found an ECS (using inulin as an indicator) of 7-14 percent. Woodward et al. (1967) found an inulin space in the rat cerebral cortex of 13.5-14.5 percent after 6 hours of perfusion. Baethman et al. (1970) calculated a thiocyanate space in rat brains of 15.4 percent after 90 min. of perfusion. Heisey (1971) found in turtle brains a RIHSA space of 4-5 percent, an inulin space that varied between 2-10 percent and a fructose space of 14 percent. He indicates that there is an inverse relationship between the molecular weight of the extracellular

marker and the calculated ECS and that there appears to be a direct relationship between the size of the inulin and RIHSA spaces and the length of perfusion. Longer perfusions apparently allowed time for greater penetration of the inulin and RIHSA from the ventricles into brain, thereby giving larger calculated brain spaces. Davson (1967) states that unless the marker is completely impermeable to the blood-brain barrier, the blood can act as a sink and drain away the tag presented to the brain by CSF. He emphasizes this point by demonstrating that a sucrose space of 10 percent is found for the rabbit brain when sucrose is presented to the brain by way of the blood and CSF (ventriculocisternal perfusion). When sucrose is presented to the brain by ventriculocisternal perfusion only, a 6 percent sucrose space is obtained.

The controversy surrounding the size of neural ECS has resulted mainly from electron micrographs of brain tissue that was abnormal due to fixation and because of the slow penetration of molecules from blood to brain. However, with the use of improved tissue fixation techniques for electron micrography and presentation of extracellular markers to the brain by way of the CSF, it appears that the size of the brain ECS is 10-15 percent of total brain weight; a value that is similar to the ECS size in other tissues.

Effect of Ionic Changes in Cerebrospinal Fluid on Cardiovascular and Respiratory Functions

Many workers have investigated the relationship between the ionic composition of the fluid environment of the CNS and various aspects of CNS function. The majority of these studies have been conducted on anesthetized mammals in which the relationship between changes in the CNS ionic environment and various autonomic functions and/or electrical activity of the brain was observed.

In general three methods have been used to alter the ionic composition of the fluid environment of the CNS and are classified as follows:

a. Intravascular injection of solutions with different ionic composition

b. Intraventricular or intracisternal injection of solutions with altered ionic composition

c. Perfusion of the CSF system with an ionically altered artificial CSF

Most studies have been performed on anesthetized mammals. All anesthetics are known to affect autonomic function (e.g., blood pressure, heart and respiratory rate) either as a result of or in addition to their generalized CNS depressing activity. In general, barbiturates (given intravenously or intraperitoneally) depress respiratory activity and moderately reduce blood pressure in mammals (Goodman and Gilman, 1965). Similarly, administration of

the inhalation anesthetics halothane and methoxyflurane is usually accompanied by varying degrees of hypotension and depressed respiration,whereas systemic arterial blood pressure generally increases moderately during cyclopropane inhalation (Emerson and Massion, 1967; Goodman and Gilman, 1965). Anesthesia affects blood pressure in birds as in mammals. Sturkie (1965) indicated that pentobarbital sodium, sodium phenobarbital, sodium barbital and urethane administered either intravenously or intraperitoneally resulted in a significantly depressed blood pressure (30 mm Hg systolic with pentobarbital sodium). Consequently, in any study of CNS control of cardiovascular function in either anesthetized birds or mammals, one must be aware of possible anesthesia influences (e.g., attenuation of responses due to experimental alterations) on the results obtained.

Hooker (1915) perfused the vascular system of the brains of dogs with blood in which the K^+ and Ca^{++} concentrations had been altered. He found that increasing the Ca^{++} concentration caused a stimulation of the respiratory center (as evidenced by a greater rate and amplitude of respiration) and increased heart rate. Conversely, increasing the K^+ concentration inhibited the respiratory center and slowed the heart rate. Rubin et al. (1943) continuously injected the femoral vein of cats with isotonic solutions of potassium chloride or calcium chloride and simultaneously recorded the electroencephalogram (EEG) and electrocardiogram

(EKG). They found K^+ or Ca^{++} produced slowing of the EEG at the time of development of intraventricular block or of cardiac arrest. They concluded that K^+ and Ca^{++} have no demonstrable effect on the EEG of the cat.

The results of injecting varying concentrations of potassium and calcium salts either into the brain ventricles or the cisterna magna of mammals are in good agreement. The following studies provide a full survey of the effects of altering the CSF K^+ and Ca^{++} concentrations by intraventricular or intracisternal injections in a variety of anesthetized mammalian species: Huggins and Hastings, 1933; Resnik et al., 1936; Mullins et al., 1937; Downman and Mackenzie, 1943; Smolik, 1943; Stern, 1945; Walker et al., 1945; Cicardo, 1949; Cicardo, 1950; Feldberg and Sherwood, 1957; and Cooper et al., 1958. In general, when a solution with a high K^+-Ca^{++} ratio (due either to excess K^+ or lowered Ca^{++}) is injected either intraventricularly or intracisternally, there is increased muscular activity, agitation, stimulation of respiration (greater rate and depth of breathing), a substantial elevation of arterial blood pressure and slowing of the heart rate. Slight elevations in the K^+ levels sometimes produced an initial fall in arterial pressure along with a bradycardia. Large elevations in CSF K^+ (around 10 times normal levels) results in apneusis associated with circulatory collapse. Decreased CSF K^+ levels were generally without effect. Increased CSF Ca^{++}

levels resulted generally in a condition of torpor with a decrease in the rate and depth of breathing, a decline in arterial pressure, and a general muscular relaxation.

Perfusion of the cerebral ventricles with an artificial CSF containing altered concentrations of K^+ and/or Ca^{++} has confirmed the results obtained with single intracisternal injection. Merlis (1940) perfused the lower spinal subarachnoid space in anesthetized dogs with the spinal cord sectioned at T10. The calcium content of the perfusion fluid was altered in these experiments. Merlis found that Ca^{++}-free solutions produced augmentation of the spinal flexion reflex, an increase in muscle tone and spontaneous twitching of the muscles in the lower half of the body. He attributed the muscle twitching to an increased responsiveness of the spinal cord neurons to normal afferent impulses from the periphery. Leusen (1950) perfused the cerebral ventricles in anesthetized dogs after bilateral vagotomy and carotid sinus isolation. He found that an excess of K^+ in the perfusate caused an increase in the arterial pressure and enhanced vasomotor reflexes. Perfusion with a K^+-free solution was without effect. Increased Ca^{++} concentration in the perfusion fluid resulted in a lowering of the blood pressure, whereas lowered Ca^{++} caused a rise in arterial pressure and augmentation of vasomotor reflexes. Devos (1951) also used a ventriculocisternal perfusion technique in anesthetized dogs. He indicated

that an excess of K^+ in the perfusate caused an increase in arterial pressure and a reflex bradycardia. Lowered K^+ did not affect blood pressure or heart rate. High Ca^{++} concentrations in the perfusion fluid caused a depression of both arterial pressure and heart rate whereas lowered Ca^{++} concentrations provoked a rise in blood pressure and lowered heart rate. Pappenheimer et al. (1962) found that lowering the Ca^{++} concentration by 60 percent in the fluid perfusing the cerebral ventricles of unanesthetized goats resulted in the animal becoming restless and excitable. Also evident were large increases in arterial pressure (at times exceeding 220 mm Hg) that remained elevated during the perfusion. This hypertension was generally accompanied by a bradycardia and an altered EKG. Horsten and Klopper (1952), using an artificial CSF with altered K^+ and Ca^{++} concentrations, perfused the cerebral ventricles of anesthetized cats while recording the cortical EEG. They found increasing the K^+ content of the perfusion fluid led to formation of spike waves in the EEG. K^+-free or Ca^{++}-elevated perfusions had no effect on the EEG. However, decreasing the Ca^{++} concentration of the perfusion fluid resulted in slow waves (with fast waves superimposed) in the EEG. These workers feel that the changes seem in the cortical EEG following alterations in the ionic composition of CSF were not due to a direct action of the ions on the cortex but rather a reflection of an effect on the brain stem.

From these studies it appears that the ionic composition of CSF plays an important role in the maintenance and variation of membrane potentials in the CNS and in the regulation of certain autonomic processes.

STATEMENT OF THE PROBLEM

The purpose of this study is to determine in the anesthetized chicken, using ventriculocisternal perfusion, the volume, pressure and rates of formation and absorption of CSF; to obtain an estimate of the size of the brain extracellular space; to investigate the movement of radioiodinated human serum albumin (RIHSA), creatinine, ^{22}Na, ^{42}K, and ^{45}Ca out of the CSF; and to investigate the effects of changes in the K^{+} and Ca^{++} concentration in CSF on cardiovascular function.

METHODS AND MATERIALS

A. Animals

Sexually mature female single-comb white leghorn chickens (weighing between 1 and 2 kg each were obtained from the Michigan State University Poultry Farm and were maintained at 25 ± 1°C with cycles of fourteen hours of light and ten hours of dark. Birds were housed one per cage with feed (MSU cage layer ration) and water given ad libitum.

B. Anesthesia

1. Phenobarbital sodium

Some birds were anesthetized with phenobarbital sodium (170 mg/kg; Merck and Co., Rahway, N.J.) given via the brachial vein. The humerus was exposed and broken to provide an auxillary route for ventilation since there is a unique connection, in birds, between the lungs (via the auxillary air sac) and the humerus air sac. This procedure was necessary because chickens anesthetized with phenobarbital often experience respiratory difficulties due to excessive mucous secretion in the trachea.

2. 2,2-dichloro-1,1-difluoroethyl methyl ether (Metofane)

The remaining animals used in these studies were anesthetized with the inhalation anesthetic, Metofane (Pitman-Moore, Washington Crossing, N.J.). These chickens were brought to surgical stages of anesthesia by placing their heads in a mask supplied with Metofane and compressed air. When an adequate surgical level was obtained (as indicated by pupillary dilation and muscle relaxation), the trachea was exposed, incised, and cannulated with a 3-4 cm length of rubber tubing. This tubing was connected to a T-tube, one arm of which was open to room air and the other to the anesthesia delivery instrument, providing a non-rebreathing system.

Compressed air, the carrier gas, was delivered through a flowmeter (Ohio Medical Products, Cleveland, Ohio) and past a vaporizer (Ohio Medical Products, Cleveland, Ohio) containing liquid Metofane. The amount of Metofane entering the carrier-gas stream was adjusted by means of an adjustable orifice in the vaporizer chamber. Metofane-air mixture was delivered to the bird at a rate (700-900 cc/min) sufficient to supply the bird's minute ventilation. The amount of Metofane delivered to the animal could not be determined because: (1) With a non-rebreathing system, an unknown amount of Metofane will escape to room air instead of being delivered to the animal; and (2) An unknown quantity of the Metofane delivered to the bird will be sequestered in the

various air sacs associated with the avian respiratory system. Pupillary dilation, response to pinching of the comb or toes and general muscle tone were used as qualitative indicators of the stage of anesthesia.

C. Surgical Procedures

The left femoral artery was cannulated with PE-90 tubing and arterial pressure monitored using a Statham pressure transducer (Model P23AC; Grass Instrument Co., Quincy, Mass.) and a Grass polygraph (Model 5; Grass Instrument Co., Quincy, Mass.). The arterial pressure transducer was calibrated using a mercury manometer; the response to pressure was linear over the range 0-200 mm Hg.

With the chicken prone, the head was secured in a stereotaxic frame (Model 4C; H. Neuman and Co., Skokie, Ill.), approximately 10 cm above heart level by means of earbars inserted into the external auditory meatus and a pin through the external nares attached to a Y-shaped yoke secured to the support stand of the stereotaxic frame. The animal's neck was acutely flexed so that the parietal surface of the skull assumed an angle approximately 30° from the horizontal. A midline incision from the posterior edge of the comb extended caudally to the fifth or sixth cervical vertebra. A portion of the parietal bone, 7 mm posterior to the bregma and 6 mm lateral to the midline, was abraded with a dental drill and burr (Model 21, Foredom Electric Co., New York,

New York) and removed with a small dental spatula, which exposed the dura overlying the left cerebral hemisphere. The atlantooccipital membrane overlying the cisterna magna was exposed by blunt dissection of the complexus and longus colli anterior muscles.

The left lateral ventricle was penetrated at a 20° angle to the dura with a short-bevel 27 gauge, 2 inch needle (Vita Needle Co., Needham, Mass.) held in a micromanipulator (Model MM-3; Eric Sobotka, Inc., Farmingdale, New York). The needle was connected to a motor-driven syringe pump (Model 940; Harvard Apparatus Co., Dover, Mass.) and a Statham pressure transducer (Model P23BC; Grass Instrument Co., Quincy, Mass.) by means of a male "T" adaptor and PC-60 tubing (Figure 1). A water reservoir (the height of which was adjustable) was used to calibrate the pressure transducer; the response to pressure was linear over the range 0-40 mm H_2O. Zero hydrostatic pressure was set at the level of the stereotaxic earbars. The perfusion pressure was continuously recorded on a Grass model 5 polygraph.

A low flow of artificial CSF was started through the needle prior to its insertion into the brain. Pressure due to the resistance of the inflow needle and tubing was recorded when the tip of the needle was placed perpendicular to and on the dura mater. As the needle was lowered slowly through the dura into brain tissue, pressure rose and

Figure 1. Diagram of brain ventricular perfusion system in reference to a saggital midline section of a chicken head (not drawn to scale).
Dorsal needle is in left lateral ventricle; posterior needle is in cisterna magna. CH, cerebral hemisphere; C, cerebellum; M, medulla; $\dot{V}_i$, perfusion inflow; $\dot{V}_o$, perfusion outflow; c_i, inflow concentration; c_o, outflow concentration.

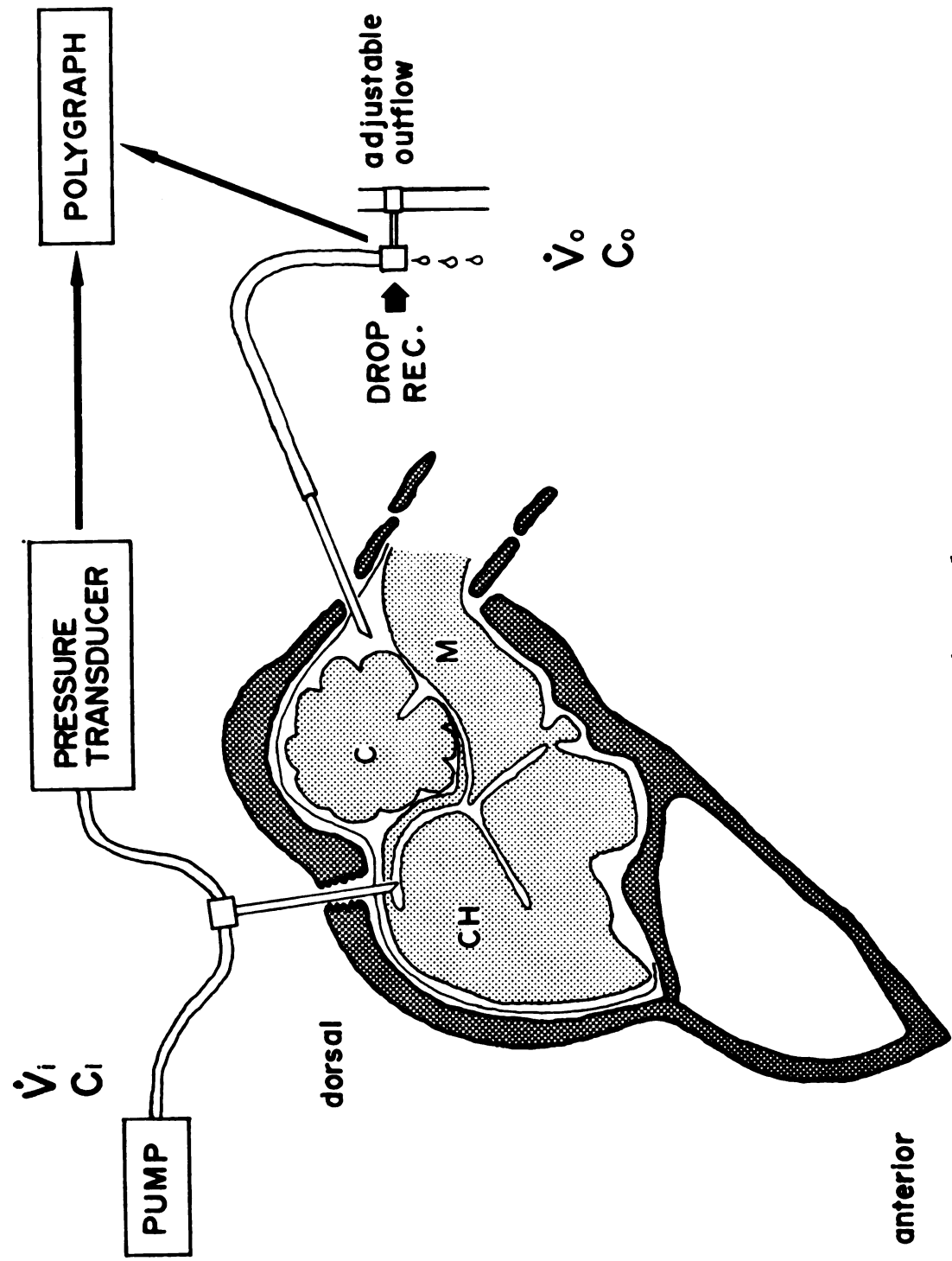
POLYGRAPH
adjustable outflow
PRESSURE TRANSDUCER
DROP REC.
$\dot{V}_o$ C_o
$\dot{V}_i$ C_i
C
M
CH
PUMP
dorsal
anterior

Figure 1

remained elevated. When the ventricle was penetrated (generally 1-2 mm below the dura), there was an abrupt fall in pressure.

The cisterna magna was punctured with a short-bevel, 23-gauge, 2 inch hubless needle (Vita Needle Co., Needham, Mass.) fixed in a micromanipulator (Baltimore Instrument Co., Baltimore, Md.) and attached by PE-50 tubing to a reservoir of artificial CSF held 20 cm above the head. The needle, directed anteriorly at a 28° angle to the atlantooccipital membrane was rapidly lowered to a depth of 8 mm below the dura. Simultaneously there was a sharp rise in intraventricular pressure; pressure dropped quickly when the tubing was disconnected from the reservoir and the cisternal needle withdrawn 3-4 mm. This fall in pressure concomitant with fluid flow through the cisternal cannula, indicated hydraulic continuity through the ventricular system. The cisternal cannula was connected by PE-50 tubing to a photoelectric drop recorder (Model PTT1; Grass Instrument Co., Quincy, Mass.), the height of which could be varied with respect to the stereotaxic earbars (Figure 1). Pressure in the ventricular system was set by the level of the outflow tubing. Intraventricular pressure was calculated by subtracting the pressure with the needle tip on the dura from that with the needle tip in the lateral ventricle.

D. Cerebral Ventricular Perfusion Technique

1. Perfusion fluid composition

In all studies, the total osmolarity of the perfusion fluid was between 280-295 mOsm per Kg H_2O and was equilibrated with 4-6% CO_2 just prior to use.

a. Normal artificial chicken CSF

The perfusion fluid used in most experiments was an artificial chicken CSF (Appendix 1) that was similar in composition to normal chicken CSF (Anderson and Hazelwood, 1969).

b. Ionically altered artificial chicken CSF

In the remaining studies, the perfusion fluid was an artificial chicken CSF with altered Na and K concentrations (Appendix 1); the other salts remaining in their original concentrations.

c. Test molecules added to the perfusion fluids

The test molecules used in these experiments were:

1. ^{22}Na (3.0 μc/ml; New England Nuclear Corp., Boston, Mass.)
2. ^{45}Ca (1.0 μc/ml; New England Nuclear Corp., Boston, Mass.)
3. ^{42}K (1.0 μc/ml; Michigan State University Triga reactor)

4. carbon-14 labelled glucose (D-glucose-^{14}C; 0.1 μc/ml; New England Nuclear Corp., Boston, Mass.)
5. carbon-14 labelled creatinine (creatinine carboxyl-^{14}C hydrochloride; 0.1 μc/ml; Nuclear Equipment Chemical Corp., Farmingdale, New York)
6. creatinine (0-2.5 mg/ml; Pfanstiehl Laboratories Inc., Waukegan, Ill.)

