injections of one or three pituitaries per chick maintained on corn-cob meal ration suggesting that a threshold response to gonadotropic hormones had been reached with injections of one rat pituitary per chick.

Summary. Day-old cockerels were kept on a corn-cob meal ration while testing homogenates of rat pituitaries for gonadotropic hormones. The chicks failure to grow made the testes weight a more sensitive indicator of gonadotropin when contrasted with the cockerels on a balanced ration. With the corn-cob meal ration the chick assay method was significant with ½ of a rat's pituitary.

1. Robinson, G. E., Jr., and Nalbandov, A. V., *J. Ani. Sci.*, 1951, v10, 469.

2. Breneman, W. R., and Mason, R. C., *Endocrinol.*, 1951, v48, 752.

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Formation, Flow, and Reabsorption of Cerebrospinal Fluid in Man.* (20659)

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On the basis of data obtained from multiple simultaneous isotopic tracer studies in man, employing various suitable combinations of Na-24, K-42, Cl-38, Br-86, P-32, HTO, D_2O and radio-iodinated human serum albumin, we have been led to a unified theory of cerebrospinal fluid (CSF) formation, flow and reabsorption which is at variance with the classical and currently held concepts. The textbook teaching(1) is that CSF is formed by the choroid plexuses (Dandy, 2) of the cerebral ventricles whence it flows into the spinal and epicerebral subarachnoid spaces. Here it is considered free to circulate until it is finally reabsorbed, presumably largely or wholly at the arachnoid villi (Weed(3)).

We wish to present evidence that CSF is elaborated throughout the cerebrospinal fluid system independently of flow from one area to another. It is derived both from the choroid plexus in the ventricles, and in the subarachnoid space from the vascular bed of the pia. The reabsorption of CSF, in so far as water and electrolytes are concerned, has similarly been demonstrated by us to occur throughout the system independently of flow. These elements of the fluid enter and leave the ventricles and subarachnoid space by direct molecular capillary exchange, and hence may be considered to be in a state of dynamic equilibrium with plasma, similar to that of other fluid compartments of the body.†

Methods of study. After a large number of tracer studies had been done in patients with normal CSF systems, it became evident that a precise compartmental analysis of the data involved equations of a fair degree of complexity, and more variables than could be adequately measured, the most troublesome in this respect being the rate of flow between compartments. Accordingly we set out to limit the variables insofar as possible and to test the validity of a number of possible compartmental models of CSF physiology in a series of critical experiments. To this end, we sought patients for study in whom it would be possible to isolate the main ventricular compartments from the subarachnoid space. Two such patients were found; and the studies presented below, on which our unified concept of the CSF system rests, were carried out in them. These patients had developed complete blockage of CSF outflow from the large lateral ventricles as the result of tumor encroachment on the third ventricle over five years ago. At that time they were restored to a normal state of CSF pressure and dynam-

* This study was aided by grants from the National Institutes of Health, U. S. Public Health Service, by the Atomic Energy Commission and by the Associated Universities, Inc.

† In this paper, the mechanism of exchange is not considered, i.e., whether by simple diffusion or some form of active transport.

