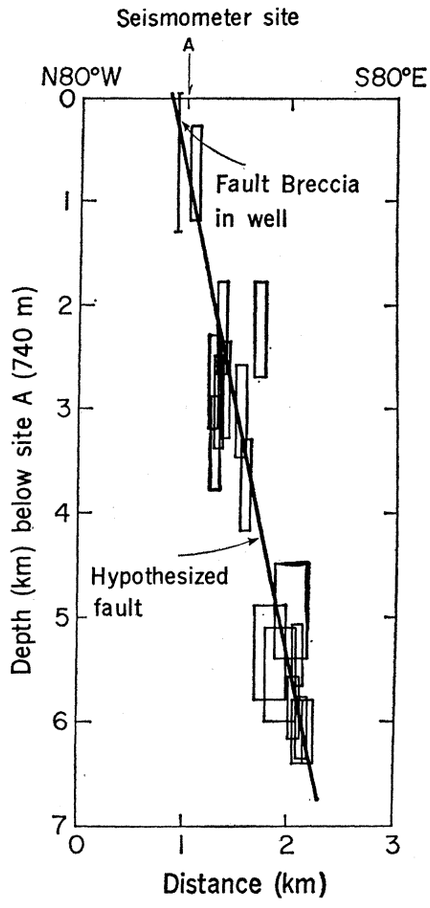

Fig. 3. Cross section through the geothermal area. Hypocenters of earthquakes are shown by rectangles. The vertical line just left of seismometer site A represents the well shown in Fig. 1.

fault directly under the best producing wells in the Ahuachapan geothermal area. Production wells in the future might best be drilled to intercept this fault. Such simple microearthquake studies provide a powerful method of mapping active faults and can, therefore, be of considerable practical and economic importance in the location and utilization of geothermal heat sources.

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References and Notes

1. See, for example, J. Oliver, A. Ryall, J. N. Brune, D. B. Slemmons, *Bull. Seismol. Soc. Am.* **56**, 899 (1966); L. Seeber, M. Barazangi, A. Nowroozi, *ibid.* **60**, 1669 (1970).
2. J. N. Brune and C. R. Allen, *ibid.* **57**, 277 (1967).
3. P. L. Ward, G. Pálmason, C. Drake, *J. Geophys. Res.* **74**, 664 (1969).
4. P. L. Ward and S. Björnsson, *ibid.* **76**, 3953 (1971).
5. For California: A. L. Lange and W. H. Westphal, *ibid.* **74**, 4377 (1969).
6. For Japan: I. Kasuga, *J. Geogr. Tokyo* **76**, 76 (1967).
7. J. W. Elder, in *Terrestrial Heat Flow*, W. H. K. Lee, Ed. (American Geophysical Union, Monogr. 8, Washington, D.C., 1965), p. 211; J. R. McKnit, *ibid.*, p. 240; G. W. Grindley, *Bull. N.Z. Geol. Surv.* **75**, 131 (1965).
8. D. M. Evans, *Geotimes* **10**, 11 (1966); J. H. Healy, W. W. Rubey, D. T. Griggs, C. B. Raleigh, *Science* **161**, 1301 (1968); C. B. Raleigh, J. Bohn, J. H. Healy, *Trans. Am. Geophys. Union* **51**, 351 (1970).
9. M. K. Hubbard and W. W. Rubey, *Bull. Geol. Soc. Am.* **70**, 115 (1959).
10. C. H. Scholz, *J. Geophys. Res.* **73**, 3295 (1968).
11. P. Molnar and L. R. Sykes, *Bull. Geol. Soc. Am.* **80**, 1639 (1969).
12. G. Pálmason, *Soc. Sci. Islandica*, No. 40 (1971).
13. J. Jónsson, "Report to United Nations DP Survey of Geothermal Resources in El Salvador" (United Nations Resources and Transport Division, Energy Section, New York, 1970).
14. P. C. Whiteford, *United Nations Symposium* (Pisa, Italy, 1970), in press; G. R. T. Clacy, *J. Geophys. Res.* **73**, 5377 (1968).
15. Contribution No. 1674 from Lamont-Doherty Geological Observatory of Columbia University. This work was supported by United Nations purchase order 9-20-11471. Special thanks to the staff of the U.N. Resources and Transport Division in El Salvador and the Comision Ejecutiva Hidroelectrica del Rio Lempa, who assisted in the fieldwork. Drs. Bryan Isacks and Jack Oliver critically reviewed the manuscript.