In all experiments either radioiodinated human serum albumin (RIHSA; 1.0 μc/ml; E.R. Squibb and Sons, Inc., New Brunswick, New Jersey) or inulin (1.0 mg/ml; Pfanstiehl Laboratories Inc., Waukegan, Ill.) was added to the perfusion fluid to measure bulk absorption of CSF.

2. Definition of perfusion period

At the start of each experiment the outflow concentration of test molecules was zero. Outflow concentrations increased with time with steady-state outflow concentrations reached in 20-30 min (Figure 2). The approach to the steady-state and three 10-20 minute collections of cisternal effluent in the steady-state constituted the first period of an individual experiment. Subsequent periods in the experiment (defined as either a change in intraventricular pressure due to a change in the height of the outflow tubing or a change in the inflow concentration of K, Ca or creatinine) were

Figure 2. Results of a single perfusion experiment from the left cerebral ventricle to the cisterna magna in a chicken. Outflow concentration of ^{14}C-glucose is expressed as a percentage of inflow concentration (ordinate) and is plotted as a function of perfusion time (minutes; abcissa). Zero time indicates introduction of test molecule to animal. Steady-state outflow concentration was approximately 84 percent of inflow concentration and was reached after about 30 minutes of perfusion.

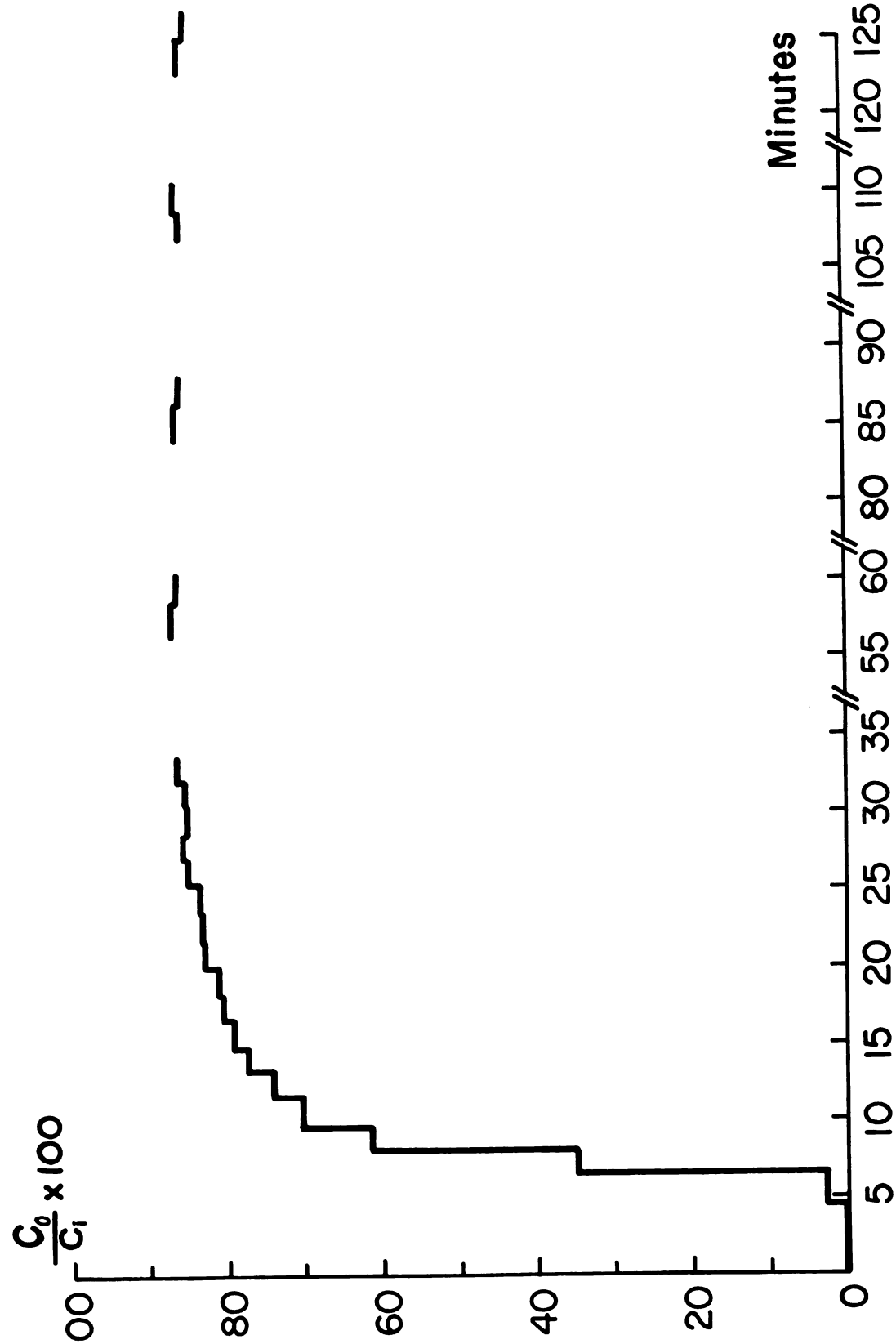
$\frac{C_0}{C_i} \times 100$
100
80
60
40
20
0
5
10
15
20
25
30
35
55
60
80
85
90
105
110
120
125
Minutes

Figure 2

made up of four collections of outflow fluid. The first outflow collection of each period was for at least 30 minutes to assure that steady-state outflow concentration of test molecules was reachieved. After the steady-state was attained, three additional 10-20 minute outflow vials were collected and it was from these vials that the outflow rate and test molecule concentration for that period was obtained.

3. Determination of inflow and outflow rates

Artificial CSF containing test substances was perfused into the lateral ventricular needle and collected from the needle in the cisterna magna. Inflow and outflow rates were determined gravimetrically for each experiment. Inflow perfusion rates varied between 38-52 µl/min but were constant for any one experiment. In most experiments inflow rate was determined by collecting fluid from the perfusion syringe over timed periods (10-15 minutes) in tared vials at the beginning and end of each experiment. For the experiments where inflow K, Ca and creatinine concentrations were varied, inflow rates were determined at the beginning and end of each period. An average outflow rate for each period was determined from three collections of cisternal effluent (in the steady-state) over timed periods (10-20 min) in tared vials.

4. Determination of test molecule concentration

a. In perfusion inflow and outflow

The inflow concentration of test molecules was determined in duplicate on 50 µl aliquots from the inflow syringe. Mean outflow concentrations for each period were determined from duplicate or triplicate 50 µl aliquots from the three outflow fluid vials collected in the steady-state for that period. Concentrations of RIHSA, ^{22}Na and ^{42}K were determined by 1-5 minute counts with a gamma well spectrometer (Model 530, University II Series; Baird-Atomic, Cambridge, Mass.). Carbon-14 and ^{45}Ca were counted integrally in 10 ml of scintillation fluid (Aquasol; New England Nuclear Corp., Boston, Mass.) with a trichannel liquid scintillation spectrometer (Model Mark I; Nuclear Chicago Corp., Des Plaines, Ill.) (Appendix 2A and 2B, respectively). Non-radioactive K and Ca concentrations were determined by flame photometry (Appendix 3) and atomic absorbance (Appendix 4), respectively. Creatinine was determined colorimetrically using an alkaline picrate method (Appendix 5); inulin by the resorcinol method (Appendix 6).

b. In brain tissue

Verification of needle placement and the perfusion path was made at the end of each experiment by perfusing methylene blue. The brain was removed, sectioned sagitally and both halves were examined for staining. The

two brain halves were then blotted, weighed, homogenized in water with a teflon pestle homogenizer (A.H. Thomas Co., Philadelphia, Pa.) and diluted to 25 ml. Duplicate 2 ml aliquots of the homogenate were counted in a gamma well spectrometer for 40 minutes for ^{131}I, ^{22}Na or ^{42}K activity. In addition, duplicate 50 µl samples from the inflow syringe and from two outflow vials (all diluted to 2 ml with water) were counted.

To determine ^{45}Ca activity, each brain half was treated as above. Triplicate 5 ml aliquots of the homogenates were pipetted into 10 ml of Aquasol scintillation fluid forming an opaque, stiff gel. These were counted integrally in a trichannel liquid scintillation spectrometer (Appendix 2B).

E. Calculations

The equations to be presented have been derived by Heisey et al. (1962). Sample calculations are shown in Appendix 7.

1. Definition of symbols

   $\dot{V}$ = rate of flow, µl/min

   i,o = subscripts referring to inflow and outflow, respectively

   f,a = subscripts referring to formation and absorption of CSF, respectively

c = concentration, quantity per unit volume

C_B = clearance of RIHSA or inulin, µl/min

2. Rate of CSF formation, $\dot{V}_f$

The fluid balance in the ventriculocisternal perfusion system is assumed to be (Literature review, p. 9).

$$\dot{V}_f + \dot{V}_i = \dot{V}_a + \dot{V}_o \quad (1)$$

Rearranging (1) gives the equation used to calculate CSF formation.

$$\dot{V}_f = \dot{V}_a + (\dot{V}_o - \dot{V}_i) \quad (2)$$

$\dot{V}_o$ and $\dot{V}_i$ are measured gravimetrically (Methods, D-3). Thus, in order to calculate $\dot{V}_f$, $\dot{V}_a$ must be determined.

3. Rate of CSF bulk absorption, $\dot{V}_a$

Evidence will be presented that loss of RIHSA and inulin from the avian ventricular system is predominantly by bulk absorption of CSF distal to the fourth ventricle (i.e., diffusion of these two molecules from the ventricular system is negligible). The calculation of $\dot{V}_a$ is then

$$\dot{V}_a = C_B = \frac{\dot{V}_i c_i - \dot{V}_o c_o}{c_o} \quad (3)$$

4. Clearance of smaller molecules from the ventricular system

For molecules smaller than RIHSA or inulin which can leave the ventricular system by simple diffusion or active transport, clearance is calculated by:

$$C_x = \frac{\dot{V}_i c_i - \dot{V}_o c_o}{\bar{c}} \tag{4}$$

where: C_x = clearance of a small molecule x, µl/min

$\bar{c}$ = mean ventricular concentration of x

The calculation of $\bar{c}$ was defined by Pappenheimer et al. (1961) as

$$\bar{c} = c_o + 0.37\ (c_i - c_o) \tag{5}$$

5. Outflux coefficient, K_D (non-bulk clearance)

The clearance of molecules smaller than RIHSA or inulin from the CSF system is made up of two components: (1) clearance due to bulk absorption; and (2) clearance due to diffusion and/or active transport. The clearance of the small molecule by bulk absorption is given by:

$$C_B \left(\frac{c_o}{\bar{c}}\right)_x \tag{6}$$

where: $\left(\frac{c_o}{\bar{c}}\right)_x$ = ratio of the cisternal outflow and mean ventricular concentrations of the small molecule, x.

Subtracting the bulk clearance of x from its total clearance gives clearance of x due to diffusion or active transport, i.e.,

$$K_{D_x} = C_x - C_B\left(\frac{c_o}{\bar{c}}\right)_x \tag{7}$$

where: K_{D_x} = non-bulk clearance of x, µl/min

K_D is also called the outflux coefficient of x. Expanding (7) gives the equation used to calculate K_D.

$$K_D = \frac{\dot{V}_i c_i - \dot{V}_o c_o - (C_B \cdot c_o)}{\bar{c}} \tag{8}$$

6. Outflux rate, $\dot{n}$

The outflux rate of x from CSF by diffusion or active transport is calculated as the difference between the rate at which x enters the ventricular system by perfusion ($\dot{V}_i c_i$) and the rate at which x leaves the CSF in the cisternal outflow ($\dot{V}_o c_o$) and by bulk absorption ($C_B \cdot c_o$). Stated mathematically:

$$\dot{n}_x = K_D \cdot \bar{c} = \dot{V}_i c_i - \dot{V}_o c_o - (C_B \cdot c_o) \tag{9}$$

where: $\dot{n}_x$ = outflux rate of x, quantity per minute

7. Mass balance determination

In order to further quantify the amount of material leaving the ventricles and to determine its final disposition (i.e., brain parenchyma or blood), a mass balance for four radioactive molecules (RIHSA, ^{22}Na, ^{45}Ca and ^{42}K) was determined using the following equations.

a. Total radioactive input

The total amount of radioactivity (I_T) added to the system from the perfusion inflow is equal to the inflow

rate ($\dot{V}_i$) times the inflow concentration (c_i) times the total perfusion time (t). Stated mathematically:

$$I_T = (\dot{V}_i c_i)\ t \tag{10}$$

b. Total radioactivity collected in perfusion effluent

The total amount of radioactivity collected from the cisternal effluent (O_T) is given by the perfusion outflow rate ($\dot{V}_o$) times the concentration in individual effluent samples (c_o) times the collection time of each sample (Δt) summed for all samples collected during an experiment (n). Stated mathematically:

$$O_T = \sum_{0}^{n} (\dot{V}_o c_o)\ \Delta t \tag{11}$$

c. Total radioactivity cleared from the ventricular system

The total radioactivity cleared from the ventricular system (C_T) is given as the product of the total CSF clearance (C_x) and mean ventricular concentration ($\bar{c}_x$) of molecule x times the clearance time of each period (Δt) summed for all clearance periods in an experiment (n). Stated mathematically:

$$C_T = \sum_{0}^{n} (C_x \bar{c}_x)\ \Delta t \tag{12}$$

d. Total radioactivity cleared from the ventricular system by bulk absorption

The total radioactivity removed from the ventricular system by bulk absorption (F_T) is given as the product of the clearance of RIHSA or inulin (C_B) and the outflow concentration of molecule x (c_{o_x}) times the clearance time for each period (Δt) summed for all clearance periods (n). Stated mathematically:

$$F_T = \sum_{0}^{n} (C_B \, c_{o_x}) \, \Delta t \tag{13}$$

e. Total radioactivity in brain tissue

The total radioactivity in brain tissue (B_T) was determined by multiplying the radioactive concentration of x in an aliquot of homogenized brain (c_{x_b}) times the dilution factor for the brain homogenate (r) times the total brain weight (W_B). Stated mathematically:

$$B_T = (c_{x_b} \cdot r) \, W_B \tag{14}$$

The calculations for the mass balance determinations were performed on the Michigan State University CDC 6500 computer (Appendix 8).

8. Distribution volume

The distribution volume (which is an estimate of the ventricular volume) of each molecule was determined by

integrating the outflow concentration approach to the steady-state with respect to time (Figure 2). Distribution volume was calculated using the following equation:

$$V_D = \frac{\sum_{0}^{n} \left[(\dot{V}_i c_{i_t} - \dot{V}_o c_{o_t} - C_x \bar{c}_t) \right] \Delta t}{\bar{c}} - V_{ds} c_i \qquad (15)$$

where:

V_D = distribution volume of x, µl

c_{i_t}, c_{o_t} = concentrations of x in the inflow and outflow, respectively at time t

$\bar{c}_t$ = mean concentration of x in CSF during time Δt (Equation 5)

$\bar{c}$ = mean ventricular concentration after distribution of x is complete

V_{ds} = volume of the perfusion needles and tubing (dead space volume), µl

n = number of samples

9. Brain space calculation

Assuming that the concentration of radioactive material in the brain was in equilibrium with the mean concentration in the ventricular system in the steady-state, brain space was calculated as:

$$S_x = \frac{R_{b_x} \cdot 100}{R_{CSF}} \qquad (16)$$

where: S_x = brain space of x, percent of brain weight

R_{b_x} = brain radioactivity, cpm/gm

R_{CSF} = CSF radioactivity, cpm/ml

F. Cardiovascular Measurements

Blood pressure was recorded on the Grass polygraph and heart rates were taken from the blood pressure records. Mean blood pressure was calculated from the following equation (R.K. Ringer, personal communication).

$$\text{mean pressure} = \frac{2\ (\text{diastolic pressure}) + \text{systolic pressure}}{3} \qquad (17)$$

Blood pressures and heart rates were measured for 30 minutes prior to and after perfusion with an unaltered artificial CSF had been initiated. The values obtained were used as control measurements, each animal serving as its own control. Blood pressure and heart rate was determined at three separate intervals during the steady-state portion of the experimental perfusion period and the results were expressed as a percent of control values.

G. Statistical Analysis

All data obtained were statistically analyzed using either continuous simple linear regression; student's "t" (paired or group comparisons) or analysis of variance (Sokal and Rohlf, 1969). A probability of 0.05 was used as the level of significance in all statistical tests.

RESULTS

Cerebrospinal Fluid Formation and Absorption Rates

The cerebral ventricular system of the chicken was perfused with an artificial chicken CSF containing either RIHSA or inulin. The clearance of either of these molecules was used to estimate the rate of CSF bulk absorption ($\dot{V}_a$; Equation 3, Methods) and CSF formation rate ($\dot{V}_f$; Equation 2, Methods). Figure 3 illustrates the relationship of CSF formation, RIHSA clearance (C_{RIHSA}) and perfusion outflow minus inflow rates ($\dot{V}_o$-$\dot{V}_i$) to intraventricular pressure (P). Data used to calculate lines in Figure 3 were obtained from 19 steady-state perfusion periods in 6 animals. Intraventricular pressure was altered by varying the height of the outflow cannula; each change in perfusion pressure represented a different clearance period (section D2, Methods). C_{RIHSA} increases linearly and ($\dot{V}_o$-$\dot{V}_i$) decreases linearly with intraventricular pressure over a range from 0 to 20 cm H_2O. Absolute values for the slopes of $\dot{V}_o$-$\dot{V}_i$) on P (-0.26 µl/min·cm) and C_{RIHSA} on P (0.22 µl/min·cm) are not statistically different ($P > 0.05$), which is interpreted to indicate that RIHSA is removed from CSF predominantly by bulk absorption thereby justifying its use in the chicken to measure