ics by means of a small plastic tube so placed surgically as to bypass the block and restore the flow of CSF from the lateral ventricles to the basal cistern of the subarachnoid space, (the so-called Torkildsen operation). In these last 5 years since operation, their intracranial pressures have remained essentially normal. The neurological signs in one of them (S.L.) had deteriorated somewhat, but the other has remained unchanged, suggesting in both cases a very slowly evolving type of lesion. In patient S.P. at the time of one of our studies, the CSF protein was within the normal range, being 13 mg% in the ventricle and 18 mg% in the lumbar subarachnoid space. The relatively normal status and CSF findings in these patients would seem to be reasonable evidence that the tumor tissue itself is making no significant contribution to the exchanges of water, electrolytes and protein. That any disturbance in the normal mechanism of exchange which may have attended their operations or prior period of hydrocephalus has had ample time for recovery in the intervening 5 years of unimpeded CSF outflow from the ventricles, is evident in the similarity between isotopic appearance curves in the ventricular CSF of these patients and those of more normal patients after intravascular injection(4). Hence, we feel warranted in the tentative conclusion that our studies in these patients provide an approximately quantitative as well as qualitative notion of the CSF mechanisms in normal man. The striking advantages offered by such patients are that first, by the simple expedient of clamping their Torkildsen bypassing tubes, the main ventricular compartments are effectively isolated from the subarachnoid space, and any communication between them by direct flow is precluded. Thorough mixing within each compartment can then be achieved by barbotage, and the volume of the compartments readily measured. Turnover constants calculated from the data are characteristic of the entire compartment, and when corrected for the volume of the compartment may be meaningfully compared. Finally, isolation of the subarachnoid space from the alleged main fountainhead of CSF, the choroid plexuses of the first three ventricles, provides a critical test of the importance of these structures in supplying the CSF of the subarachnoid space, or alternatively, evidence for the existence of some other important source.

Our experimental technic consisted of fixing small bore needles in the lateral ventricles and lumbar subarachnoid space, leaving them in position for the duration of the study, to obviate leakage from the puncture sites. The isotopic solutions were prepared in small volumes with physiologically insignificant amounts of carrier, and were injected either intravascularly or directly into the CSF system. After thorough mixing of the compartment, samples were withdrawn in less than 0.5 cc quantities, and metered out with calibrated Kinsey pipettes. The radioactivity of these CSF samples and of plasma samples was assayed in a well-type scintillation counter, and compared with a standard prepared in known dilution from an aliquot of the injection solution. K-42 and Cl-38 were chosen as being the most expedient isotopes for studying simultaneously major intracellular and extracellular ions. The 38 minute half-life of Cl-38 and 12.4 hour half-life of K-42 are sufficiently diverse to permit the assay of both isotopes from a single sample by serial counting before and after the chloride has decayed to inappreciable levels. We have measured ventricular volumes by isotopic dilution methods and checked these by dye dilution using indigo carmine and by planigraphic measurement of the ventricles on X-rays taken during pneumoventriculography. The volume of the subarachnoid space was measured in patient S.P. by the dilution of a known activity of I^{131} - human serum albumin immediately after protracted barbotage. Because of the short life of Cl-38, it was necessary to carry out much of this work at Brookhaven National Laboratory.‡ where Cl-38 is produced in the atomic pile.

Results. A. *Electrolytes and water.* Fig. 1 depicts the type of CSF appearance curves

‡ We are greatly indebted to Dr. Lee Farr, Director of the Medical Department, and his associates Drs. Dahl, Robertson, and Stickley, for their extraordinary cooperation and generosity in aiding the performance of these studies.