29 March 1971

Cerebrospinal Fluid Production by the Choroid Plexus and Brain

Abstract. *The production of cerebrospinal fluid and the transport of ^{24}Na from the blood to the cerebrospinal fluid were studied simultaneously in normal and choroid plexectomized rhesus monkeys. Choroid plexectomy reduced the production of cerebrospinal fluid by an average of 33 to 40 percent and the rate of appearance of ^{24}Na in the cerebrospinal fluid and its final concentration were proportionately reduced. In both normal and plexectomized animals, ^{24}Na levels were found to be markedly greater in the gray matter surrounding the ventricles and in the gray matter bordering the subarachnoid space. That sodium exchanges in these two general areas of the brain may be linked to the formation of the cerebrospinal fluid is discussed here.*

Although numerous studies (*1–3*), including the classical experiments of Dandy and Blackfan (*4*, *5*), have pointed to the choroid plexuses as the exclusive site of cerebrospinal fluid (CSF) secretion, this view has not gone unquestioned (*6–8*). Evidence has been advanced, for example, that at least a limited amount of CSF can be formed extrachoroidally in the cerebral ventricles (*6*), the aqueduct of Sylvius (*7*), and the subarachnoid space (*8*). In a recent study, the production of CSF after choroid plexectomy was estimated to be far more substantial than previously reported (*9*). On the basis of studies on 76 choroid plexectomized rhesus monkeys, these conclusions were reached: (i) The production of CSF rostral to the fourth ventricle (determined by ventriculo-aqueductal perfusion of the CSF with [^{14}C]-inulin) was reduced by only one-third after choroid plexectomy; (ii) the composition of the CSF after choroid plexectomy was unchanged; and (iii) the surgical obstruction of the choroid plexectomized ventricles resulted in acute and progressive hydrocephalus that was only slightly less severe than that occurring in nonplexectomized ventricles with similar obstructions (*9*).

In this study, we have compared the production of CSF and the transport of ^{24}Na from the blood to the CSF in normal and choroid plexectomized rhesus monkeys. The animals ranged in age between 1½ to 2 years and varied in weight between 2 and 3 kg. In six animals, the choroid plexuses of both lateral ventricles had been removed 3 to 9 months before the current experiments [the technique of choroid plexectomy and the histological consequences of the procedure have been reported elsewhere (*9*)]. Eleven animals served as normal, nonplexectomized controls. In all animals, the fourth ventricle was obstructed with an inflatable balloon at the beginning of each experiment (Fig. 1). A lateral ventricle-to-lateral ventricle perfusion was then arranged, in which a Harvard pump apparatus with an inflow rate of 0.191 ml/min was used. The ventricular perfusate consisted of artificial CSF to which the following tracers were added: (i) ^{14}C-labeled inulin (20 μc per 30 ml of perfusate); (ii) dextran 2000 (15 mg per 30 ml of perfusate), and (iii) ^{3}H-labeled sucrose (15 μc per 30 ml of perfusate). Simultaneously, an intravenous infusion of ^{24}Na (0.4 mc per experiment) was administered so as to establish a steady-state level of the isotope in the blood (± 3 to 8 percent variation from mean counts per minute per microliter). During each experiment, serial samples of plasma and outflow perfusate were taken at 15-minute intervals. Inflow samples were obtained at the beginning and end of each perfusion period, and

duplicate 50-μl samples of inflow, outflow, and plasma were placed in liquid scintillation counting vials. At the conclusion of the experiments, the animals were killed with pentobarbital, and the brains were rapidly removed and partially frozen in liquid nitrogen. Coronal sections were made, and 1-mm blocks of tissue running perpendicularly from the wall of the lateral ventricles to the surface of the brain were taken. All tissue and fluid samples were digested by pipetting 0.5 ml of NCS (Amersham-Searle) into the sample vials. The vials were then incubated in a water bath for 8 to 12 hours at 40°C. When digestion was completed, 18 ml of Liquifluor (New England Nuclear) was added to each vial and the ^{24}Na activity was immediately determined in a high energy–open window of a liquid scintillation spectrometer. After the ^{24}Na had decayed to background levels (10 to 14 days), the samples were recounted by liquid scintillation spectroscopy with the conventional double label method for separating ^{14}C and ^{3}H activity. Appropriate decay (^{24}Na) and quench (^{14}C and ^{3}H) corrections were applied, and all data were subsequently calculated as counts per minute per microliter (fluid samples) or counts per minute per milligram (tissue samples).