Figure 3. Mean cerebrospinal fluid (CSF) formation rate, RIHSA clearance, and difference between ventricular outflow and inflow rates as a function of intraventricular pressure. Mean CSF formation rate ($\dot{V}_f$; µl/min; left ordinate), RIHSA clearance (C_{RIHSA}; µl/min; left ordinate), and difference between ventricular outflow and inflow rates ($\dot{V}_o - \dot{V}_i$; µl/min.; right ordinate) plotted as a function of intraventricular pressure (P; cm H_2O; abscissa). Equations for lines were calculated by the method of linear regression and are:

$$\dot{V}_f = -0.04\ (\pm\ 0.03)\ P + 1.63$$

$$C_{RIHSA} = 0.22\ (\pm\ 0.05)\ P + 0.54$$

$$\dot{V}_o - \dot{V}_i = -0.26\ (\pm\ 0.05)\ P + 1.09$$

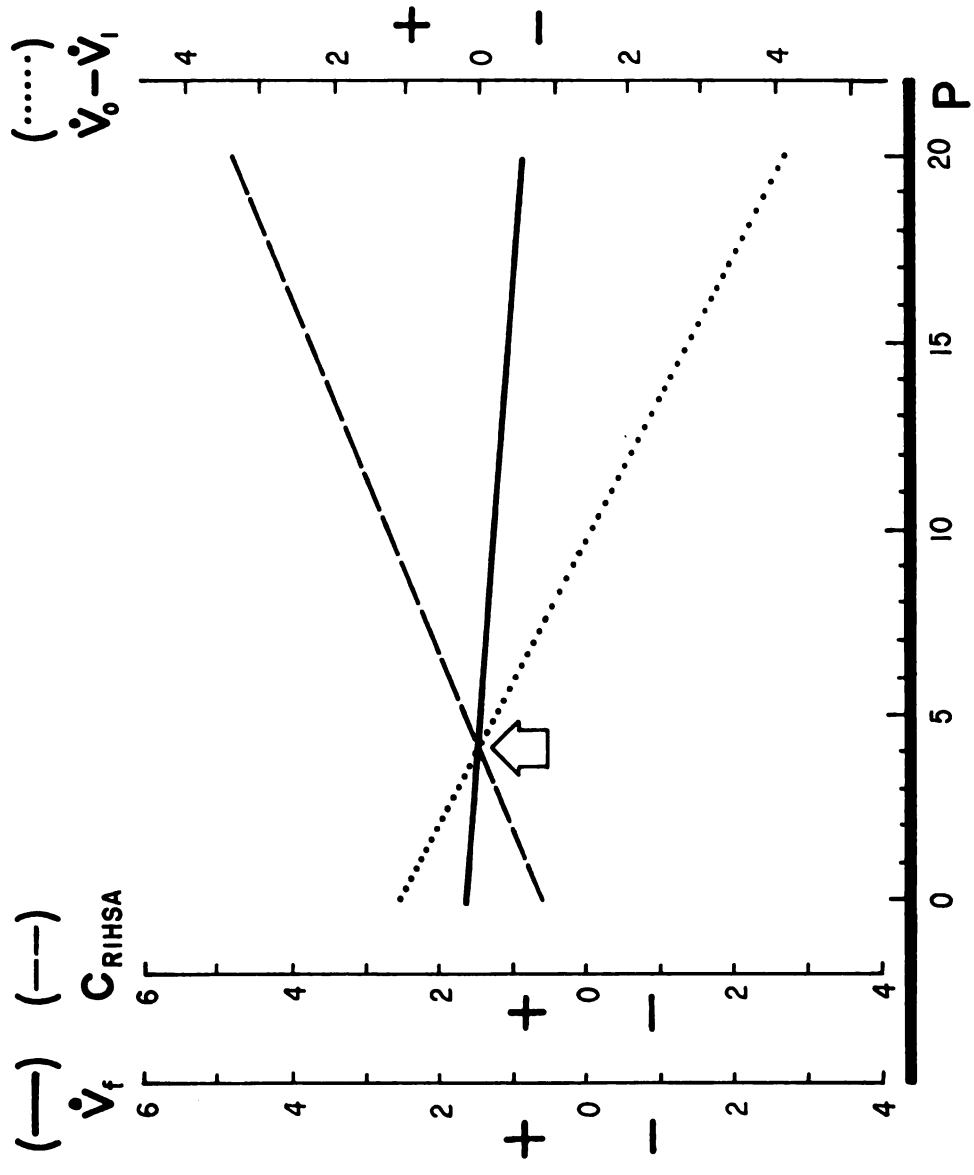

Figure 3

CSF bulk absorption. The regression line relating $\dot{V}_f$ to P was not different from zero ($P > 0.05$) over the range 0-20 cm H_2O, the implication being that intraventricular pressure had no effect on CSF formation rate in chickens. Where the regression lines for CSF formation and absorption are superimposed, the point of intersection (Figure 3; arrow) represents the normal rate of formation (i.e., where formation and absorption are equal) and the normal physiological pressure in the CSF system. A value for intraventricular pressure (4 cm H_2O) determined in this way coincides with that of CSF pressure measured directly from the cisterna magna in 20 experiments (4.2 ± 0.3 cm H_2O). Mean rate of CSF formation (calculated from 56 steady-state perfusion periods in 21 animals) was 1.4 ± 0.1 µl/min.

Molecular Flux from Cerebrospinal Fluid to Brain and Blood

The artificial CSF used in these studies contained (in addition to RIHSA or inulin) ^{22}Na, ^{42}K, ^{45}Ca or creatinine in order to evaluate the permeability characteristics of the avian cerebral ventricular system. Evidence given previously (Figure 3) indicates that the ependymal linings of the chicken ventricular system are essentially impermeable to large molecules like RIHSA and inulin and that these molecules are removed from CSF by a pressure-dependent bulk flow process. For smaller molecules (like ^{22}Na and creatinine) my data indicate that they are also removed from CSF by a

pressure-dependent process but, in addition, they exit by simple diffusion and/or active transport. Data presented in Figure 4 (from 14 steady-state perfusion periods in five animals) illustrate the separation of total ^{22}Na clearance (C_{Na}; Figure 4B; Equation 4, Methods) into bulk absorption (Figure 4C; Equation 6, Methods) and non-bulk clearance (Figure 4A; Equations 7 and 8, Methods) components. The difference between C_{Na} and the ^{22}Na absorbed in bulk with RIHSA or inulin is the outflux coefficient ($K_{D_{Na}}$) for ^{22}Na (Figure 4A). The regression line relating $K_{D_{Na}}$ to P is not significantly different from zero ($P > 0.05$), indicating that over the pressure range 0-20 cm H_2O, $K_{D_{Na}}$ is independent of intraventricular pressure. This shows the pressure-sensitive part of C_{Na} to be bulk absorption. Non-bulk clearance accounts for most of the ^{22}Na lost from the CSF system at low intraventricular pressures. At higher pressures, the amount of ^{22}Na lost by both routes is approximately the same.

Data in Figure 5 (from 16 steady-state perfusion periods in five animals) show the division of total creatinine clearance (C_{cr}; Figure 5B) into bulk clearance ($\dot{V}_a$; Figure 5C) and non-bulk clearance ($K_{D_{cr}}$; Figure 5A) components. The regression line of $K_{D_{cr}}$ vs P is not different from zero ($P > 0.05$) indicating that, like $K_{D_{Na}}$, $K_{D_{cr}}$ is independent of intraventricular pressure over the range 0.20 cm H_2O. At low intraventricular pressures, the amount of creatinine lost from the CSF system by bulk absorption

Figure 4. ^{22}Na non-bulk clearance, total ^{22}Na clearance, and ^{22}Na bulk absorption versus intraventricular pressure. Ordinate. A: ^{22}Na non-bulk clearance ($K_{D_{Na}}$; µl/min), B: Total ^{22}Na clearance (C_{Na}; µl/min.), and C: ^{22}Na bulk absorption $\left[\dot{V}'_a = C_{RIHSA}\ (c_o/\bar{c})_{Na}\right.$; µl/min.$\left.\right]$ vs. intraventricular pressure (P; cm H_2O; abscissa). Equations for lines were calculated by method of linear regression and are:

$$K_{D_{Na}} = 0.09\ (\pm\ 0.06)\ P + 2.86$$

$$C_{Na} = 0.31\ (\pm\ 0.09)\ P + 3.28$$

$$\dot{V}'_a = 0.22\ (\pm\ 0.06)\ P + 0.46$$

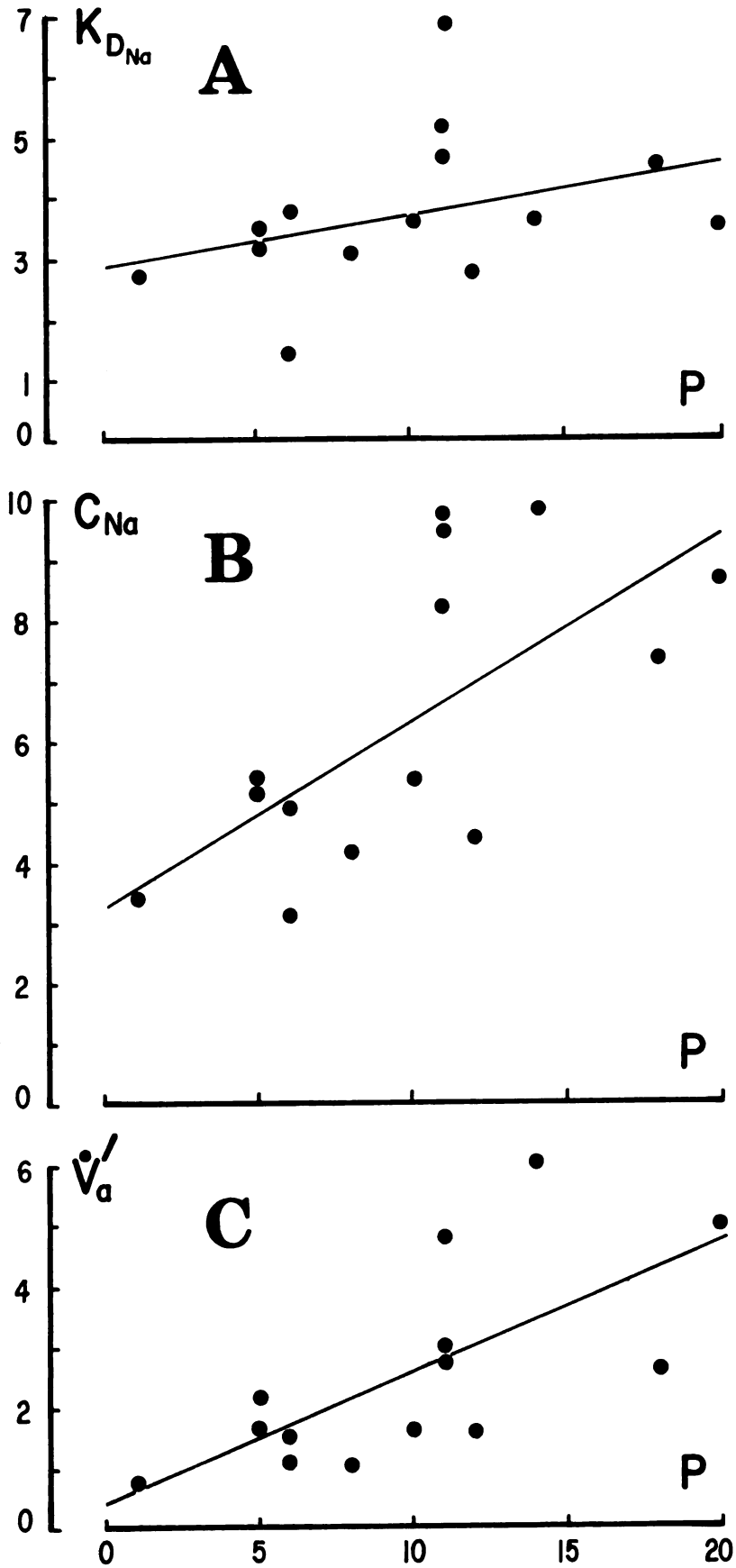

Figure 4

Figure 5. Creatinine non-bulk clearance, total creatinine clearance and creatinine bulk absorption versus intraventricular pressure. Ordinate. A: Creatinine non-bulk clearance ($K_{D_{cr}}$, µl/min.), B: Total creatinine clearance (C_{Na}, µl/min.) and C: Creatinine bulk absorption $\left[\dot{V}_a' = C_{RIHSA}\ (c_o/c_i)_{cr}\right.$; $\left.\mu l/min.\right]$ vs. intraventricular pressure (P; cm H_2O; abscissa). Equations for lines were calculated by method of linear regression and are:

$$K_{D_{cr}} = -0.06\ (\pm\ 0.06)\ P + 7.4$$

$$C_{cr} = 0.15\ (\pm\ 0.08)\ P + 7.7$$

$$\dot{V}_a = 0.21\ (\pm\ 0.04)\ P + 0.3$$

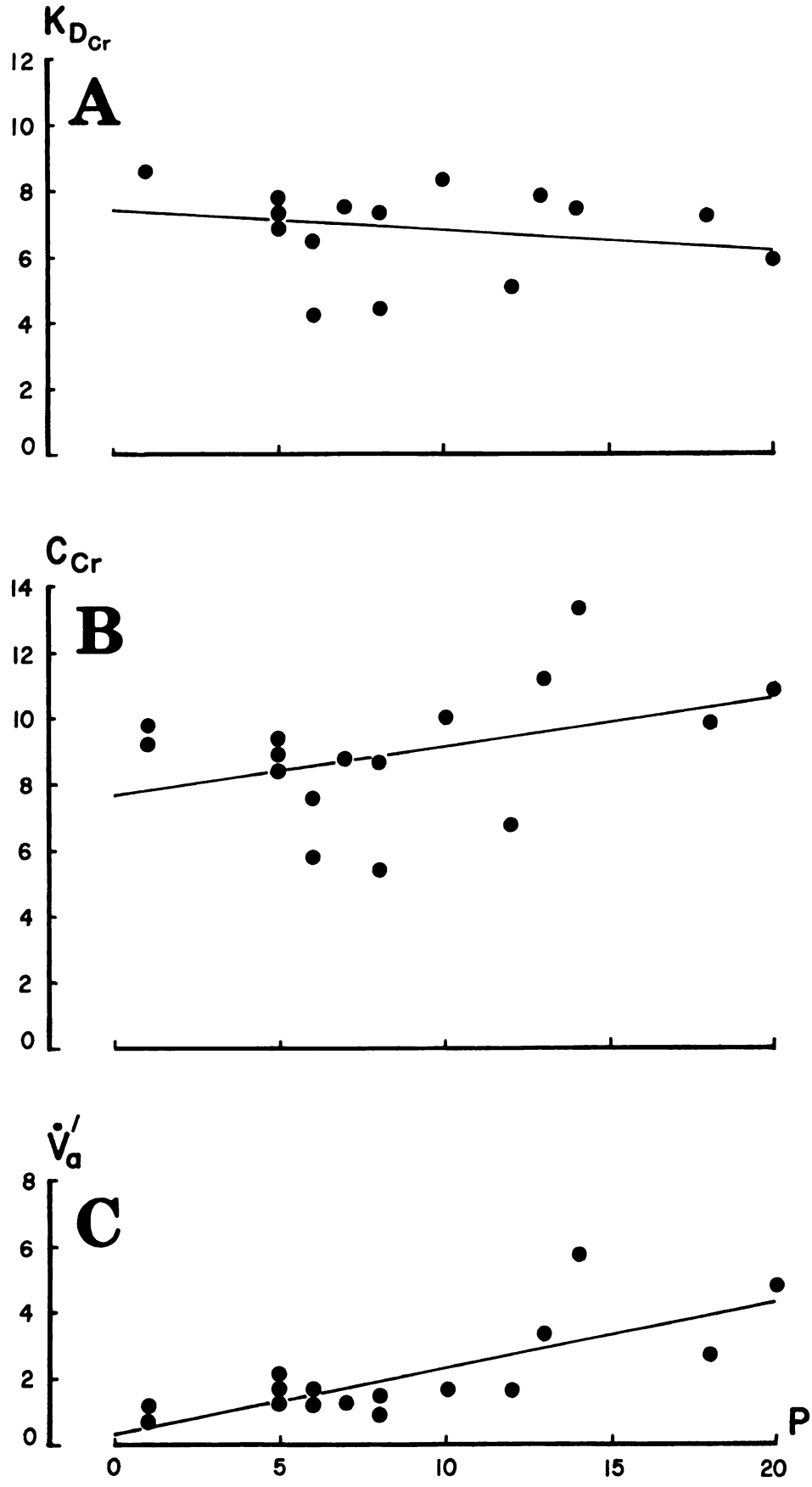

Figure 5

is negligible as compared with that removed by non-bulk means. Even at higher pressures, creatinine lost by non-bulk clearance exceeds that lost by bulk absorption.

While evidence presented in Figures 4 and 5 reveals that the non-bulk movement out of the CSF system for both an electrolyte and non-electrolyte was not influenced by intraventricular pressure, it gives no information as to whether this non-bulk clearance is by simple diffusion or active transport. The perfusion fluid inflow concentrations of creatinine, ^{42}K and ^{45}Ca were raised to see if the outflux of these molecules from CSF exhibited saturation kinetics as evidenced by changes in the outflux coefficients (K_D) and efflux rates ($\dot{n}$; Equation 9, Methods) of these three molecules.

Figure 6 is a plot of the creatinine outflux coefficient ($K_{D_{cr}}$) as a function of the ventricular perfusion inflow creatinine concentration ($[cr]_i$) from 21 steady-state perfusion periods in 10 animals. The regression line is not different from zero ($P > 0.05$) indicating that over the $[cr]_i$ range 0-225 mg/100 ml, $K_{D_{cr}}$ is not affected by $[cr]_i$. A concentration-independent K_D is consistent with but does not conclusively prove simple diffusion.

Creatinine outflux rate ($\dot{n}_{cr}$) as a function of inflow creatinine concentration ($[cr]_i$) is shown in Figure 7. Over Over the concentration range 0-225 mg/100 ml, $\dot{n}_{cr}$ increases linearly with $[cr]_i$ illustrating a concentration dependent

Figure 6. Creatinine outflux coefficient plotted as a function of perfusion inflow creatine concentration. Ordinate. Creatinine outflux coefficient ($K_{D_{cr}}$; µl/min.). Abscissa. Inflow creatinine concentration ($[cr]_i$; mg/100 ml). The equation for the line was calculated by the method of linear regression and is:

$$K_{D_{cr}} = -0.017\ (\pm\ 0.02)\ [cr]_i + 16.9$$

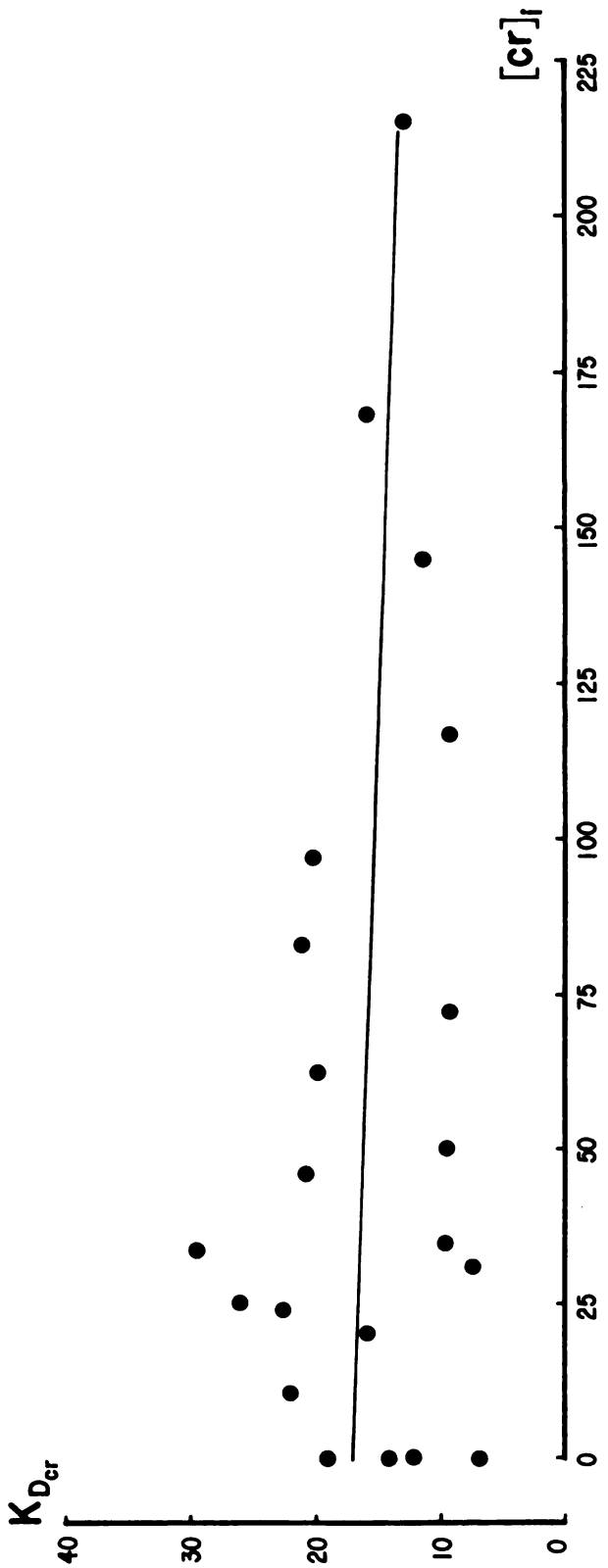

Figure 6

Figure 7. Creatinine outflux rate plotted as a function of perfusion inflow creatinine concentration. Ordinate. Creatinine outflux rate ($\dot{n}_{cr}$; mg/min.). Abscissa. Inflow creatinine concentration ($[cr]_i$; mg/100 ml). The equation for the line was calculated by linear regression and is:

$$\dot{n}_{cr} = 1.1\ (\pm\ 0.08)\ [cr]_i + 7.25 \times 10^{-4}$$

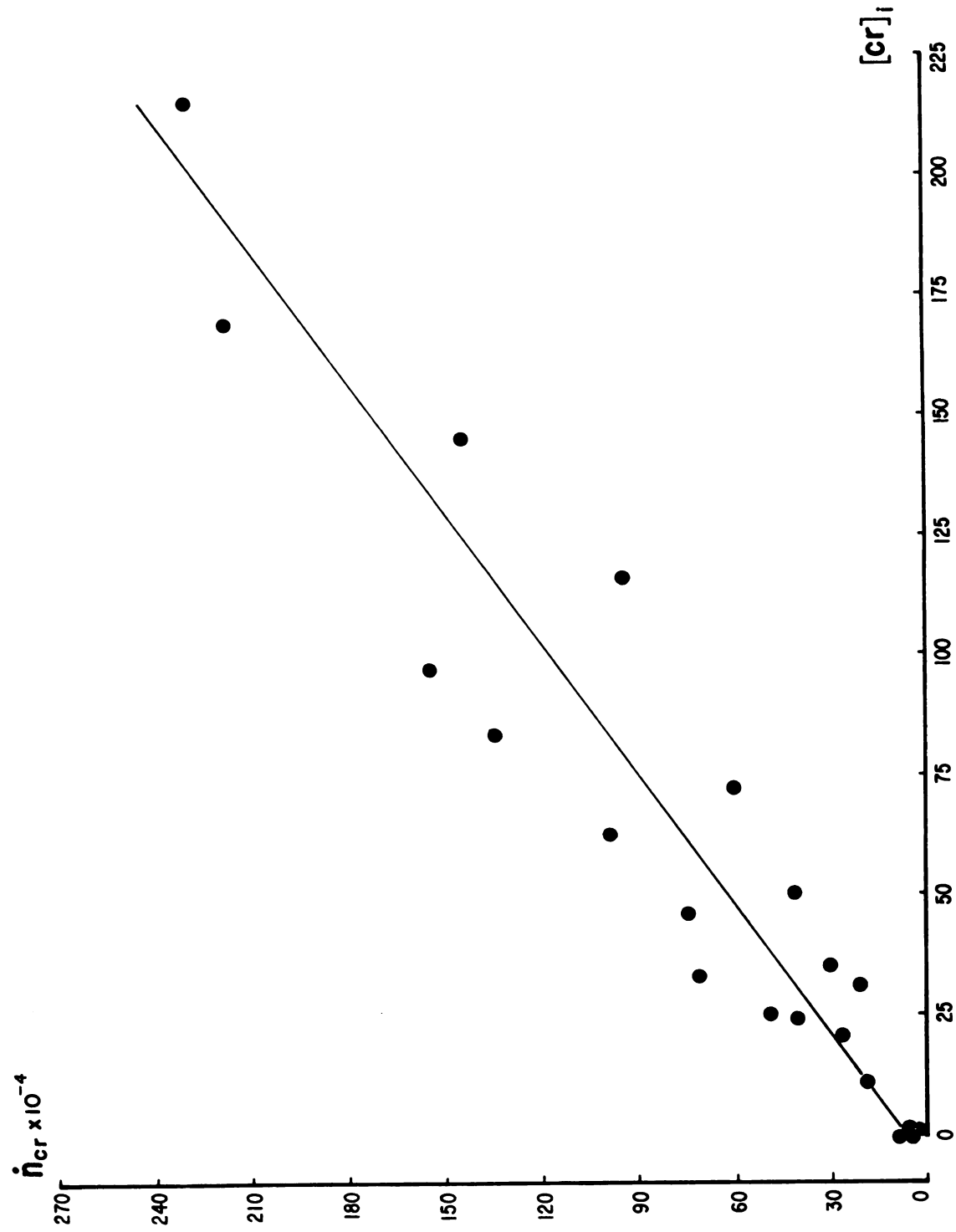
$\dot{n}_{cr} \times 10^{-4}$
270
240
210
180
150
120
90
60
30
0
0
25
50
75
100
125
150
175
200
225
$[cr]_i$

Figure 7

flux of creatinine from CSF. That $\dot{n}_{cr}$ is not saturable at high $[cr]_i$ implies either creatinine efflux from CSF is by non-carrier mediated diffusion, the carrier has not been saturated, or the carrier is not located at the ventricular ependyma.

Figure 8 is a plot of ^{42}K outflux coefficient (K_{D_K}) as a function of the inflow potassium concentration ($[K]_i$) in the perfusion fluid for 16 steady-state perfusion periods in six animals. The regression line is not different from zero ($P > 0.05$) suggesting that ^{42}K exits the CSF system by passive diffusion at least over the $[K]_i$ range 0-31 mEq/L.

Further indication of the passive ^{42}K outflux from CSF is shown in Figure 9 which is a plot of the ^{42}K outflux rate ($\dot{n}_K$) versus the inflow potassium concentration ($[K]_i$). The $\dot{n}_K$ increases proportionately to $[K]_i$ revealing no saturation of ^{42}K outflux from the avian ventricular system up to 31 mEq/L.

The ^{45}Ca outflux coefficient ($K_{D_{Ca}}$) and calcium outflux rate ($\dot{n}_{Ca}$) as a function of inflow calcium concentration ($[Ca]_i$) for 8 steady-state perfusion periods in three animals appear in Figures 10 and 11, respectively. The regression line relating $K_{D_{Ca}}$ and $[Ca]_i$ (Figure 10) is not significantly different from zero indicating that over the concentration range 0-5 mEq/L, $K_{D_{Ca}}$ is independent of $[Ca]_i$. ^{45}Ca flux from CSF is a positive linear function of $[Ca]_i$ (Figure 11).

Figure 8. Potassium outflux coefficient versus perfusion inflow potassium concentration. Potassium outflux coefficient (K_{D_K}; µl/min; ordinate). Inflow potassium concentration ($[K]_i$; mEq/L; abscissa). The equation for the line was calculated by the method of linear regression for 16 steady-state clearance periods in 6 animals and is:

$$K_{D_K} = -0.15\ (\pm\ 0.09)\ [K]_i + 15.9$$

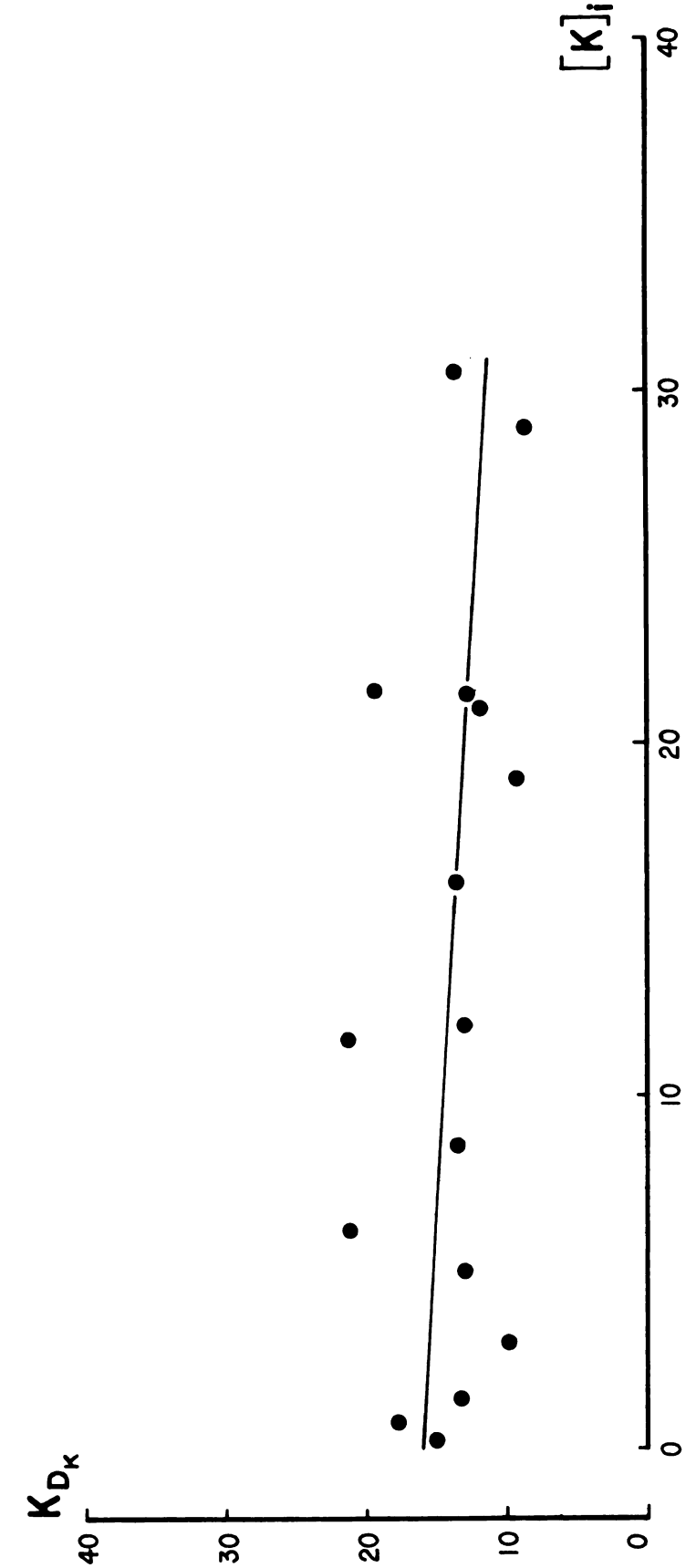

Figure 8

Figure 9. Potassium outflux rate as a function of perfusion inflow potassium concentration. Potassium outflux rate ($\dot{n}_K$; μM/min.; ordinate). Inflow potassium concentration ($[K]_i$; mM/L; abscissa). The equation for the line was calculated by the method of linear regression and is:

$$\dot{n}_K = 0.92\ (\pm\ 0.12)\ [K]_i + 3.3 \times 10^{-2}$$

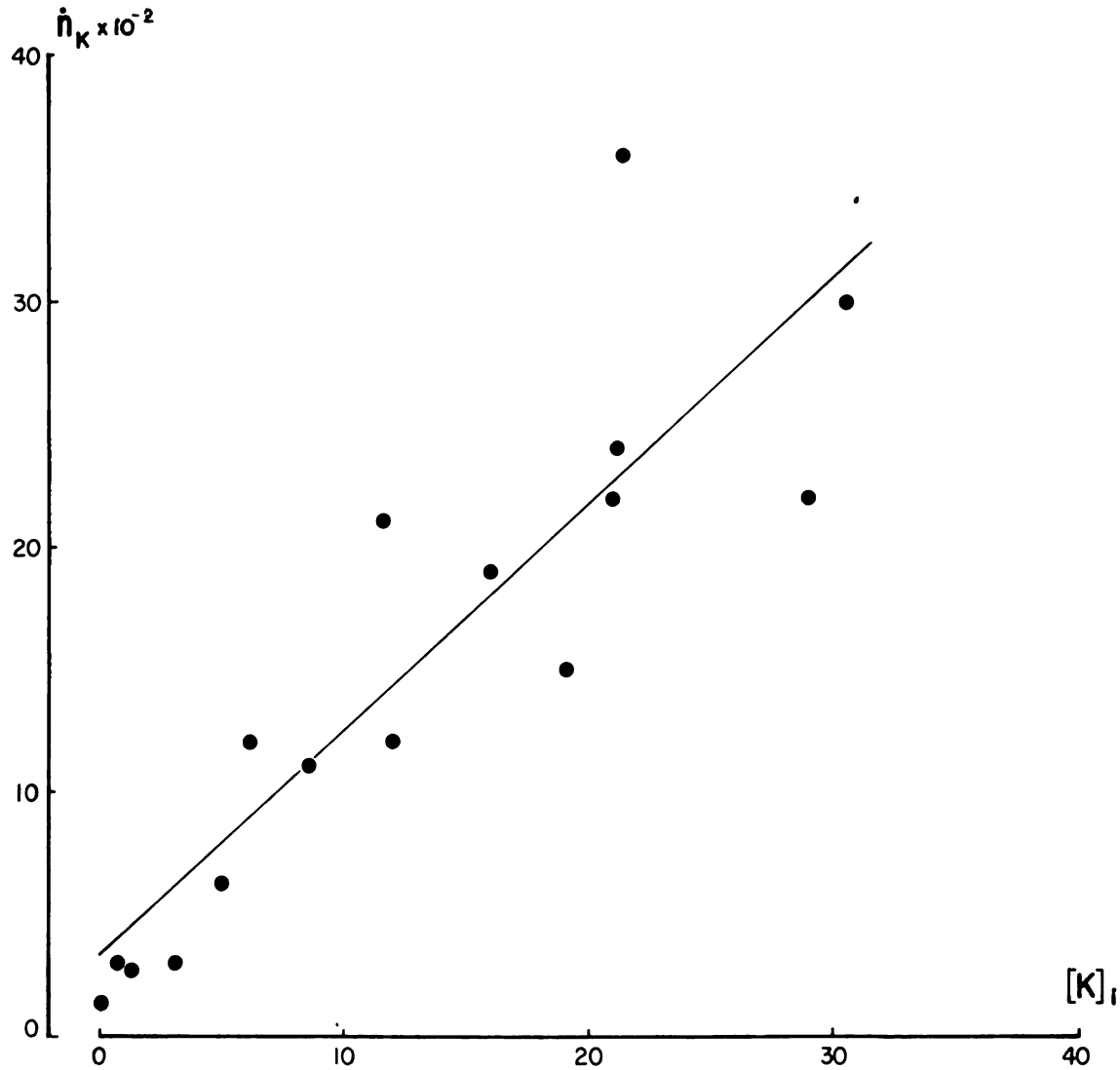

Figure 9

Figure 10. Calcium outflux coefficient plotted as a function of perfusion inflow calcium concentration. Ordinate. Calcium outflux coefficient ($K_{D_{Ca}}$, µl/min.). Abscissa. Inflow calcium concentration ($[Ca]_i$, mEq/L). Equation for the line was calculated by the method of linear regression and is:

$$K_{D_{Ca}} = -0.04\ (\pm\ 0.4)\ [Ca]_i + 4.6$$

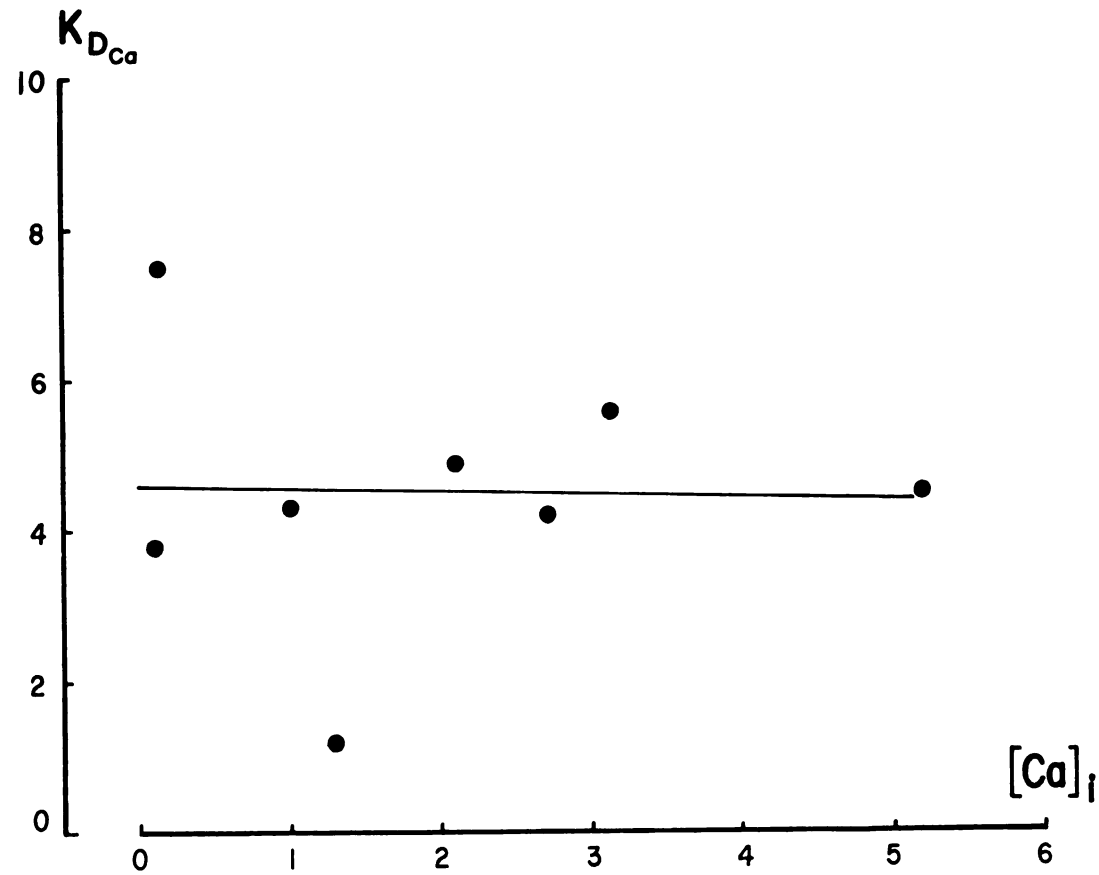

Figure 10

Figure 11. Calcium outflux rate as a function of perfusion inflow calcium concentration. Ordinate. Calcium outflux rate ($\dot{n}_{Ca}$, μEq/min.). Abscissa. Inflow calcium concentration ($[Ca]_i$; mEq/L). Equation for the line was calculated by linear regression and is:

$$\dot{n}_{Ca} = 56.8\ (\pm\ 1.5)\ [Ca]_i - 0.39 \times 10^{-4}$$

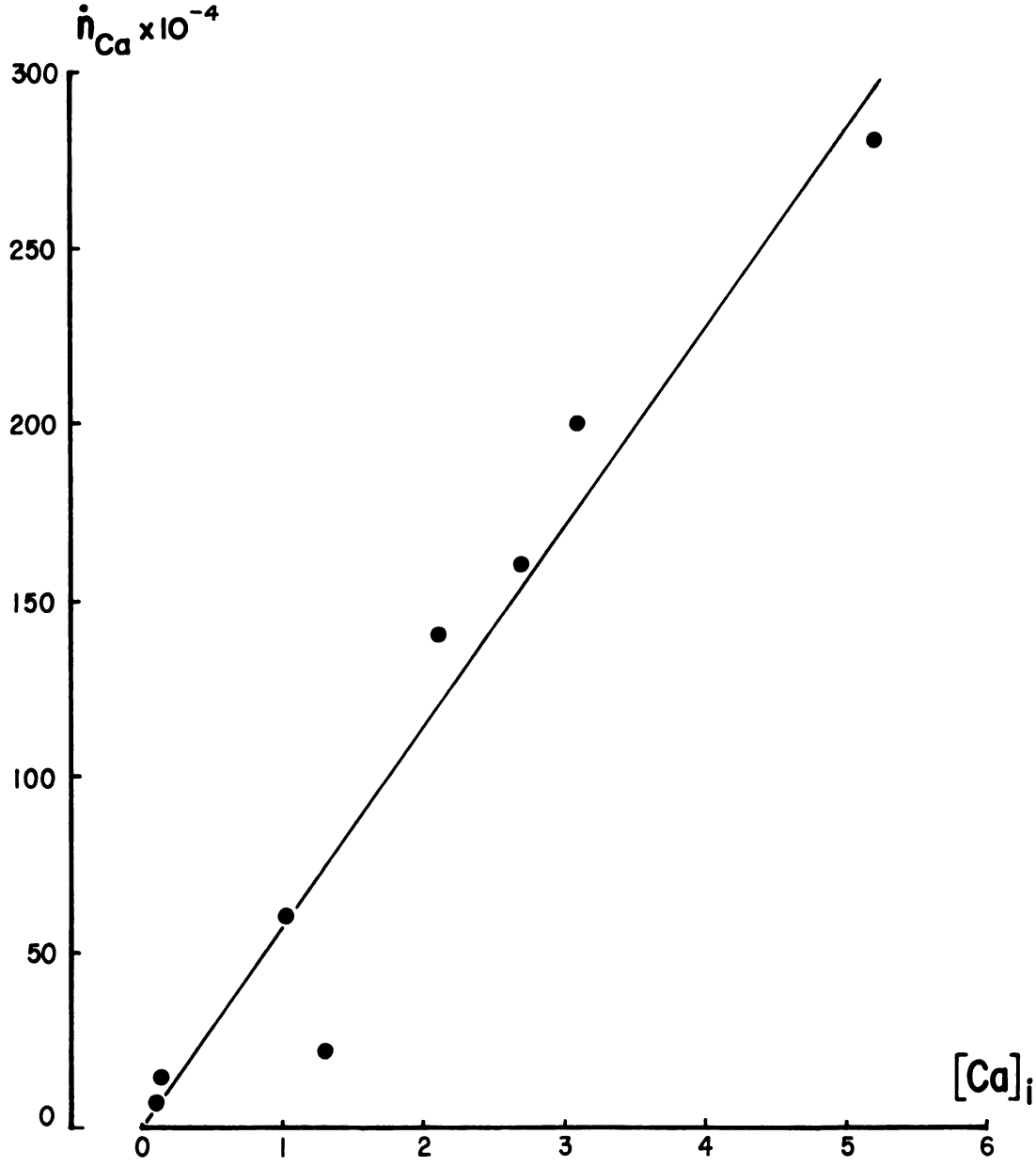

Figure 11

The data are interpreted to indicate that ^{45}Ca efflux from the ventricular system is by passive diffusion.