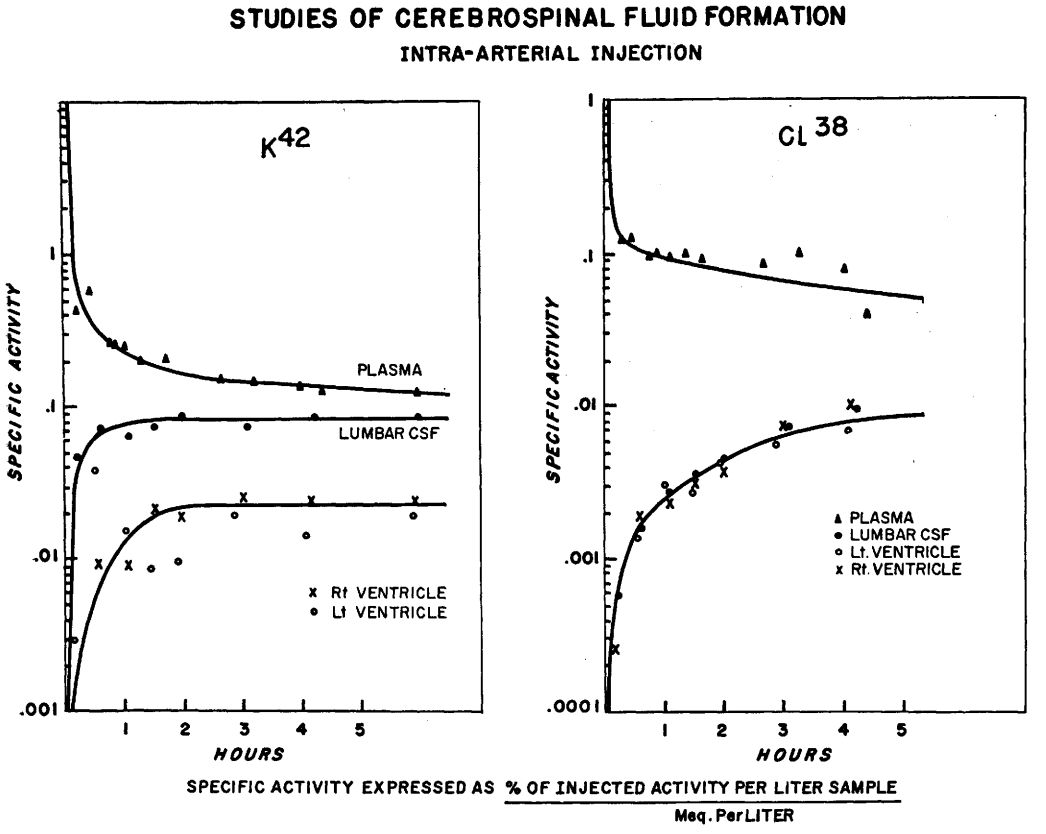

FIG. 1. Lateral ventricular volumes: Right, 301 cc, left, 320; volume of subarachnoid space: 83 cc.

obtained after intravascular injection of K-42 and Cl-38 with the ventricular compartments isolated from the subarachnoid space. One sees that in the case of potassium there is actually a far more rapid rise in isotopic concentration in the lumbar CSF than in the ventricles, and that chloride appears at the same rate in all 3 sites. We consider this to be decisive evidence for a rapid and direct exchange of electrolytes between blood and the subarachnoid space which is independent of flow from the choroid plexus. That a similar situation exists for water we have demonstrated in a previous communication (5). In this, the rate of appearance of D_2O was measured at various points in the CSF system of patients entirely normal in this respect following intravenous injection. D_2O in the cisternal CSF was seen to reach equilibrium much more rapidly than it did in the ventricles —an impossible situation if cisternal fluid is assumed to be derived from the ventricles by flow.

That reabsorption of water and electrolytes also occurs throughout the system we have demonstrated by studies of the disappearance of isotopes injected into the isolated ventricular compartments and into the subarachnoid space. The results of such a study employing again K-42 and Cl-38 are shown in Fig. 2. It may be seen that both of these substances pass rapidly out of the ventricles according to a simple exponential function, with turnover half-times of 4.3 hours and 8.2 hours for K and Cl respectively. The only possible routes of egress here are a retrograde passage through the choroid plexus supplemented by diffusion through the ependymal wall into the brain. D_2O injected into the ventricles of this patient at the same time as the electrolytes behaved