In ventricular perfusion experiments, the CSF production rates are determined by measuring the dilution of a large, inert marker material (such as [^{14}C]inulin or blue dextran) between the inflow and outflow sites. The equation for this calculation is

$$\dot{V}_F = \dot{V}_I \left(\frac{C_I}{C_0} - 1 \right) \qquad (1)$$

in which V_F is the CSF production rate ; V_I, the perfusion rate; C_I, concentration of the marker in the inflow; and C_0, concentration of the marker in the outflow (*10*, *11*). In the current experiments, the average CSF production of the lateral ventricles, third ventricle, and aqueduct of Sylvius in the normal, nonplexectomized group was 19.2 μl/min $\pm$ 2.2 (standard deviation). In the plexectomized group, the production rate was 13.3 μl/min $\pm$ 2.7. This represented an overall decrease in CSF production of 31 percent following choroid plexectomy. If it can be assumed that the choroid plexus of the third ventricle produces CSF in proportion to its size and weight [9 percent of the combined weight of the plexuses from the lateral and third ventricles (*9*)], then no more than 33 to 40 percent of the newly formed CSF in the nonplexectomized perfused system could be accounted for by the choroid plexuses.

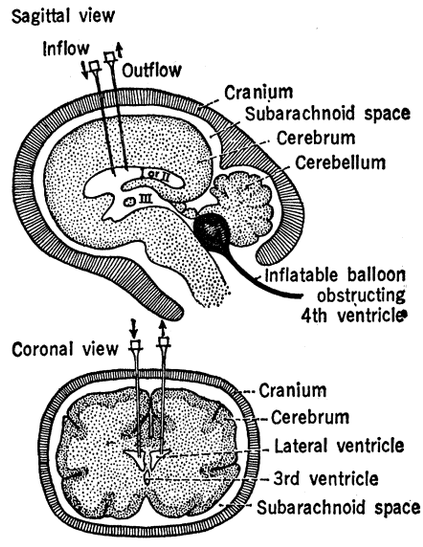

Fig. 1. Ventricular perfusion system.

The rate of appearance of intravenous ^{24}Na in the CSF, and the CSF concentration curves of the isotope in the plexectomized and nonplexectomized groups during the 3-hour infusion interval, are shown in Fig. 2. In general, the transport of ^{24}Na from the blood to the CSF was slightly delayed in the plexectomized group and the outflow concentration curves of the isotope were consistently lower. The ratios of ^{24}Na outflow to plasma at the end of each 3-hour perfusion averaged 0.102 in the control group and 0.062 in the plexectomized group.

Figure 3 illustrates the intracerebral distribution of ^{24}Na at the conclusion of each experiment. The amount of tracer sodium was markedly greater around the cerebral ventricles (the zone of periventricular gray matter) and adjacent to the cerebral subarachnoid space (the zone of cortical gray matter). In general, there was no difference in the intracerebral distribution of ^{24}Na in the plexectomized and nonplexectomized groups (this suggests that cerebral capillary permeability 3 to 9 months after choroid plexectomy was essentially normal). Of particular interest was the finding that the counts of ^{24}Na per milligram of tissue in the periventricular gray matter were invariably higher than the counts of ^{24}Na per microliter of CSF (one and a half to two times higher). Provided that the endogenous sodium spaces in the monkey approximate those in the rabbit [104 percent for the CSF, 35 percent for the brain (*3*)], then it would appear that the effective concentration of ^{24}Na in the periventricular tissue was five to six times greater than that in the CSF.

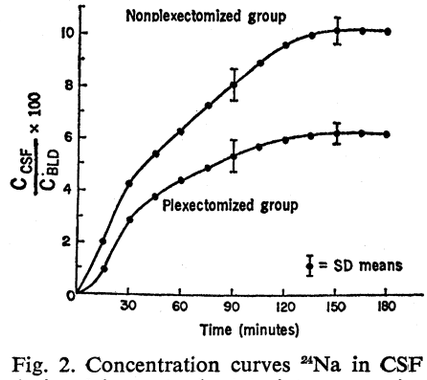

Fig. 2. Concentration curves ^{24}Na in CSF during 3-hour steady-state intravenous infusion of ^{24}Na. The production of CSF for the nonplexectomized group was 19.2 μl/min ($\pm$ 2.2, standard deviation); for the plexectomized group, 13.3 μl/min ($\pm$ 2.7). *C* is concentration.