The effect of perfusion time on the outflux coefficients for ^{22}Na, ^{45}Ca, ^{42}K and creatinine are summarized in Table 3. Data in this table reveal that extended perfusion time has no effect on the K_D's of ^{22}Na, ^{45}Ca, ^{42}K or creatinine and indicates that passive permeability of the ventricular ependyma was not altered by perfusion.

Table 3. Effect of perfusion time on the efflux coefficients for sodium, calcium, potassium and creatinine.

Perfusion Time (hrs.)	Outflux Coefficients (µl/min)			
	Sodium	Calcium	Potassium	Creatinine
0-2	4.0 ± 0.8 (6)	5.3 ± 1.1 (3)	15.6 ± 1.4 (6)	12.4 ± 2.2 (14)
2-4	4.0 ± 0.5 (5)	3.0 (2)	13.0 ± 1.8 (6)	13.2 ± 2.0 (11)
4-6	4.6 ± 0.4 (5)	4.6 ± 0.5 (3)	13.1 ± 1.7 (5)	14.3 ± 2.6 (6)

Note: Values are means ± SEM.

Number of observations given in parentheses.

Data in Table 3 show that there is no difference ($P > 0.05$) between $K_{D_{Na}}$ and $K_{D_{Ca}}$ or between K_{D_K} and $K_{D_{cr}}$ but the latter two K_D's are approximately three times greater than those for sodium and calcium. This shows that the

chicken cerebral ventricles are more permeable to potassium and creatinine than to either sodium or calcium.

Mass Balance Determination

In an attempt to determine the extent to which test molecules enter either blood or brain tissue from CSF, the total amount of radioactivity presented to the animal, the total amount recovered in the perfusion outflow and the total amount cleared from CSF were calculated in addition to measuring the total amount of radioactivity recovered from brain tissue (Methods, Section D4b). Table 4 is a radioactivity balance sheet for RIHSA, ^{22}Na, ^{45}Ca and ^{42}K. Values are expressed as a percent of either total input (Equation 10, Methods) or total cleared from CSF (Equation 12, Methods). Rows 1 and 2 are total radioactivity collected in the perfusion effluent (Equation 11, Methods) and total radioactivity cleared from CSF (Equation 12, Methods), respectively, and both are expressed as a percent of total input. It is assumed that all radioactivity presented to the animal by perfusion inflow will either be collected in the cisternal effluent or will be cleared from CSF into brain or blood. Thus, the sum of row 1 and 2 for each molecule should equal 100 percent; that they do not reflects errors associated with measurement of inflow and outflow rates and concentrations and errors in the estimation of clearance. Approximately 97 percent of the radioactivity (for all test

Table 4. Ventricular perfusion mass balance for RIHSA, ^{22}Na, ^{45}Ca, and ^{42}K.

	Radioactivity Recovered	RIHSA (9)	^{22}Na (5)	^{45}Ca (2)	^{42}K (6)
1	Total collected in perfusion effluent as % of total input	90.4 ± 1.2	80.9 ± 1.7	80.8	70.8 ± 2.1
2	Total cleared as % of total input	6.8 ± 1.2	15.7 ± 1.6	15.4	26.7 ± 1.8
3	Total cleared in bulk as % of total input	7.3 ± 1.0	7.7 ± 1.5	8.4	3.3 ± 0.5
4	Total non-bulk cleared as % of total input	-0.5 ± 0.3	7.9 ± 0.8	7.0	23.4 ± 1.6
5	Total in brain as % of total cleared	15.6 ± 2.5	22.7 ± 7.2	26.9	49.5 ± 2.5

Note: Percentages expressed as mean ± SEM.

Number of animals shown parenthetically.

molecules) introduced at the perfusion inflow could be accounted for in that collected in effluent fluid and that cleared indicating a 3 percent measurement error.

The total clearance of molecules from the CSF is made up of a bulk and non-bulk clearance. Total bulk clearance (Equation 13, Methods) expressed as a percent of total input appears in row 3 (Table 4). Non-bulk clearance (expressed as a percent of total input, row 4) was calculated as the difference between total clearance and bulk clearance and the sum of values in rows 3 and 4 for each molecule should equal the value in row 2. The data show that approximately 50 percent of the ^{22}Na and ^{45}Ca cleared from CSF is by bulk absorption whereas only about 12 percent of the total ^{42}K cleared is by this route; most ^{42}K exiting by non-bulk means. There is no difference ($P > 0.05$) in total RIHSA clearance and that absorbed in bulk, providing additional justification for using RIHSA clearance to measure CSF bulk absorption in the chicken. The negative non-bulk RIHSA clearance (Row 4) is probably due to errors in measurement of inflow and outflow rates and concentrations.

Row 5 (Table 4) shows the uptake of RIHSA, ^{22}Na, ^{45}Ca and ^{42}K by brain tissue as a percent of the total clearance from the perfusion fluid. The results show that brain uptake accounts for approximately 50 percent of ^{42}K clearance, 27 percent of ^{45}Ca clearance and 23 percent of ^{22}Na clearance from CSF, the remainder presumably going into

blood. While ^{42}K flux out of CSF appears to be divided equally between brain and blood, approximately three-fourths of ^{22}Na and ^{45}Ca efflux is into blood. Approximately 16 percent of the total RIHSA clearance is into brain tissue. It is interesting to speculate how this occurs since the non-bulk clearance of RIHSA has been shown to be essentially zero (Figure 3; Table 4, row 4). The data suggest that RIHSA is cleared into blood from the ventricular system by bulk absorption but then in some manner enters brain tissue from blood.

Cerebrospinal Fluid Volumes and Brain Spaces

The cerebral ventricular volume was estimated from the volumes of distribution of RIHSA, ^{22}Na and ^{14}C-glucose as shown in Figure 12. The calculation of the volume of distribution of any substance depends upon the amount of the substance remaining in CSF after a steady-state concentration is attained, taking into account the amount of the substance cleared from CSF and the amount in the perfusion tubing (Equation 15, Methods). There is no difference ($P > 0.05$) in the three distribution volumes (approximately 140 µl), indicating that this volume is independent of the test molecules' weight and size. Total CSF volume in the chicken is approximately 350 µl and is estimated from the maximum volume that can be aspirated from the cisterna magna.

Figure 12. Mean distribution volumes of RIHSA, ^{22}Na and ^{14}C-glucose in cerebral ventricular system of the chicken (µl, ordinate). The number of animals used is shown in parenthesis and SEM is designated by the vertical lines.

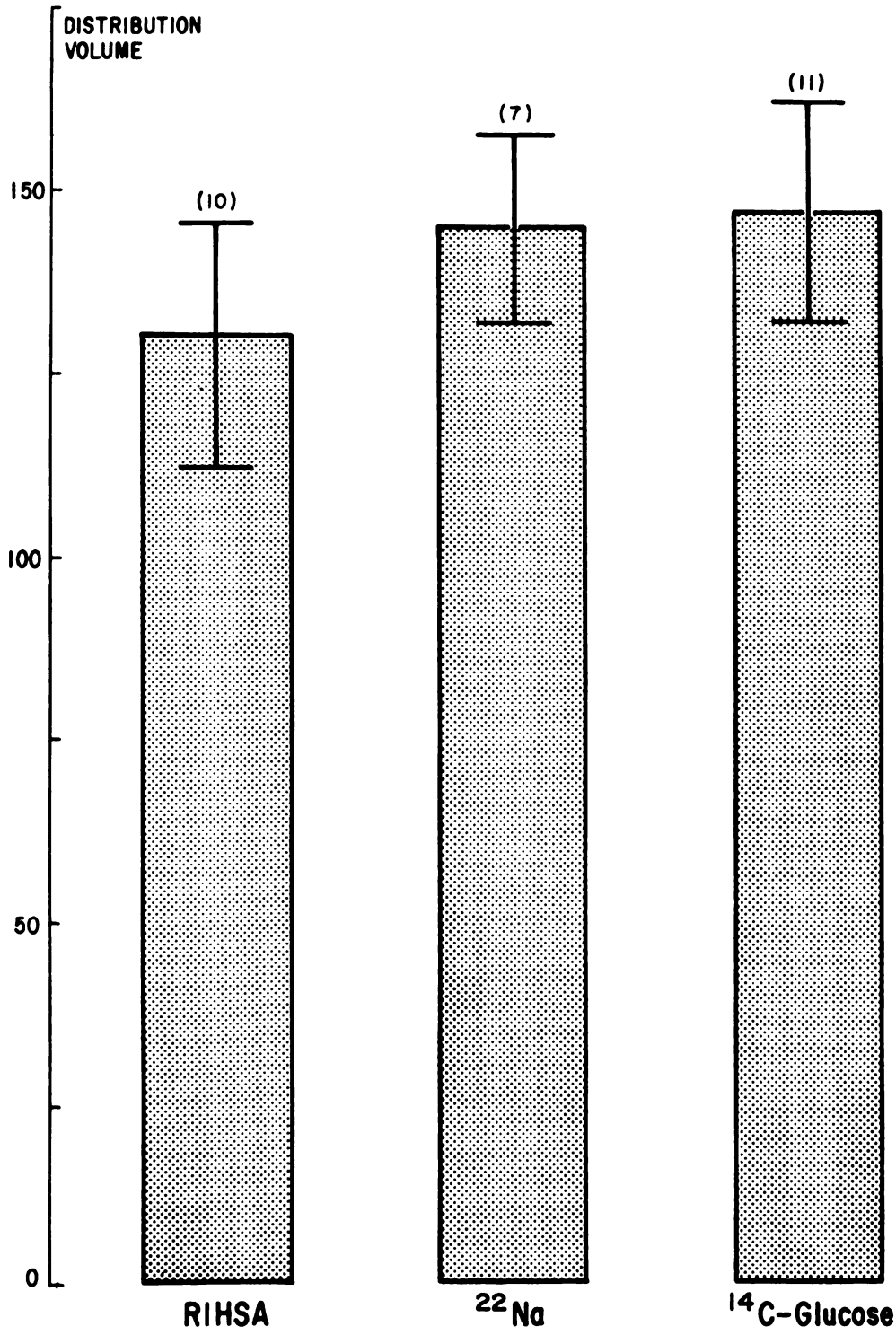
DISTRIBUTION VOLUME
150
100
50
0
(10)
(7)
(11)
RIHSA
22Na
14C-Glucose

Figure 12

Data reported in Table 5 show that brain spaces ranged from 4 percent of brain weight (RIHSA) to 91 percent of brain weight for ^{42}K. Brain spaces determined with different molecules were different ($P < 0.05$) from each other. In general, there appears to be an inverse relationship between the molecular size of the test molecule and the calculated brain space, however, this alone cannot fully explain the four different brain spaces obtained.

Table 5. Distribution of RIHSA, ^{22}Na, ^{45}Ca, and ^{42}K in the chicken brain.

Test Molecule	Number of Animals	Brain Space* (% of brain weight)
RIHSA	10	4.0 ± 0.3
^{22}Na	7	10.8 ± 1.0
^{45}Ca	3	27.5 ± 3.8
^{42}K	6	91.3 ± 10.7

*Mean ± SEM.

Effects of Altered Cerebrospinal Fluid Potassium and Calcium Concentrations on Cardiovascular Functions

The role of CSF ionic composition in cardiovascular function has been extensively investigated in anesthetized mammals. In order to study this in the chicken, the cerebral ventricles were perfused with solutions containing altered concentrations of potassium and calcium. I first had to determine if ventricular perfusion affected cardiovascular parameters. Data in Table 6 illustrate the effect of ventriculocisternal perfusion on blood pressure and heart rate in chickens anesthetized with either sodium phenobarbital or Metofane. Blood pressure (in mm Hg) are shown as systolic over diastolic and as the mean (Equation 17, Methods). Systolic, diastolic and mean blood pressure and heart rate in animals anesthetized with sodium phenobarbital were lower ($P < 0.05$) than those anesthetized with Metofane both before and after ventricular perfusion had begun. Ventricular perfusion caused an increase ($P < 0.05$) in the systolic, diastolic, and mean blood pressure in sodium phenobarbital-anesthetized chickens but did not alter heart rate ($P > 0.05$). In animals anesthetized with Metofane, ventricular perfusion produced no significant change ($P > 0.05$) in blood pressure or in heart rate.

In Figure 13 systolic (panel A), diastolic (panel B), and mean (panel C) blood pressure responses (expressed as a percent of control values; Section F, Methods) are plotted

Table 6. Effect of anesthesia and cerebral ventricular perfusion on blood pressure and heart rate in chickens.

Anesthetic	Before Ventricular Puncture			After Ventricular Puncture		
	Blood Pressure (mg Hg)		Heart Rate (beats/min)	Blood Pressure (mg Hg)		Heart Rate (beats/min)
	systolic / diastolic	mean		systolic / diastolic	mean	
Sodium Pheno-barbital	104 ± 5 / 69 ± 4 (22)	81.4 (23)	226 ± 5 (23)	119 ± 4 / 84 ± 5 (13)	95 ± 5 (13)	229 ± 5 (13)
Metofane	132 ± 5 / 90 ± 4 (21)	104 ± 4 (21)	262 ± 7 (16)	142 ± 5 / 95 ± 4 (21)	111 ± 5 (21)	257 ± 7 (14)

Note: Values expressed as means ± SEM.

Number of animals shown in parentheses.

Figure 13. Experimental systolic, diastolic and mean blood pressure (expressed as a percent of control blood pressure) plotted as a function of perfusion inflow potassium concentration. Ordinate. Experimental systolic (A), diastolic (B), and mean (C) blood pressures (all expressed as a percent of control blood pressure). Abscissa. Inflow potassium concentration ($[K]_i$; mEq/L). Equations for the lines were calculated by the method of linear regression and are:

$$\% \text{ of control (systolic)} = 0.44\ (\pm\ 0.2)\ [K]_i + 92.3$$

$$\% \text{ of control (diastolic)} = 0.60\ (\pm\ 0.3)\ [K]_i + 88.2$$

$$\% \text{ of control (mean)} = 0.43\ (\pm\ 0.3)\ [K]_i + 92.8$$

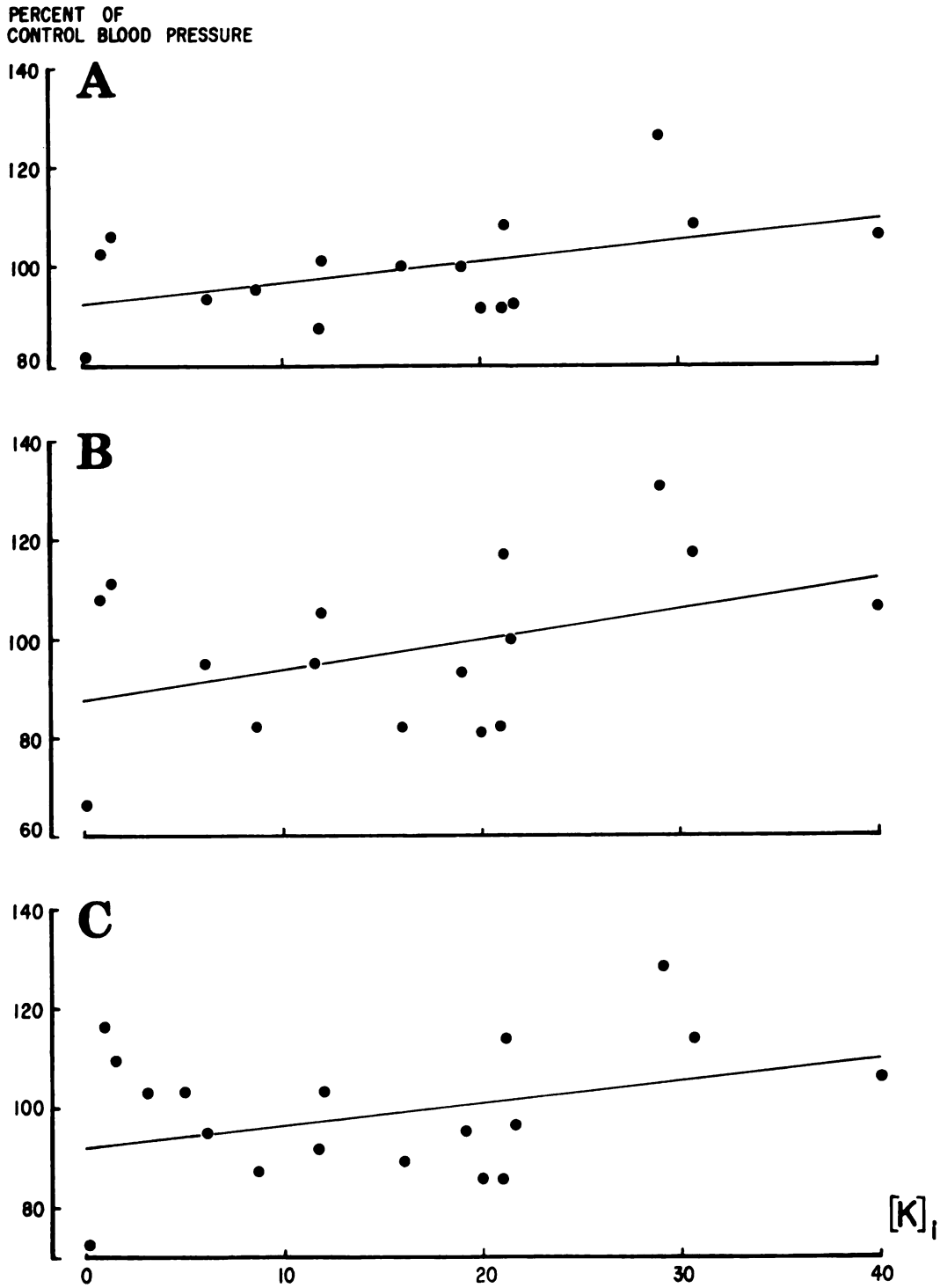
PERCENT OF
CONTROL BLOOD PRESSURE
A
B
C
140
120
100
80
60
0
10
20
30
40
[K]i

Figure 13

as a function of inflow potassium concentration ($[K]_i$). Data are from 18 steady-state periods in eight animals anesthetized with Metofane. Although all three regression lines have positive slopes (suggesting an increase in blood pressure with an increasing $[K]_i$), none are significantly different from zero. These data indicate that CSF potassium concentrations in the range 0-40 mEq/L did not affect blood pressure in anesthetized chickens.