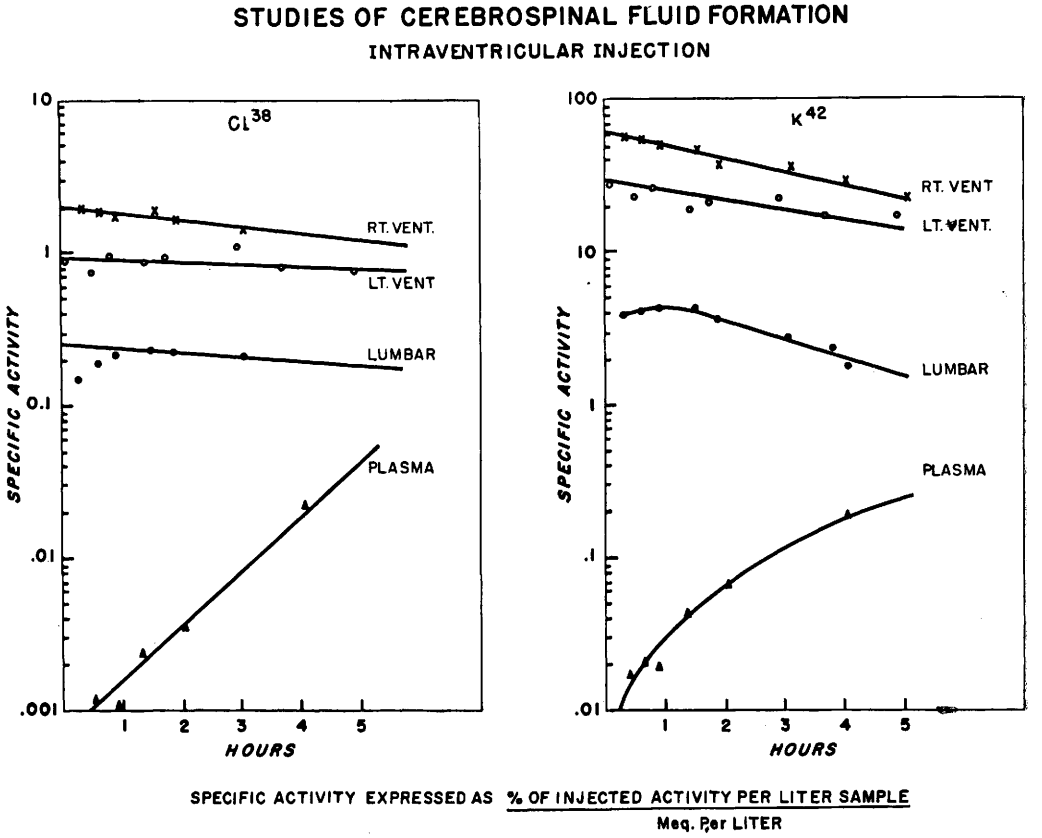

FIG. 2. Ventricular volumes: Right 220 cc, left 220 cc. The intraventricular inj. in this patient was carried out with the Torkildsen tube open. This permitted a small quantity of the isotopes to pass into the subarachnoid space; hence the rise during the first hour of isotopic concentrations in the lumbar region. The tube was closed during the last 4½ hr of study.

similarly with an average turnover half-time for ventricular water of 40 minutes and in subarachnoid space of 72 minutes.

A third feature of the behavior of electrolytes is that they move in and out of the CSF independently and each at its own characteristic rate. This is evident in the foregoing data and is supported by other multiple tracer studies done with various combinations of Na-24, Br-86, Cl-38 and P-32 in other patients.

It is thus seen that the water and electrolytes of CSF are in dynamic equilibrium with plasma, and that CSF is renewed by local exchange therewith in every region. Hence any net increase in volume within any compartment capable of initiating flow must be dependent either on a small secretion pressure (active transport) or on some alteration in the balance of hydrostatic and osmotic forces.