In view of the foregoing observations, it is apparent that the production of CSF is not abolished in the rhesus monkey 3 to 9 months after choroid plexectomy. Indeed, in the current experiments, the production of CSF in the chambers rostral to the fourth ventricle was reduced to about two-thirds of the normal rate. This does not necessarily mean that in the normal nonplexectomized animal more than 60 percent of the CSF is formed at extrachoroidal sites. Such a high production of extrachoroidal fluid might be unique to the plexectomized model (that is, secretion induced by choroid plexectomy). On the other hand, there is evidence that the CSF formed by the plexectomized ventricular system is in no way different from normal (*9*, *12*). This indicates, at least, that sites other than the choroid plexus can elaborate a fluid whose composition of water, electrolytes, and protein is similar to that of normal CSF. Regardless of its origin, the production of extrachoroidal fluid may be important clinically. It is well known, for example, that cauterization or removal of the choroid plexuses is rarely a curative treatment for hydrocephalus. The data reported here indicate that a continued production of CSF from extrachoroidal sites may explain some of these failures.

It is interesting to examine the differences in ^{24}Na transport in the plexectomized and nonplexectomized animals (Fig. 2). In both groups of animals, the intravenously infused ^{24}Na entered the CSF rapidly and reached significant concentrations during the 3-hour infusion interval. In the plexectomized animals, however, the tracer sodium entered the CSF slightly later and never

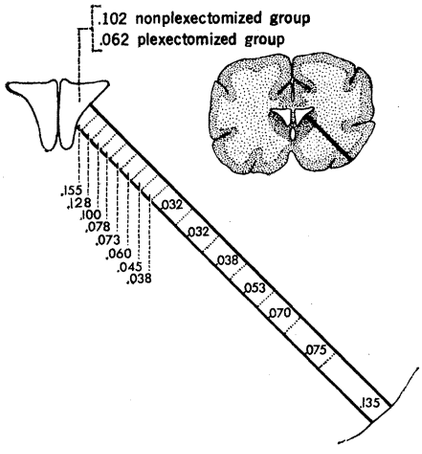

Fig. 3. Distribution of ^{24}Na in the brain and CSF at conclusion of 3-hour steady-state intravenous infusion of ^{24}Na. The radioactivity is given in counts per minute per milligram of tissue (or counts per minute per microliter of CSF or plasma). No corrections have been made for differences in vascular volume, capillary density, or endogenous sodium spaces in the various areas of the brain. The counts from all experiments have been averaged. There was no difference in the intracerebral distribution of ^{24}Na in the plexectomized and nonplexectomized groups.

reached the same concentrations as in the control animals. A comparison of the entry of ^{24}Na into the ventricular fluid and the rate of CSF secretion can be made by using a simple mathematical description of the system. If it is assumed that ^{24}Na enters the ventricular fluid only in the secreted CSF (that is, no diffusional or molecular transport of labeled sodium occurs between ventricular fluid and blood or brain, or both) and that the concentration of the ^{24}Na in the secreted CSF water equals that in the plasma water, then the rate of change of the amount of tracer sodium in the perfused ventricular system is given by the following equation:

$$\frac{dn}{dt} = \dot{V}_F N_p - \left(\dot{V}_F + \dot{V}_I \right) N_0 \quad (2)$$

where N is the amount of labeled sodium; t, time; N_p, concentration of labeled sodium in the plasma (and secreted CSF); and N_0, concentration of labeled sodium in the outflow. When the amount of ^{24}Na reaches a steady-state in the ventricular system ($dn/dt = 0$), Eq. 2 can be reduced and rearranged to

$$\frac{\dot{V}_F}{\dot{V}_I + \dot{V}_F} = \frac{N_0}{N_p} \quad (3)$$

Substituting the values for the secretion and perfusion rates from this study into Eq. 3, the calculated tracer sodium ratio (N_0/N_p) would be $19.2/(191 + 19.2) = 0.0915$ for the normal animals and $13.3/(191 + 13.3) = 0.065$ for the plexectomized animals. The experimental averages for the ratio of ^{24}Na in the outflow water to that in the plasma water were 0.097 and 0.060, respectively (ratios of ^{24}Na outflow to plasma in Fig. 2 are not corrected for water). The agreement between the calculated and experimental values is good and indicates that both the tracer flux and CSF secretion were similarly affected by choroid plexectomy. Overall, the data in Fig. 2 suggest (i) that ^{24}Na is transported from the blood to the CSF across extrachoroidal as well as choroidal boundaries, and (ii) that the transport of ^{24}Na into the ventricular fluid is proportional to the rate of intraventricular CSF production.