In Figure 14, experimental systolic (panel A), diastolic (panel B) and mean (panel C) blood pressures (expressed as a percent of control pressures) are plotted as a function of inflow calcium concentration ($[Ca]_i$) for 11 steady-state perfusion periods in five Metofane-anesthetized animals. All three regression lines have similar negative slopes; however, only the one for systolic blood pressure (Figure 14, panel A) is different from zero ($P < 0.05$). The data suggest that altering CSF calcium concentration affected blood pressure but variability in the responses did not permit statistical significance to be achieved for diastolic and mean blood pressure.

Figure 14. Experimental systolic, diastolic, and mean blood pressure (expressed as percent of control blood pressure) versus perfusion inflow calcium concentration. Ordinate. Experimental systolic (A), diastolic (B), and mean (C) blood pressures (all expressed as percent of control blood pressure). Abscissa. Inflow calcium concentration ($[Ca]_i$; mEq/L). Equations for lines were calculated by the method of linear regression and are:

$$\% \text{ of control (systolic)} = -4.4\ (\pm\ 1.9)\ [Ca]_i + 97.0$$

$$\% \text{ of control (diastolic)} = -4.9\ (\pm\ 2.6)\ [Ca]_i + 98.6$$

$$\% \text{ of control (mean)} = -4.7\ (\pm\ 2.3)\ [Ca]_i + 98.0$$

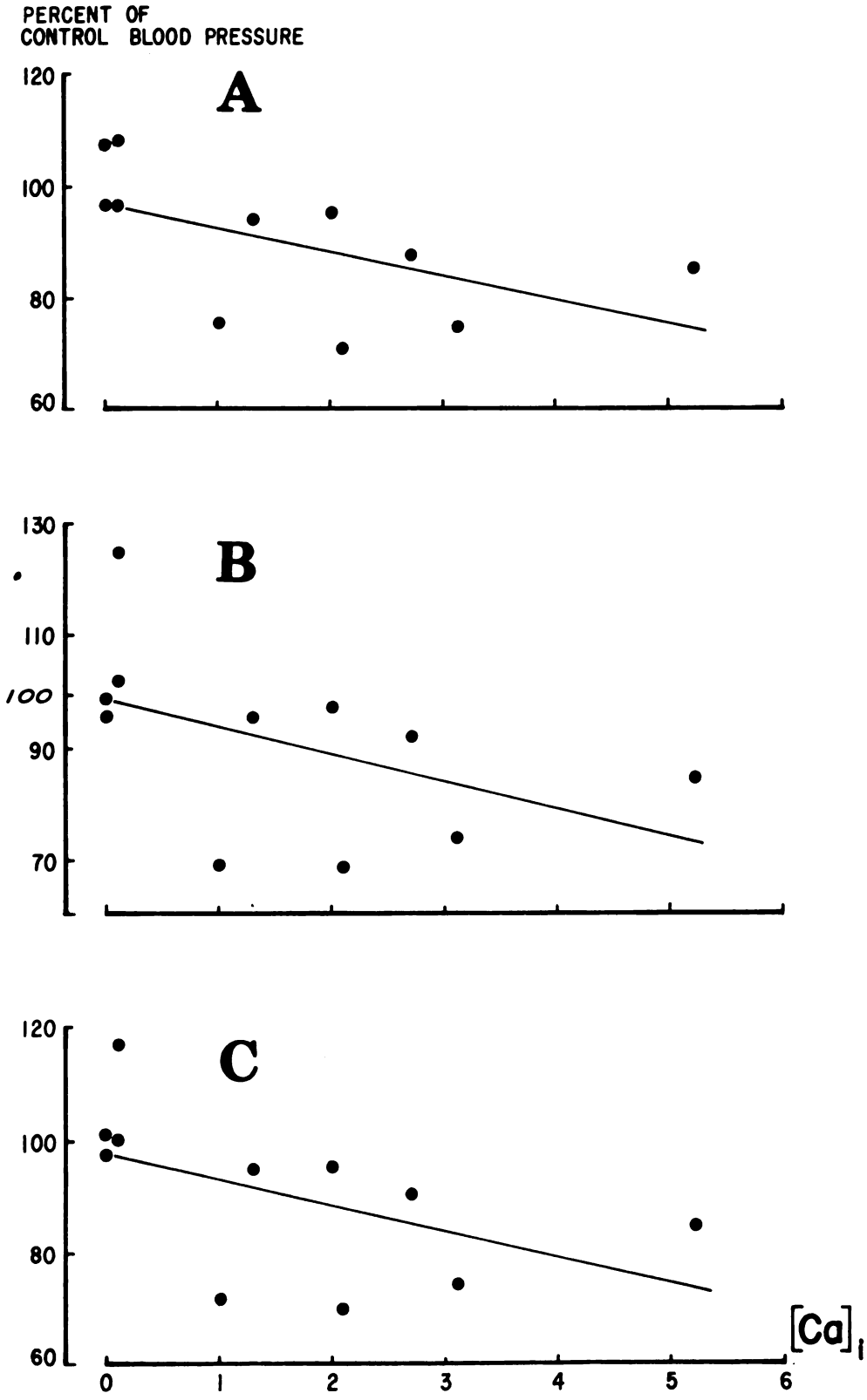
PERCENT OF
CONTROL BLOOD PRESSURE
A
120
100
80
60
B
130
110
100
90
70
C
120
100
80
60
0
1
2
3
4
5
6
[Ca]i

Figure 14

DISCUSSION

Cerebrospinal Fluid Formation and Absorption

Cerebrospinal fluid (CSF) formation has been measured either by draining CSF from the cisterna magna over a measured time period or by measuring the time required to restore CSF pressure to pre-withdrawal levels after removal of a known volume of fluid. The major weakness of such methods is that at normal intraventricular pressures, not all of the fluid formed will be collected; an unknown amount will be lost to blood by bulk absorption. CSF formation, determined by free drainage techniques, was determined at low intraventricular pressures and CSF loss by bulk absorption was assumed to be negligible. However, low intraventricular pressures do not guarantee the complete cessation of bulk absorption. Validity of the values for CSF formation obtained using free drainage techniques are questionable.

The technique of ventriculocisternal perfusion has provided the most precise and, probably, the most physiological method of studying CSF formation and absorption. One advantage of this method is that bulk CSF absorption can be estimated. Thus, normal CSF formation and absorption rates (at physiological intraventricular pressure) can be

determined in addition to studying the effects of altering intraventricular pressure on both parameters.

In this study on chickens as in previous studies on one reptilian species (Heisey and Michael, 1971) and various mammalian species (Heisey et al., 1962; Bering and Sato, 1963; Cutler et al., 1968; Katzman and Hussy, 1970), the rate of CSF bulk absorption was linearly related to intraventricular pressure (Figure 3). However, the CSF bulk absorption rate in chickens is less than reported for mammals (Cutler et al., 1968) and turtles (Heisey and Michael, 1971). Resistance to bulk absorption (the reciprocal of the slope of C_{RIHSA} plotted as a function of P (Cutler et al., 1968) in the chicken (4,545 cm·min/ml) was 12-350 times the resistance reported for mammals (Cutler et al., 1968) and 2.5 times that reported for turtles (Heisey and Michael, 1971). These latter workers suggested that the large resistance to bulk absorption in the turtle might be caused by the absence of an arachnoid membrane with associated "valves." However, Hansen-Pruss (1923) has demonstrated that birds possess an arachnoid membrane. The high resistance in chickens may indicate either a lack of valve-like structures in the arachnoid villi, as described for mammals (Welsh and Friedman, 1960), or presence of high resistance pathways in the arachnoid membrane.

CSF formation rate in the chicken was found to be independent of intraventricular pressure (Figure 3)

paralleling results previously reported for the goat (Heisey et al., 1962), cat (Hochwald and Wallenstein, 1967; Katzman and Hussy, 1970), dog (Bering and Sato, 1963), human (Cutler et al., 1968) and turtle (Heisey and Michael, 1971). The mean value of CSF secretion in 21 chickens was 1.4 µl/min. Total choroid plexus weight was 3.6 ± 0.4 mg in 7 chickens. Thus, formation rate per unit weight of choroid plexus was 0.39 µl/min per mg tissue, a value that is similar to values obtained in other species (Table 1). In addition, the CSF turnover rate in the chicken was 0.4 percent/minute, a value which is comparable to those calculated for other species (Table 1) and which reveals that the ventricular CSF volume (140 µl) is replaced every 1.7 hours and that the total CSF volume (350 µl) is replenished every 4 hours.

Heisey et al. (1962) estimated that choroid plexus blood flow in the goat would be about 3 µl/min per mg tissue to get a CSF secretion rate of 0.3 µl/min per mg tissue assuming 20 percent of the plasma flow through the choroid plexuses was lost forming CSF. Welch (1963) measured a choroid plexus blood flow of 2.86 µl/min per mg tissue, a blood volume loss of 13.3 percent during transit through the plexuses and calculated from these values a CSF formation rate of 0.37 µl/min per mg tissue for the rabbit. In the chicken, for a CSF formation rate of 0.39 µl/min per mg tissue (assuming that, like the rabbit, 13.3 percent of the blood flow through the choroid plexuses is secreted as

CSF) choroid plexus blood flow would be 2.9 µl/min per mg tissue, a value which is essentially the same as that reported for the rabbit and goat. Choroid plexus blood flow in all three animals is about 5 times greater than to whole brain and 1.4 times less than to the kidney, indicating that choroidal tissue has a comparatively large blood flow.

Molecular Flux from Cerebrospinal Fluid To Brain and Blood

Development of the technique of ventriculocisternal perfusion has provided a useful method for the quantitative study of the transport of substances among blood, brain and CSF. The technique has been used in this study to investigate the movement of creatinine, ^{42}K and ^{45}Ca from CSF to brain and blood. All test molecules investigated had a clearance from the perfusion fluid which was greater than inulin or RIHSA, indicating that, in addition to their loss from CSF by bulk absorption, these molecules left the ventricular system by simple diffusion and/or active transport.

Before molecular flux of materials from the perfusion fluid can be studied, it is necessary to determine what effects perfusion has on the integrity of the ventricular walls. Cserr (1965) has shown, in dogs, a doubling of the creatinine outflux coefficient after 4-6 hours of perfusion. She indicated that this increase in creatinine outflux was

due to a progressive increase in ventricular permeability resulting from deterioration of the preparation with time. There was no change in $K_{D_{Na}}$, $K_{D_{Ca}}$, K_{D_K}, and $K_{D_{cr}}$ with time (Table 3) suggesting that cerebral ventricular perfusion (of up to 6 hours) did not alter the passive permeability of the chicken ventricles.

Permeability of porous membranes to inert, lipid-insoluble molecules varies inversely with molecular size. If any lipid-insoluble substance is cleared from the ventricular system at a rate greater than that predicted for diffusion based on molecular size, this may be taken as presumptive evidence that the molecule is actively transported although further experimental evidence is required to prove the point conclusively (Heisey et al., 1962). These workers found that, in goats, the outflux coefficient of creatinine (MW=113) was less than that of smaller molecules and was greater than that of fructose (MW=160). Bering and Sato (1963) found that the urea outflux coefficient in the dog was three times greater than that for creatinine. Cserr (1965) reported for both rats and dogs, ^{22}Na and ^{42}K efflux coefficients which exceeded that of creatinine. Results from all three studies indicate that creatinine movement from mammalian CSF is by passive diffusion. These results are contrary to data of Bierer (1972) who found that, in dogs, the creatinine outflux coefficient exceeded that of para-aminohippuric acid (PAH), a molecule

which he demonstrated to be actively transported from the ventricular system. He suggested that creatinine may be actively transported from CSF.

Ventricular permeability to creatinine, in the chicken, is the same as that for ^{42}K and is approximately three times larger than that for ^{45}Ca or ^{22}Na. This suggests either that some mechanism retards the movement of the ions from CSF or that creatinine may be actively transported from the avian ventricular system. If ^{42}K, ^{22}Na and ^{45}Ca movement from CSF is against an electrical gradient, their movement could be impeded so that the outflux coefficient of a non-electrolyte (like creatinine) might appear large by comparison. However, Held et al. (1964) and Bradbury and Šťulcová (1969) demonstrated that in mammals CSF was electropositive with respect to blood, providing an electric field that would accelerate the passage of these cations from CSF. Assuming that, like mammals, the CSF of chickens is electrically positive with respect to blood (although it has never been measured) would suggest that the large creatinine outflux coefficient cannot be explained by an electrical gradient hindering ionic outflux.

In the present study, the creatinine outflux coefficient was independent of creatinine concentration in CSF (Figure 6) and creatinine outflux was a linear function of CSF creatinine concentrations (Figure 7) suggesting (but not conclusively proving) that creatinine apparently exits

CSF by passive diffusion. This indicates that creatinine outflux is not limited by competition for sites on a carrier at the concentrations (--20 mM/L) used in this study. These results confirm those of Bradbury and Davson (1964) who perfused the ventricular system of rabbits with "low" (1.7 mM/L) and "high" (34 mM/L) concentrations of creatinine. They found no evidence of a saturable system involved in creatinine movement from CSF and concluded that non-carrier mediated diffusion described creatinine movement. The large creatinine outflux coefficient (relative to that for ^{42}K, ^{22}Na, and ^{45}Ca) might suggest either an active transport process that does not involve carrier mediation, a carrier which is saturated at creatinine concentrations above 20 mM/L, or the carrier is not located at the ventricular ependyma. Transport of creatinine from the kidney tubules of chickens has been postulated by Shannon (1938), Sykes (1960) and Rennick (1967) who reported that the ratio of creatinine clearance to inulin clearance was greater than one but approached unity with increasing plasma creatinine concentrations. In addition, Rennick demonstrated that this renal tubular transport could be competitively inhibited. Further experimentation with metabolic and competitive inhibitors will be required to determine if creatinine movement from chicken CSF involves active transport.

Data presented in Table 3 show that ventricular permeability to ^{42}K is 3-4 times that for ^{22}Na. The lack

of change in the ^{42}K outflux coefficient with increasing CSF potassium levels (Figure 8) coupled with the dependence of ^{42}K outflux on concentration (Figure 9) suggests that removal of ^{42}K is by simple diffusion. Passive movement of ^{42}K out of CSF has previously been demonstrated in various mammalian species (Bradbury and Davson, 1965; Bradbury and Šťulcová, 1969; Katzman, 1965; and Cserr, 1965), and in addition, Heisey et al. (1962) and Cserr (1965) found that ^{42}K outflux always exceeded that for ^{22}Na by approximately four times.

It is likely that the large ^{42}K outflux observed in chickens and mammals results from brain cells acting as a "sink" for ^{42}K. Because of the high concentration of potassium in brain cells relative to that in interstitial fluid or CSF (Bradbury and Šťulcová, 1970), there is probably a large capacity for ^{42}K exchange with the unlabelled intracellular potassium. Data in Table 4 show that 50 percent of the ^{42}K and 23 percent of the ^{22}Na leaving CSF enters brain tissue, confirming findings in the dog (Cserr, 1965) and cat (Katzman et al., 1965). This intracerebral potassium pool presumably acts as a "sink" for ^{42}K and is one factor that is responsible for the large ^{42}K outflux coefficient (relative to ^{22}Na outflux coefficient) observed. In addition, molecular size may contribute to the difference in ventricular permeability to ^{22}Na and ^{42}K. When ions are in solution, water molecules become intimately associated with the ion resulting in solvation or hydration

(Dowben, 1971). Ions with the smallest radii have the largest and most strongly held layer of water molecules. The sodium ion has a smaller radius than the potassium ion, but with its shell of water molecules, its diameter is 1.2 times larger than that of the potassium ion. Sodium ions should diffuse from the ventricles more slowly than potassium which would contribute to the difference between ^{22}Na and ^{42}K outflux coefficients.

Data presented in Figures 10 and 11 demonstrate that, like creatinine and ^{42}K, ^{45}Ca movement from chicken CSF does not exhibit saturation kinetics and confirms findings in dogs (Oppelt et al., 1963) and cats (Graziani et al., 1965). This suggests that ^{45}Ca efflux is by diffusion unless a carrier for calcium is unsaturated at twice the normal calcium concentration in CSF.

Cerebrospinal Fluid Volume and Brain Spaces

The avian brain contains bilateral cerebral and optic lobe ventricles, a third and fourth ventricle, and a small cerebellar ventricle. While most mammalian ventricles can be described as well-formed invaginations (more or less centrally located in their respective brain areas), chicken ventricles are little more than narrow slits. The cerebral ventricles are located superficially under the posterior surface of the cerebral hemispheres as evidenced by their penetration at a point 1-2 mm below the dura. The

ventricular volume of chickens averaged 140 µl which is less than half the total CSF volume (350 µl) estimated for these animals (Results). Total ventricular volume (expressed per unit weight of brain tissue) was 45 µl/gm of brain and falls within the range of values reported for several mammalian species (Table 2) indicating that even though the avian brain has a greater number of CSF cavities than the mammalian brain, it contains a similar volume of ventricular CSF.

The wide variance in brain spaces calculated for RIHSA, ^{42}K, ^{22}Na and ^{45}Ca (Table 7) is probably due to: (1) differences in ventricular permeability to the test substances; (2) the extent to which the different molecules enter brain tissue or blood from CSF; and (3) the degree to which the molecules enter cells.

Heisey (1971) reported a RIHSA brain space in turtles that was 3-5 percent of brain weight and which varied directly with perfusion time. This range of values encompasses the RIHSA brain space calculated for chickens (Table 5). However, in chickens, there was no relationship between calculated RIHSA space and perfusion time. The small RIHSA space is probably due to the low permeability of the ventricular ependyma to RIHSA (Figure 3, Table 6).

It has been suggested previously that the rapid ^{42}K movement from CSF occurs because brain cells, with high intracellular potassium concentration, act as a "sink" for ^{42}K. Consequently, ^{42}K molecules entering brain tissue

would exchange with this intracellular potassium. Combined with the large flux of ^{42}K from CSF into brain tissue (Table 4), distribution of ^{42}K molecules into the intracellular space of brain is apparently responsible for the large ^{42}K brain space.

Tschirgi (1960) assumed that sodium is contained primarily in the extracellular fluid of brain tissue and reported a 30-35 percent sodium brain space in mammals, a value substantially larger than the sodium brain space (11 percent) measured in the chicken (Table 5). Data in Table 4 indicate that only about 20 percent of the ^{22}Na leaving CSF is recovered in brain tissue; presumably most of the ^{22}Na enters blood. Perhaps blood acts as a "sink" for ^{22}Na leaving CSF and continually drains it away from brain parenchyma. The superficial location of the lateral ventricles within the cerebral hemispheres may result in long diffusional pathways to the bulk of brain tissue and could cause incomplete distribution of ^{22}Na in brain ECF. Both mechanisms would result in an underestimation of the ^{22}Na brain space.

^{45}Ca brain space determination is subjected to the same source of error associated with incomplete distribution between ventricular fluid and brain extracellular fluid as ^{22}Na, yet the ^{45}Ca brain space exceeds that of ^{22}Na by 2.5 times (Table 5). Data in Tables 3 and 4 reveal that there is no difference in ventricular ependymal permeability to

^{22}Na and ^{45}Ca and the flux of these two molecules from CSF into brain tissue is the same. Giese (1968) has reported that calcium can readily bind with intra- and extracellular proteins. This suggests that the size of the ^{45}Ca brain space (relative to that for ^{22}Na) may be influenced by ^{45}Ca binding to proteins.