B. *Protein.* In clinical or experimental (Dandy(2)) instances where outflow from the ventricles is blocked for any protracted period of time, a slight unbalance in the equilibrium condition appears actually to occur since over a period of days or weeks increased pressure and an expanded ventricular volume results—clinically known as hydrocephalus. By analogy to the situation in the systemic circulation where the constant sweeping away of protein by the lymphatics is essential to the balance of transcapillary exchange, it occurred to us that a slow rate of flow away from the ventricles is necessary to prevent the accumulation of protein and the resulting shift in equilibrium due to the increased osmotic tension. In order to test this hypothesis, we have studied the rate of reabsorption of

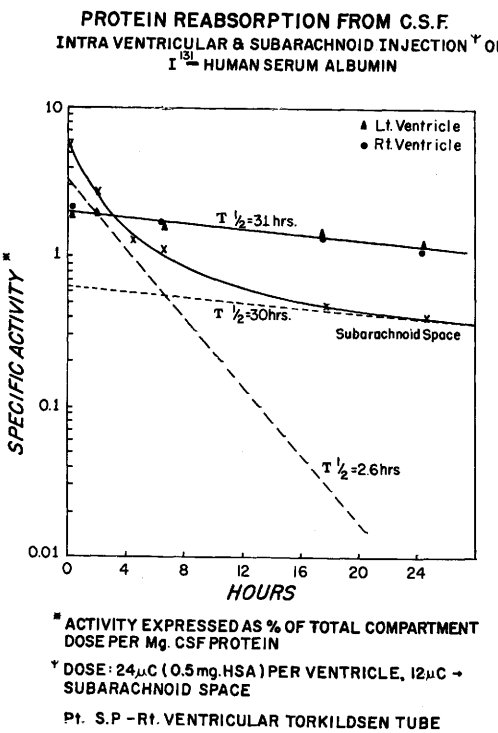

FIG. 3.

radio-iodinated human serum albumin from the isolated ventricles and subarachnoid space. Fig. 3 illustrates one set of results. Albumin was reabsorbed from the ventricles very slowly as a simple exponential function with a turnover half-time in this patient, of 31 hours. In sharp contrast to this, the rate of disappearance from the subarachnoid space was considerably more rapid and could be resolved into two exponential components: one with a half-time of 30 hours, essentially the same as the ventricular rate, and the other with a turnover half-time of 2.6 hours. This provides direct evidence for our theory that in contrast to the water and electrolytes of the CSF, protein is absorbed largely from the sub-arachnoid space. Based on abundant evidence in the older literature that the integrity of the arachnoidal villi is essential to the prevention of hydrocephalus (Weed(3)) and on demonstrations that certain large negatively charged molecules such as gelatin (Key and Retzius(6)) and ferrocyanide (Weed (7,8)) are specifically absorbed by the arachnoidal villi, it is our belief that these specialized structures subserve the special and essential function of protein reabsorption from the CSF. In this function, the arachnoidal villi become analogous to the lymphatics of the general circulation. To imbue them however, with the entire task of CSF absorption as in traditional theory is just as unsound as it would be to ascribe the entire return of capillary filtration to the lymphatics.§

There is a corollary to the facts that electrolytes and water of the CSF exchange everywhere freely whereas protein is absorbed largely from the subarachnoid space—namely that the site and rate of protein reabsorption is an important determinant of the direction and rate of flow of CSF within the system. We have attempted to determine the order of magnitude of net CSF formation and flow from the ventricles in these patients whose by-passing tubes from the lateral ventricles to the cisterna magna we could block. During the study, the patient was kept in a semi-reclining position at an angle such that the initial intraventricular pressure within the tube blocked was only 10- 20 mm above the vertex of the head. Over the 24 hour period of the study, withdrawal of 0.35 cc samples at less than hourly intervals for the assay of isotopic concentrations sufficed to keep the CSF pressure virtually constant. This was measured periodically, without withdrawal of fluid, between the times of sampling. At the conclusion of the 24 hours in one patient, the final pressure was a few millimeters of water lower than the initial pressure during which time a total of about 15 cc of fluid had been withdrawn and the patient's clinical condition and arterial blood pressure had remained in the same excellent state. Since at the end of the study the intraventricular pressure was slightly lower than initially, it seems improbable that any increase in ventricular volume occurred. Thus our figure of 15 cc withdrawn over the 24 hours must represent a fair estimate of the net amount of CSF elaborated in the lateral ventricles of this patient per