Finally, any conclusions regarding the extrachoroidal sites of CSF production must remain speculative. The pattern of ^{24}Na distribution in the brain parenchyma (Fig. 3) suggests that there are differences in the sodium exchange kinetics between tissue adjacent to the CSF (periventricular and cortical gray matter) and the deep tissue (white matter). The ^{24}Na tissue profile could be the result of variations in the (i) vascular volume, blood supply, and capillary permeability; (ii) endogenous sodium space; (iii) rate of intracellular uptake or binding, or both; and relative proportions of intracellular and extracellular compartments between these two general zones of brain tissue. However, all of these possibilities taken together do not seem adequate to explain the four- to fivefold variation in the tissue levels. This suggests that other, more complex mechanisms may play a role in the sodium exchange kinetics of brain tissue. In view of the agreement between the rate of CSF production and the entry of ^{24}Na into the CSF in both normal and plexectomized animals, it is reasonable to suspect that the brain tracer profiles are related, in part, to the parenchymal formation and flow of fluid (extrachoroidal CSF). Needless to say, with the data available in this study, no firm conclusions can be drawn about the brain ^{24}Na profile or the mechanisms involved in its development.

In summary, we conclude that the choroid plexus is but one site of cerebrospinal fluid production. We are unable at this time, however, to arrive at any definitive opinions regarding the extrachoroidal source of the CSF. Our findings indicate that sodium ions in the blood distribute in much higher amounts in the brain tissue bordering the ventricles and subarachnoid space. Whether such sodium exchanges are linked to the formation of the cerebrospinal fluid awaits further proof.

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References and Notes

1. K. Welch, *Am. J. Physiol.* **205**, 617 (1963).
2. J. D. Rougemont, A. Ames, F. B. Nesbitt, H. F. Hofmann, *J. Neurophysiol.* **23**, 485 (1960); A. Ames, M. Sakanowe, S. Endo, *ibid.* **27**, 672 (1964); A. Ames, K. Higashi, F. B. Nesbitt, *J. Physiol.* **181**, 506, 516 (1965).
3. H. Davson, *Physiology of the Cerebrospinal Fluid* (Little, Brown, Boston, 1967).
4. W. E. Dandy, *Ann. Surg.* **70**, 129 (1919).
5. ——— and K. D. Blackfan, *Am. J. Dis. Child.* **8**, 406 (1914).
6. G. B. Hassin, *J. Neuropathol.* **7**, 432 (1948).
7. M. Pollay and F. D. Curl, *Am. J. Physiol.* **213**, 1031 (1967); F. D. Curl and M. Pollay, *Exp. Neurol.* **20**, 558 (1968).
8. E. A. Bering and O. Sato, *J. Neurosurg.* **20**, 1050 (1963); O. Sato and E. A. Bering, *Brain Nerve (Japan)* **19**, 883 (1967).
9. T. H. Milhorat, *Science* **166**, 1514 (1969).
10. S. R. Heisey, D. Held, J. R. Pappenheimer, *Am. J. Physiol.* **203**, 775 (1962).
11. For the measurement of the CSF production rate to be valid the following conditions must obtain: (i) the volume of the ventricular system does not change; (ii) the concentration of the marker in the system reaches a steady state; and (iii) the diffusional or molecular loss of the marker material from the perfusion fluid is negligible. In the current experiments, the CSF production rates were calculated on the basis of the dilution of [^{14}C]inulin (approximate molecular weight, 5×10^3). Technical difficulties were encountered with the initial photometric determinations of blue dextran (approximate molecular weight, 2×10^6) and for this reason the dextran data were not used. Although there is preliminary evidence that the *absolute* CSF production rates are more accurately determined by the dextran dilution technique [CSF losses of dextran 2000 are apparently less than for the smaller inulin molecule: H. Davson and M. B. Segal, *J. Physiol.* **209**, 131 (1970)], it is likely that the *relative* differences in CSF production between the plexectomized and nonplexectomized animals can be accurately determined by the inulin dilution technique.
12. E. A. Bering, *Workshop in Hydrocephalus* (Univ. of Pennsylvania Press, Philadelphia, 1965), pp. 9–19.

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