Effects of Altered Cerebrospinal Fluid Potassium and Calcium Concentrations on Cardiovascular Functions

Effects of CSF potassium and calcium concentrations on the mammalian cardiovascular system has been investigated by several authors and has been extensively reviewed by Tschirgi (1960) and Winterstein (1961). A marked rise (up to 180 mm Hg above control) in systemic blood pressure concomitant with a reflex slowing of the heart is the response to either raising the potassium concentration or reducing the calcium concentration in CSF. Raising the CSF calcium concentration above normal values usually causes a significant decline in blood pressure whereas lowering the CSF potassium levels was without effect on blood pressure. These cardiovascular responses to changes in CSF composition are considered to be due to a direct action of these ions on the medullary vasomotor center (Tschirgi, 1960; Winterstein, 1961).

The blood pressures, following changes in the CSF potassium concentrations (Figure 13), were not different

from control values ($P > 0.05$) indicating a lack of influence of CSF potassium on the blood pressure of chickens. Systolic blood pressure was depressed ($P < 0.05$) by increased CSF calcium concentrations (Figure 14, Panel A) but no changes in diastolic and mean blood pressure were detected ($P > 0.05$). With the exception of the systolic blood pressure response to altered CSF calcium levels, these results on chickens do not confirm previous findings in mammals. Absence of a blood pressure response in birds could occur because: (1) the avian brain does not possess a vasomotor center controlling blood pressure; (2) changes in CSF ionic composition were not reflected in the interstitial fluid surrounding the vasomotor center; (3) the avian vasomotor center is normally insensitive to changes in the ionic composition of CSF; or (4) the anesthetic used in these studies reduced the responsiveness of the vasomotor center to alterations in CSF ionic composition. It is difficult to determine which of the above reasons might be responsible for the lack of cardiovascular response in birds to changes in the ionic composition of CSF.

Systolic blood pressure measured from a cannulated carotid artery in unanesthetized, restrained adult white leghorn hens averaged 160 mm Hg with a pulse pressure of 25 mm Hg while mean heart rate in these animals was 357 beats per minute (Sturkie, 1965). These blood pressure values may underestimate "normal" values as restraint per se

has been shown to reduce blood pressure in white leghorn hens (Whittow et al., 1965). Sturkie's values are considerably higher than those presented in Table 6 for chickens anesthetized with either sodium phenobarbital or Metofane. Sodium phenobarbital lowered blood pressure more so than Metofane suggesting that Metofane does not depress central nervous system activity as much as sodium phenobarbital. Since chickens anesthetized with Metofane had blood pressures and heart rates that more closely approximated unanesthetized values, all the cardiovascular studies were performed on chickens anesthetized with Metofane in an attempt to minimize any masking effect by anesthesia of cardiovascular responses to changes in CSF ionic composition.

Sturkie (1965) presumes that, like mammals, the avian brain has a vasomotor center located in the medulla although he states that no conclusive experiments on this have been conducted. Rodbard and Tolpin (1947) found a positive relationship between body temperature and blood pressure in the chicken and inferred from previous studies on turtles (Rodbard, 1947) that neural control of blood pressure was present in chickens. Birds are known to possess a "neural pool" located in the medulla that is responsible for the central control of respiration (Marshal, 1960; Sturkie, 1965). Sturkie (1965) indicates that anesthetics can either stimulate or inhibit this center depending on the anesthetic used. The reduction of blood pressure and

heart rate in the anesthetized chickens of the present study (Table 6) compared with unanesthetized chickens (Sturkie, 1965) might be interpreted as additional evidence suggesting some central control of blood pressure and heart rate. Recently, Cohen et al. (1970) using the technique of retrograde degeneration, discovered that the cell bodies of vagal cardio-inhibitory fibers in the pigeon reside in the dorsal motor nucleus. Electrical stimulation of this nucleus resulted in bradycardia, a response that was abolished by bilateral vagotomy (Cohen and Schnall, 1970). Since there appears to be central control of heart rate and respiration in birds, it seems likely that the brains of these animals would possess the capability for control of blood pressure.

It is impossible to determine if changes in the CSF ionic composition were reproduced in the interstitial fluid surrounding the vasomotor center. The fact that both ^{42}K and ^{45}Ca had measurable fluxes into brain and appreciable brain spaces suggests that calcium and potassium entered brain tissue. If the vasomotor center was in proximity to the path of the perfusion fluid, presumably changes in CSF ionic composition were reflected in the extracellular fluid around this center.

It appears unlikely that the avian vasomotor center is normally insensitive to changes in the CSF ionic composition as increased calcium concentrations in the perfusion fluid caused a moderate but significant decline in systolic

blood pressure (Figure 14, Panel A). The inability to statistically detect responses in diastolic and mean blood pressure (Figure 14, Panels B and C, respectively) to changes in the CSF calcium levels or in systolic, diastolic and mean blood pressure (Figure 13, Panels A, B and C, respectively) following alteration in the CSF potassium concentration is probably due to the variability of the response as indicated by the scatter of points around the regression lines in Figures 13 and 14. Similarly, in pilot studies on chickens anesthetized with sodium phenobarbital, the blood pressure and heart rate responses to changes in the CSF potassium and calcium levels were also variable and inconsistent. Downman (1943) reported that in rabbits anesthetized with either urethane or sodium phenobarbital, intracisternal injection of potassium phosphate increased blood pressure in some cases and depressed it in others, even in the same animal. He attributed this variability of response to the use of anesthetics in their experiments. Mullins et al. (1938) found, in dogs, that increases in blood pressure following intracisternal injection of calcium-free solutions or solutions with elevated potassium concentrations were greater in unanesthetized than in barbital or sodium pentobarbital anesthetized animals. In Rodbard and Tolpin's (1947) study in chickens, the use of anesthetics (sodium phenobarbital) was discontinued because it tended to attenuate changes in blood pressure that resulted from changes in

body temperature. The inability to show changes in blood pressure as a result of CSF ionic alternations is probably due to the variability of the blood pressure response that may have resulted from anesthetic interference with the sensitivity of the vasomotor center. Perhaps chickens that were deeply anesthetized did not respond to the same degree as more lightly anesthetized animals. To answer this question conclusively, studies with unanesthetized animals or animals where the circulating anesthetic concentrations can be monitored and adjusted are required.

SUMMARY

1. Chicken brain ventricles were perfused with an artificial CSF yielding data on CSF formation and absorption rates, molecular movement from the CSF, size of the ventricular and brain extracellular spaces, and effects of CSF ionic changes on cardiovascular functions.
2. Clearance of RIHSA and inulin from CSF was a positive linear function of intraventricular pressure and was a measure of CSF bulk absorption.
3. CSF formation rate was 1.4 ± 0.1 µl/min and was independent of intraventricular pressure from 0-20 cm H_2O.
4. $K_{D_{cr}}$ and $K_{D_{Na}}$ were independent of intraventricular pressure from 0 to 20 cm H_2O.
5. $K_{D_{Na}}$, $K_{D_{Ca}}$, K_{D_K} and $K_{D_{cr}}$ were unaffected by perfusion time indicating perfusion did not alter the permeability of the brain ventricular walls.
6. $K_{D_{Na}}$ and $K_{D_{Ca}}$ were of the same order of magnitude as were K_{D_K} and $K_{D_{cr}}$, however the latter two K_D's exceeded those for sodium and calcium by three times indicating that chicken cerebral ventricles were more permeable to potassium and creatinine than to either sodium or calcium.

7. $K_{D_{cr}}$, K_{D_K} and $K_{D_{Ca}}$ were each independent of their perfusion inflow concentration while $\dot{n}_{cr}$, $\dot{n}_K$ and $\dot{n}_{Ca}$ were positive linear functions of inflow perfusion concentration.
8. The mechanism of creatinine efflux was not determined. Creatinine outflux coefficient (K_D) was independent of concentration which implies diffusion whereas the large K_D for creatinine (relative to $K_{D_{Na}}$, $K_{D_{Ca}}$, and K_{D_K}) is presumptive evidence for active transport.
9. That ^{42}K and ^{45}Ca outflux did not exhibit saturation kinetics was interpreted to indicate that these ions leave CSF by simple diffusion.
10. Chicken brain ventricular volume was approximately 140 µl and total CSF volume was estimated to be 350 µl.
11. Brain spaces (expressed as a percent of brain weight) for RIHSA, ^{22}Na, ^{45}Ca, and ^{42}K were 4 percent, 10.8 percent, 27.5 percent, and 91.3 percent, respectively. Size of the calculated brain spaces depended on: differences in the ventricular permeability to the test molecules; the extent to which the different molecules enter brain tissue or blood; and the degree to which the molecules enter brain cells.
12. With the exception of a significant ($P < 0.05$) decline in systolic blood pressure as a result of increasing CSF calcium levels, variability of the blood pressure response prevented any statistical detection of blood pressure changes that occurred following alterations

in the CSF calcium or potassium concentrations in anesthetized chickens. This suggests that chickens are normally capable of responding to variations in the ionic composition of CSF, but perhaps anesthetics attenuated the blood pressure response.

APPENDICES

APPENDIX I

PREPARATION OF ARTIFICIAL CEREBROSPINAL FLUID

APPENDIX I

PREPARATION OF ARTIFICIAL CEREBROSPINAL FLUID

Artificial chicken CSF contains:

Cations		Anions	
Na^+	155 mEq/L	Cl^-	140 mEq/L
K^+	3.7 mEq/L	HCO_3^-	23 mEq/L
Ca^{++}	2.5 mEq/L		
Mg^{++}	2.1 mEq/L		

Total osmolality = 287 mOsm/Kg H_2O

Reagents:

1. NaCl, analytical reagent (A.R.)
2. KCl, A.R.
3. $NaHCO_3$, A.R.
4. $CaCl_2$, A.R.
5. $MgCl_2 \cdot 6\ H_2O$, A.R.
6. Dextrose (Fisher Scientific Co., Fairlawn, N.J.).

Stock Solutions

1. Normal CSF

   Dissolve 7.72 gm NaCl, 0.2759 gm KCl and 1.93 gm $NaHCO_3$ in distilled water; q.s. 1 liter.

2. Ionically altered CSF

   Dissolve 4.77 gms NaCl and 1.93 gm $NaHCO_3$ in distilled water; q.s. 1 liter.

3. $MgCl_2$

   Dissolve 21.35 gms $MgCl_2 \cdot 6 H_2O$ in distilled water; q.s. 100 ml.

4. $CaCl_2$

   a. Dissolve 13.87 gms $CaCl_2$ in distilled water; q.s. 100 ml.

   b. Dissolve 11.10 gms $CaCl_2$ in distilled water; q.s. 100 ml.

Perfusion fluids

A. Artificial chicken CSF (normal composition)

   One-tenth ml of solutions #3 and #4a and 125 mg dextrose was added to 100 ml of solution #1.

B. Artificial chicken CSF (altered calcium concentration)

   Perfusion fluid containing 0-5 mEq/L Ca was made by adding 0 to 0.025 ml of solution #4b, 0.01 ml of solution #3 and 12.5 mg dextrose to 10 ml of solution #1.

C. Artificial chicken CSF (altered potassium concentration)

   Changing the potassium concentration of the perfusion fluid between 0 and 40 mEq/L would result in the perfusion fluid becoming either hypo- or hyperosmotic.

When KCl was added in excess of normal concentration (3.7 mEq/L), an equal amount of NaCl was removed to maintain isotonicity. Solution #2 was potassium-free and contained 40 mEq/L less NaCl than that in normal CSF perfusion fluid (solution #1). Crystalline KCl and NaCl were added to solution #2 so that their combined contribution was 40 mEq/L. This was accomplished by adding 0-29.8 mg KCl (0-40 mEq/L K^+) 0-23.4 mg NaCl, 12.5 mg dextrose and 0.01 ml of solutions #3 and #4 to 10 ml of solution #2. The osmolality of this fluid was 280-295 mOsm/kg H_2O.

APPENDIX II

LIQUID SCINTILLATION COUNTING

APPENDIX II

LIQUID SCINTILLATION COUNTING

Reference: Instruction Manual, Mark I liquid scintillation counter Model 6860, Nuclear Chicago Corp., DesPlaines, Ill.

Principle:

Liquid scintillation counting is a method of detecting radioactivity by means of a solution of fluors (liquid scintillator) and a photomultiplier tube. The energy emitted by the radioactive material is converted to light energy by the liquid scintillator which is then detected by the photomultiplier tube connected to amplifiers and a scaler circuit. The radioactive sample is placed close to the fluor by dissolving, immersing, or suspending it in the liquid scintillator. Consequently, this technique is particularly well-suited for use with low energy beta emitters such as tritium and carbon-14.

A. Carbon-14 detection

In these studies carbon-14 was counted alone. In two experiments it was determined that the quenching of all samples was equal. The counts per minute (cpm) and not the calculated disintegration rate (dpm) was used to determine carbon-14 concentrations.

B. Calcium-45 detection

Calcium-45 activity was measured in both the perfusion fluid and brain tissue. The quenching of ^{45}Ca activity was approximately five times greater with brain tissue than with perfusion fluid samples. To compare ^{45}Ca activity in both perfusion fluid and brain, it was necessary to eliminate quenching effects and get ^{45}Ca activity in terms of its actual disintegration rate (dpm). ^{45}Ca standards, in both the perfusion fluid and in brain tissue were prepared and ^{45}Ca counting efficiency determined in both media. Knowing the efficiency, the disintegration rate of ^{45}Ca activity in the unknown samples was calculated from the counts per minute.

1. Preparation of calcium-45 standards

   a. Perfusion fluid standards.

      Five standards were prepared by adding 0.05 µc (111,000 dpm) of ^{45}Ca in 0.05 ml of artificial chicken CSF (perfusion fluid A, Appendix 1) to 10 ml of scintillation fluid (Aquasol; New England Nuclear Corp., Boston, Mass.)

   b. Brain tissue standards

      Five standards were prepared by adding 0.05 µc (111,000 dpm) of ^{45}Ca in 5 ml of homogenized chicken brain tissue (Methods, part D4b) to 10 ml of Aquasol forming a stiff, opaque gel.

2. Counting procedure and calculations

The efficiency is calculated using the equation:

$$c_e = \frac{N_s \cdot 100}{D_s}$$

where: c_e = efficiency of calcium-45 counting (%)

N_s = net count rate of calcium-45 standards (cpm)

D_s = known disintegration rate of calcium-45 standards (dpm)

The disintegration rate of all unknown samples was determined using the equation:

$$D_u = \frac{N_u}{c_e}$$

where: D_u = disintegration rate of unknown samples (dpm)

N_u = net count rate of unknown samples (cpm)

APPENDIX III

FLAME PHOTOMETRY

APPENDIX III

FLAME PHOTOMETRY

Reference: Instruction Manual, Model 105 flame photometer, Beckman Instruments Inc., Fullerton, California

Principle:

The electrons of certain neutral atoms (such as sodium and potassium) can be raised from the ground state to an excited state by heat from propane-oxygen flame. In returning to the ground state, the excited electrons emit radiation of a specific wave-length which is characteristic for each atom. The intensity of the radiation is proportional to the number of atoms excited. Detection of the emitted radiation is by a phototube which is connected to amplifiers and a readout meter.

Reagents:

1. Lithium concentrate (18 mg/ml, Li^+; Beckman Instruments Inc., Fullerton, Calif.)
2. Potassium standard solutions (0,5,10 mEq/L K^+; Beckman Instruments Inc., Fullerton, Calif.)
3. KCl, A.R.

Solutions:

A. Lithium working solution

Mix 5 ml of reagent 1 with deionized water; q.s. 1 liter. This solution is used to dilute all standards and unknown samples.

B. Potassium working standards

1. Add 0.5 ml each of potassium (0,5 and 10 mEq/L) standard solutions (0,5 and 10 mEq/L) to solution A; q.s. 100 ml. These are 1/200 dilutions of 0,5 and 10 mEq/L potassium standards, respectively.
2. Two additional potassium standards (20 and 40 mEq/L) were prepared by adding 0.1492 and 0.2984 gm KCl to solution A; q.s. 100 ml. The reproducibility of these standards was used to verify the commercial standards (B-1).

Instrument Standardization:

1. Zero mEq/L potassium working standard (B-1) was aspirated into the atomizer for 5 seconds and the instrument adjusted to read zero mEq/L potassium. The procedure was repeated in triplicate.
2. The remaining potassium working standards (B-1; 5 and 10 mEq/L) were used to set the output span of the instrument and to determine the linearity of the response.

Procedure:

Duplicates of unknown samples were diluted 1/200 with solution A. Samples containing between 11-20 mEq/L potassium along with the 20 mEq/L potassium standard were diluted an additional 2-fold (final dilution, 1/400) while samples with 21-40 mEq/L potassium (and the 40 mEq/L potassium standard) were diluted an additional 4-fold (final dilution, 1/800) with solution A. All samples were aspirated into the atomizer for 5 seconds and their potassium concentrations (in mEq/L) were determined from the standards.

APPENDIX IV

ATOMIC ABSORPTION SPECTROSCOPY

APPENDIX IV

ATOMIC ABSORPTION SPECTROSCOPY

Reference: Instruction Manual, Model 290B Atomic Absorbance Spectrophotometer, Perkin-Elmer Co., Norwalk, Conn.

Principle:

Atomic absorption spectroscopy is based on the principle that neutral atoms of certain elements (such as calcium and magnesium) can absorb energy. The energy is supplied from a hollow cathode lamp, the cathode of which is constructed of the element under study. Energy emitted by the excited atoms from the cathode is characteristic of the metal of which the cathode is made. As a result, a calcium cathode will emit energy of a wavelength that only calcium atoms can absorb. The function of the flame in an atomic absorption instrument is to isolate the neutral, ground state atoms in the sample. The amount of radiation absorbed is detected by a phototube and is proportional to the number of atoms present in the sample.

Reagents:

1. Lanthanum oxide, La_2O_3 (American Potash and Chemical Corp., West Chicago, Ill.)

2. Brook standard calcium solution (1 mg/ml $CaCO_3$; Aloe Scientific, St. Louis, Mo.)
3. $CaCl_2$, A.R.

Solutions:

A. Lanthanum stock solution

Mix 58.64 gm La_2O_3 in 50 ml of deionized water. Slowly add 250 ml 0.8N HCl to dissolve the La_2O_3. Dilute to 1 liter with deionized water.

B. Lanthanum working solution

Dilute 30 ml of solution A with deionized water; q.s. 1 liter. This solution is used for diluting all standards and unknown samples.

C. Calcium working standards

1. Add 0.5, 1.0 and 2 ml of the calcium standard solution (reagent 2) to solution B; q.s. 200 ml. These are 2.5, 5 and 10 mEq/L calcium standards, respectively. Solution B was used as the zero calcium standard.
2. Two additional 5 and 10 mEq/L calcium standards were prepared by adding 0.02775 and 0.0555 gm of $CaCl_2$ to solution B q.s. 100 ml. The reproducibility of these standards was used to verify the commercial standards (C-1).

Instrument Standardization:

1. Solution B was aspirated through the nebulizer and the instrument was zeroed. The procedure was repeated in triplicate.
2. The other calcium working standards (C-1; 2.5, 5 and 10 mEq/L) were used to set the output span of the instrument and to determine the linearity of the response.

Procedure:

Duplicates of unknown samples were diluted 1/200 with solution B, aspirated for 20 seconds through the nebulizer and their calcium concentrations (in mEq/L) were determined from the standards.

APPENDIX V

CREATININE ASSAY

APPENDIX V

CREATININE ASSAY

Modified from S. Natelson, 1961

Principle:

Picric acid forms a colored complex in alkaline solution with creatinine, with maximum absorbancy at 490 mμ. Color intensity is proportional to the concentration of creatinine. In addition, color intensity is dependent on the temperature, concentration of alkali and picric acid and time, but these factors were maintained constant in the analysis.