§ Professor A. Baird Hastings has repeatedly emphasized to us his opinion that the CSF might well turn out to be "the lymphatic fluid of the central nervous system". We are indebted to him for much aid in this work.

day. This estimate is substantially lower than the previous figures given in the literature for man: 300 to 400 cc/day by Dandy(2), and 600-700 cc/day given by Boyd(9). It is also proportionately lower considering the size of the species, than Flexner's(10) figure, for the cat of 12 cc/day flowing from cannulas in the aqueduct of Sylvius.

Summary. 1. Water and electrolytes enter the cerebrospinal fluid rapidly both in the ventricles and throughout the subarachnoid space. The formation of the fluid is thus not an exclusive prerogative of the choroid plexus. 2. Water and electrolytes leave directly into the blood from both the ventricles and the subarachnoid space, doing so at roughly similar rates. 3. CSF does not suddenly come into being fully formed at any point with all its constituents in their proper ratios, but each constituent is exchanging with blood at its own characteristic rate. 4. In contrast to water and electrolytes, protein is absorbed largely from the subarachnoid space, presumably from the arachnoidal villi. By virtue of this specific role played by the arachnoid villi, they serve a function in the CSF system analogous to the lymphatics of the general circulation. 5. The site and rate of protein reabsorption is an important determinant of the direction and rate of CSF flow. 6. The net amount of CSF elaborated per day in the ventricles, over and above the large and equivalent-volume exchanged, is small, and is of the order of 10 to 20 cc or less, per day in man.

1. Merritt and Fremont-Smith, *The Cerebrospinal Fluid,* Saunders, 1937.

2. Dandy, W. E., *Ann. Surg.,* 1919, v70, 129.

3. Weed, L. H., Studies on CSF, *J. Med. Res.,* 1914, v26, 51.

4. (Paper in preparation).

5. Sweet, W. H., Selverstone, B., Soloway, S, Stetten, D., *Surgical Forum Clinical Congress, Am. Coll. Surg.,* 1950, p376.

6. Key and Retzius, *Studien in der Anatomie des nervensystems unds des Bindegewebes,* Stockholm, 1875-76.

7. Weed, L. H., *Am. J. Anat.,* 1922-23, v31, 191.

8. ———, *Physiol. Rev.,* 1922, v2, 171.

9. Boyd, W., *Brit. Med. J.,* 1914, v1, 961.

10. Flexner, L. B., and Winters, H., *Am. J. Physiol.,* 1932, v101, 697.

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Natural Abundance of N^{15} in Hemin and Plasma Protein from Normal Blood.* (20660)

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Geren, Bendich, Bodansky and Brown(1) found the abundance of N^{15} in nitrogen of biological origin to be slightly higher than that of tank nitrogen used as a reference standard in the case of urinary urea, ammonia and uric acid. Urea isolated from the urine of normal individuals analysed 0.0044 $\pm$ 0.0005 atom per cent higher than tank nitrogen, and the total nitrogen, ammonia and uric acid from normal urines had N^{15} concentrations of about the same magnitude.

In connection with studies of erythropoiesis in the anemia of thermal injury(2) and of the metabolic fate of N^{15}-labelled erythrocytes transfused into normal man(3), we have had occasion to determine N^{15} abundance in a number of samples of hemin and plasma protein isolated from blood before administration of N^{15}-glycine. We have observed in hemin and plasma protein an increased abundance of N^{15} of the same magnitude found in compounds of urinary origin. For calculation of the amount of N^{15} incorporated from N^{15}-

* Studied in connection with projects conducted under contract between the Office of the Surgeon General, Department of the Army, Washington, D.C. and the Medical College of Virginia and by a grant from the U. S. Public Health Service to Dr. E. I. Evans, Director of the Laboratory for Surgical Research, Medical College of Virginia.