Reagents:

1. Picric acid (J.T. Baker Chemical Co., Phillipsburg, New Jersey)
2. NaOH, A.R.
3. HCl
4. Creatinine (Pfanstiehl Laboratories Inc., Waukegan, Ill.)

Solutions:

A. Picric acid (1.0%)

Dissolve 10.0 gm picric acid in distilled water; q.s. 1 liter

B. NaOH (10%)

Dissolve 100.0 gm NaOH in distilled water; q.s. 1 liter

C. HCl (0.1 N)

Dilute 8.5 ml conc. HCl in distilled water; q.s. 1 liter

Creatinine standard solutions:

Creatinine is placed in a desiccator over calcium chloride or heated at 100°C in an oven to remove moisture. 200 mg of the dried creatinine is dissolved in 0.1 N HCl q.s. 100 ml (2.0 mg/ml creatinine). Dilute 8.75, 7.50, 6.25, 5.00, 3.75, 2.50 and 1.25 ml of 2.0 mg/ml creatinine solution to 10 ml with 0.1 N HCl obtaining 1.75, 1.50, 1.25, 1.00, 0.75, 0.50 and 0.25 mg/ml creatinine standards, respectively. Standards are layered with toluene, stoppered and stored at 4°C to prevent their deterioration.

Procedure:

Mix 8 parts of solution A with 2 parts of solution B forming alkaline picrate. Allow mixture to stand for 10 minutes before use. To duplicate 0.025 ml water blanks, standards and unknown samples add 2.5 ml alkaline picrate and mix in pyrex test tubes. After 10 minutes add 2.5 ml distilled water and mix. Read optical density (O.D.) of all samples against the water blank at 490 mμ within

15 minutes in a spectrophotometer (Model DB; Beckman Instruments, Inc., Fullerton, Calif.).

Calculations:

Optical density at 490 mμ plotted as a function of creatinine concentration in the standards yields a straight line over the range 0-2.0 mg/ml creatinine. Creatinine concentration in the unknown samples is calculated by multiplying the optical density of the unknown samples by the slope of the standard curve.

$$C_u = \left[\frac{C_s}{OD_s}\right]_a \times OD_u$$

where: C = concentration

OD = optical density

s = standard

u = unknown

a = average

APPENDIX VI

INULIN ASSAY

APPENDIX VI

INULIN ASSAY

Direct Resorcinal Method Without Alkali Treatment.
Modified from H. W. Smith, 1956, p. 209.

Principle:

Inulin is hydrolyzed to fructose by heating in acid and the fructose molecules combine with resorcinol to yield a colored complex. The intensity of the color is proportional to the amount of fructose present. Maximum absorbancy of the complex is 490 mμ.

Reagents:

1. Resorcinol (Fisher Sci. Co., Fairlawn, New Jersey)
2. Ethanol (95%)
3. HCl
4. Inulin (Pfanstiehl Laboratories, Inc., Waukegan, Ill.)

Solutions:

A. Resorcinol (1.0 mg/ml)
   Dissolve 100 mg resorcinol in 95% alcohol; q.s. 100 ml.

B. HCl (approximately 10 N)

Add 776 ml of concentrated HCl to 224 ml of distilled water

Inulin standard solutions:

Dissolve 200 mg of inulin in distilled water; q.s. to 100 ml (2.0 mg/ml). Dilute 7.5, 5.0, 4.0, 3.0, 2.0, and 1.0 ml of 2.0 mg/ml inulin solution to 10 ml with distilled water obtaining 1.5, 1.0, 0.8, 0.6, 0.4 and 0.2 mg/ml inulin standards, respectively.

Storage and satbility of solutions:

Stopper all standards and store in refrigerator at 0-4°C. Resorcinol (solution A) is prepared fresh daily. HCl (solution B) is stable indefinitely at room temperature.

Procedures:

To duplicate 0.05 ml water blanks, inulin standards and unknown samples, add 1.0 ml solution A and 2.5 ml solution B and mix under a hood in pyrex test tubes. A glass marble is placed on the top of each tube and the tubes are incubated for 25 minutes at 80°C in a water bath. The tubes are cooled to room temperature and the optical density at 490 mμ determined against the water blank within one hour in a spectrophotometer (Model DB; Beckman Instruments, Inc., Fullerton, Calif.).

Calculations:

Optical density at 490 mμ plotted as a function of inulin concentration in the standards yields a straight line over the range 0-2.0 mg/ml inulin. Inulin concentration in unknown samples is calculated by multiplying the optical density of the unknown samples by the slope of the standard curve.

$$C_u = \left[\frac{C_s}{OD_s}\right]_a \times OD_u$$

where: C = concentration

OD = optical density

s = standard

u = unknown

a = average

APPENDIX VII

SAMPLE CALCULATIONS OF CEREBROSPINAL FLUID FORMATION AND ABSORPTION RATES, OUTFLUX COEFFICIENT, AND OUTFLUX RATE

APPENDIX VII

SAMPLE CALCULATIONS OF CEREBROSPINAL FLUID FORMATION AND ABSORPTION RATES, OUTFLUX COEFFICIENT, AND OUTFLUX RATE

Data in Table 7-1 are measured quantities from a ventriculocisternal perfusion experiment which allow calculation of CSF formation and bulk absorption rates, CSF clearance and outflux coefficients for sodium and creatinine, and flux rate of creatinine.

Table 7-1. Primary data for chicken 3c.

Perfusion Flow Rates		Perfusion Inflow and outflow concentrations					
		RIHSA		^{22}Na		Creatinine	
$\dot{V}_i$ µl/min	$\dot{V}_o$ µl/min	C_i cpm/µl	C_o cpm/µl	C_i cpm/µl	C_o cpm/µl	C_i µg/µl	C_o µg/µl
38	37	161.8	156.6	92.4	82.3	2.21	1.79

Calculations from primary data

1. CSF bulk absorption (Equation 3, Methods)

$$\dot{V}_a = C_{RIHSA} = \frac{\dot{V}_i c_i - \dot{V}_o c_o}{c_o}$$

$$= \frac{(38)(161.8) - (37)(156.6)}{156.6} = 2.3 \text{ µl/min}$$

2. CSF formation (Equation 2, Methods)

$$\dot{V}_f = \dot{V}_a + (\dot{V}_o - \dot{V}_i) = 2.3 + (37-38) = 1.3 \text{ µl/min}$$

3. ^{22}Na calculations

a. Mean ventricular concentration (Equation 5, Methods)

$$\bar{c} = c_o + 0.37\ (c_i - c_o) = (82.3) + 0.37\ (92.4-82.3)$$

$$= 86.0 \frac{\text{cpm}}{\text{µl}}$$

b. ^{22}Na clearance (Equation 4, Methods)

$$C_{Na} = \frac{\dot{V}_i c_i - \dot{V}_o c_o}{\bar{c}}$$

$$= \frac{(38)(92.4) - (37)(82.3)}{86.0} = 5.4 \text{ µl/min}$$

c. ^{22}Na outflux coefficient (Equation 8)

$$K_{DNa} = \frac{\dot{V}_i c_i - \dot{V}_o c_o - (C_{RIHSA} \cdot c_o)}{\bar{c}}$$

$$= \frac{(38)(92.4) - (37)(82.3) - (2.3(82.3)}{86.0}$$

$$= 3.2 \text{ µl/min}$$

4. Creatinine calculations

a. mean ventricular concentration, $\bar{c}$ (Equation 5, Methods) =

$$1.79 + 0.37(2.21-1.79) = 1.95 \ \mu g/\mu l$$

b. creatinine clearance, C_{cr} (Equation 5, Methods) =

$$\frac{(38)(2.21) - (37)(1.79)}{1.95} = 9.1 \ \mu l/min$$

c. creatinine outflux coefficient, $K_{D_{cr}}$ (Equation 8, Methods) =

$$\frac{(38)(2.21) - (37)(1.79) - (2.3)(1.79)}{1.95}$$

$$= 7.0 \ \mu l/min$$

d. creatinine flux rate, $\dot{n}_{cr}$ (Equation 9, Methods)

$$\dot{n}_{cr} = K_{D_{cr}} \cdot \bar{c} = \dot{V}_i c_i - \dot{V}_o c_o - (C_{RIHSA} \cdot c_o)$$

$$= (7.0)(1.95) = (38)(2.21) - (37)(1.79)$$

$$- (2.3)(1.79) = 13.7 \ \mu g/min$$

APPENDIX VIII

EXTENDED FORTRAM PROGRAM FOR THE CALCULATION OF MASS BALANCE

```
PROGRAM     BSHEET                    CDC 6400 FTN V3.0-L292 OPT=1  08/23/72 .12.41.41.      PAGE      1

                PROGRAM BSHEET (INPUT,OUTPUT)
                DIMENSION T(25) , P(10) , S(5)
                LINEX = 55
                NPAGE = 0
 5            1 SUBAC1 = 0.
                SUBAC2 = 0.
                SUBAC3 = 0.
                SUBAC4 = 0.
                 GRAAC1 = 0.
10              GRAAC2 = 0.
                GRAAC3 = 0.
                GRAAC4 = 0.
                TOTCPM = 0.
          C
15        C  READ HEADER OR END CARD
          C
              4 READ  5, ID, NSAM, BLEFT,BRIGHT,(T(I),I=1,25)
              5 FORMAT(2I1, 8X, 2F10.8 , 25A2 )
                IF( ID.EQ.9 ) GO TO 999
20              IF( ID.EQ.1 ) GO TO 10
                PRINT 6, ID, NSAM, BLEFT,BRIGHT,(T(I),I=1,25)
              6 FORMAT( 29H BAD HEADER - END OF RUN CARD , 2X ,
               1       2I1, 8X, 2F10.0 , 25A2 )
                GO TO 4
25            999 STOP
          C
          C  PRINT SET-UP FOR THIS EXP
          C
             10 NPAGE = NPAGE + 1
30              NBB1 = 0
                PRINT 12,         (T(I), I = 1,25)
          C     PRINT 12, NPAGE, (T(I), I = 1,25)
          C  12 FORMAT( 1H1, /, 120X, 4HPAGE, I3,/,
             12 FORMAT( 1H1, /,                  /,
35             1                  52X, 33HMSU PHYSIOLOGY DEPT - DR. HEISEY ,/,
               256X, 24HCPM BALANCE CALCULATIONS ,/, 43X, 25A2)
                NLINE = 5
          C
          C    READ PERIOD CARD
40        C
             19 READ 20, ID, PERTIME, VI, CI,CLEAR,BBCLEAR,(P(I),I=1,10)
             20 FORMAT( I1, 9X, 5F10.0, 10A2 )
                IF( ID.EQ.8 ) GO TO 200
                IF ( ID.EQ.2) GO TO 25
45              PRINT 22,ID, PERTIME, VI, CI,CLEAR,BBCLEAR,(P(I),I=1,10)
             22 FORMAT( 30H BAD PERIOD OR END OF EXP CARD  ,
               1 2X,    I1, 9X, 5F10.0, 10A2 )
                GO TO 19
             25  BCLEAR = BBCLEAR
50              BB1 = ABS( BCLEAR )
                IF( BB1.GT. 1.E-6 ) NBB1 = 1
                LINE = 10
                IBACK = 1
                GO TO 900
55           29 PRINT 30, (P(I),I=1,10), PERTIME,VI,CI,CLEAR ,BCLEAR
```

```
      30 FORMAT( / , 68 ( 2H * ) , / , 63X, 10A2,
     1                  //, 53X, 29HCONSTANT DATA FOR THIS PERIOD ,//,
     2   15X, 6HPERIOD,   19X, 6HINFLOW, 19X, 6HINFLOW, 18X,
     3 9HCLEARANCE, 13X, 15HBULK ABSORPTION ,
60   4                /, 16X, 4HTIME, 21X, 4HRATE ,21X, 4HCONC, 22X,
     5 4HRATE , 20X, 4HRATE ,/,14X,F7.2,3(18X,F7.2),17X, F7.2,/ )
         SUBCPM = PERTIME * VI*CI
         TOTCPM = TOTCPM + SUBCPM
         IF( NSAM.EQ.1 ) GO TO 40
65       LINE = 5
         IBACK = 2
         GO TO 900
      34 NLINE = NLINE - 1
         PRINT 35
70    35 FORMAT( 62X, 11HSAMPLE DATA,//,
     16X, 6HSAMPLE, 4X,6HSAMPLE,5X, 10HCOLLECTION, 5X,7HOUTFLOW, 5X,
     2 7HOUTFLOW , 4X,23H- - -SAMPLE TOTALS- - - ,3X, 7HAVERAGE,
     3 5X, 21H - TOTAL  CLEARED -    ,/, 8X,3HNO. ,6X, 4HSIZE , 9X,
     4 4HTIME ,9X, 4HCONC,  9X, 4HRATE , 5X,23HCOUNTS   BULK CLEARANCE,
75   5 4X, 4HCONC,12X , 8HCONSTANT   ,/ )
C
C  READ SAMPLE CARD
C
      40 READ 45, ID, (S(I),I=1,5), SSIZE,STIME,SCPM
80    45 FORMAT( I1, 9X, 5A2, 3F10.0 )
         IF( ID.EQ.7 ) GO TO 100
         IF( ID.EQ. 3) GO TO 50
         IF( ID.EQ. 8 ) GO TO 200
         PRINT 47,ID, (S(I),I=1,5), SSIZE,STIME,SCPM
85    47 FORMAT( 18H SAMPLE CARD ERROR  ,
     1  2X,   I1, 9X, 5A2, 3F10.0 )
         GO TO 40
      50 NTEMP = SSIZE *SCPM    +.5
         TSCPM = NTEMP
90       SUBAC1 = SUBAC1 + TSCPM
         GRAAC1 = GRAAC1 + TSCPM
         NTEMP=(SCPM + .37 * ( CI -SCPM) ) * 10.  + .5
         CBAR = NTEMP
         CBAR  = CBAR / 10.
95        NTEMP = CBAR * CLEAR * STIME  + .5
         SCLEAR = NTEMP
         SUBAC2 = SUBAC2 + SCLEAR
         GRAAC2 = GRAAC2 + SCLEAR
         ORATE = (SSIZE/STIME) * 10. + .5
100      NTEMP = ORATE
         ORATE = NTEMP
         ORATE = ORATE / 10.
         SBCLEAR = 0.
         IF( BCLEAR - .001 ) 54,54, 52
105   52 NTEMP = BCLEAR * STIME * SCPM  + .5
         SBCLEAR = NTEMP
         SUBAC4 = SUBAC4 + SBCLEAR
         GRAAC4 = GRAAC4 + SBCLEAR
      54 IF( NSAM.EQ.1 ) GO TO 40
110      LINE = 1
```

```
                IBACK = 3
                GO TO 900
             56 PRINT 55, (S(I),I=1,5) ,SSIZE, STIME,SCPM,ORATE, TSCPM,SBCLEAR,
               1  CBAR, SCLEAR
115          55 FORMAT(4X, 5A2,F7.1,6X,F7.2,6X,F7.1,6X,F7.1,4X, F8.0, 4X,F8.0,
               1  6X, F7.1,11X,F8.0)
                GO TO 40
           C
           C  END OF PERIOD
120        C
             100 LINE = 7
                IBACK = 4
                GO TO 900
             101 PRINT 105
125          105 FORMAT( /, 49X, 5( 2H* ) , 16HPERIOD SUBTOTALS , 5( 2H * ) , / )
                PRINT 115, SUBCPM
             115 FORMAT( 12X, 11HTOTAL INPUT , 14X,F20.2 , 5H CPMS )
                SCOLL = (SUBAC1/SUBCPM) * 100.
                PRINT 120, SUBAC1, SCOLL
130          120 FORMAT( 12X,  15HTOTAL COLLECTED, 10X, F20.2,
               1  5H CPMS, F13.2, 23H PERCENT OF TOTAL INPUT )
                CLBA =  (SUBAC2/SUBCPM) * 100.
                PRINT 125, SUBAC2, CLBA
             125 FORMAT(          12X,  13HTOTAL CLEARED , 12X, F20.2,
135            1  5H CPMS, F13.2, 23H PERCENT OF TOTAL INPUT )
                IF( 981    - .001 ) 140,140,126
             126 P2 = (SUBAC4/SUBAC2) *100.
                PRINT 135, SUBAC4,P2
             135 FORMAT( 12X,18HTOTAL BULK CLEARED, 7X,F20.2,5H CPMS, F13.2 ,
140            1 25H PERCENT OF TOTAL CLEARED )
                P2 = ( SUBAC4 / SUBCPM ) * 100.0
                PRINT 136 , SUBAC4, P2
             136 FORMAT( 12X,18HTOTAL BULK CLEARED, 7X,F20.2,5H CPMS, F13.2 ,
               1 23H PERCENT OF TOTAL INPUT   )
145          140 SUBAC1 = 0.
                SUBAC2 = 0.
                SUBAC3 = 0.
                SUBAC4 = 0.
                GO TO 19
150        C
           C  END OF EXP - PRINT GRAND TOTALS
           C
             200 LINE = 10
                IBACK = 5
155             GO TO 900
             201 PRINT 205
             205 FORMAT( /, 52X, 5( 2H* ) , 12HGRAND TOTALS ,   5( 2H *) , / )
                PRINT 115 ,TOTCPM
                BLEFT = BLEFT + BRIGHT
160             BRIGHT =  ( BLEFT/TOTCPM ) * 100.
                PRINT 210, BLEFT , BRIGHT
             210 FORMAT( 12X , 14HTOTAL IN BRAIN ,11X, F20.2,
               1  5H CPMS, F13.2, 23H PERCENT OF TOTAL INPUT )
                TCOLL = (GRAAC1/TOTCPM ) * 100.
165             PRINT 120 , GRAAC1 , TCOLL      /
```

```
      TCLBA = (GRAAC2/TOTCPM ) * 100.
      PRINT 125 , GRAAC2 , TCLBA
      IF( NBB1.EQ.0 ) GO TO 220
  215 BULKN = GRAAC2 - GRAAC4
      P1 = (BULKN/TOTCPM) * 100.0
      PRINT 216, BULKN, P1
  216 FORMAT( 12X, 18HNON-BULK CLEARANCE, 7X, F20.2, 5H CPMS,
     1 F13.2 ,26H PERCENT OF TOTAL INPUT     )
      P2 = (GRAAC4/GRAAC2) * 100.
      PRINT 135, GRAAC4,P2
      P2 =  (GRAAC4 / TOTCPM ) * 100.0
      PRINT 136, GRAAC4, P2
      P1 =  (BLEFT / BULKN ) * 100.0
      PRINT 219, BLEFT,  P1
  219 FORMAT( 12X, 14HTOTAL IN BRAIN, 11X,F20.2,5H CPMS,F13.2,
     1  28H PERCENT OF NON-BULK CLEARED   )
  220 P2 = (BLEFT/GRAAC2) * 100.
      PRINT 225, BLEFT, P2
  225 FORMAT( 12X, 14HTOTAL IN BRAIN, 11X,F20.2,5H CPMS,F13.2,
     1  25H PERCENT OF TOTAL CLEARED )
      GO TO 1
C
C  PAGE AND LINE CONTROL
C
  900 NLINE = NLINE + LINE
      IF( NLINE. LT. LINEX ) GO TO 910
      NPAGE = NPAGE +1
      NLINE = 5 + LINE
C     PRINT 12, NPAGE, (T(I),I = 1,25)
      PRINT 12,        (T(I),I = 1,25)
      IF ( IBACK.EQ.1 ) GO TO 920
  910 GO TO ( 29, 34, 56, 101, 201) , IBACK
  920 PRINT 921, (P(I),I = 1,10 )
  921 FORMAT(/58X, 10A2, // )
      PRINT 35
      NLINE = NLINE + 7
      GO TO 910
      END
```

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