One would expect no lasting effect of the oxygen deficiency if its action were only direct. On the other hand, if it produces some biochemical modifications which are injurious to malignant growths, these modifications might affect also a cancer which is inoculated subsequent to the cessation of the low pressure exposure. One of our more recent experiments seems to prove that malignancy acquired by pretreated rats may run a different course than in untreated animals.

Eleven normal rats were exposed to a 300 mm. pressure and inoculated with sarcoma on the first or second day after their removal from the tank. The control series comprised 9 rats which were inoculated at the same time with fragments from the same sarcoma. The takes were in both series 100%. The tumors grew at first at an equally rapid rate. Later 3 of the pretreated neoplasms retrogressed completely, as compared with one retrogression among the controls. The other control tumors grew to a huge size and presented an almost solid mass of normal sarcoma tissue. On the other hand, the tumors of the pretreated animals became very soft and the section of them showed that they had liquified, leaving only a thin rim of cancer tissue of doubtful normality.

4830

A Comparison of Ultrafilterable Serum Calcium and Cerebrospinal Fluid Calcium in Humans.*

DAVID M. GREENBERG. With the Assistance of H. E. Ballard and J. B. Dalton.

From the Division of Biochemistry, University of California Medical School, Berkeley.

Cameron and Moorhouse[1] have proposed using the cerebrospinal fluid calcium as a measure of the diffusible calcium of the blood plasma, arguing that the choroid plexus as a living, colloid impermeable membrane gives us a more perfect distribution of the diffusible constituents of the blood than can be obtained in any *in vitro* manner. This implies both that the cerebrospinal fluid is formed by a process of diffusion rather than of active secretion and also that the fluid is continuously in diffusion equilibrium with the constit-

* Presented at the Ann Arbor meeting of the Society of Biological Chemists, April, 1928.

[1] Cameron, A. T., and Moorhouse, V. H. K., *J. Biol. Chem.*, 1925, lxiii, 687.

uents of the plasma. Whether the cerebrospinal fluid is formed by an active process of secretion or is simply a dialysate of the blood plasma is still a mooted question.

Histological data and evidence from the mechanics of circulation, favoring the dialysis theory have recently been reviewed by Fremont-Smith.[2] It has been pointed out by Updegraff, Greenberg and Clark[3] and by Fremont-Smith,[2] that the published data of various authors for the amounts of uric acid, urea, glucose, magnesium and inorganic phosphate present in plasma and spinal fluid respectively are difficult to reconcile with the diffusion theory.

Even if it is granted that the spinal fluid is a dialysate of the blood plasma, there still remains the important point of whether the spinal fluid is continuously in equilibrium with and gives a true picture of the changing states of the constituents of the blood plasma, or if it is only rarely in actual diffusion equilibrium with the blood plasma. If there is diffusion equilibrium, the distribution of the ionic constituents between plasma and spinal fluid is expected to conform to the relations governing a Donnan membrane equilibrium. Hamilton[4] arrived at the conclusion: " . . . . an equilibrium of the Donnan type may, at least partly, govern the distribution of electrolytes between serum and spinal fluid, but it seems probable that the equilibrium is modified by unknown factors." Van Slyke[5] takes the viewpoint that the deviations in the electrolyte distribution from the Donnan theory are probably due to the spinal fluid not being usually in diffusion equilibrium with the blood plasma. Further, anatomical considerations hardly seem to favor the view that the bulk of the spinal fluid is continuously in diffusion equilibrium with the blood coursing through the capillaries of the choroid plexus. In view of the conflicting findings and opinions it seems that a great many more experimental data will be required before the questions of the manner of origin and the relationship between spinal fluid and blood can be definitely settled.

Experimental: To test the hypothesis that the spinal fluid calcium is a measure of the diffusible calcium, we have carried out parallel determinations of the diffusible calcium and the spinal fluid calcium on blood and spinal fluid obtained simultaneously from human subjects. The diffusible calcium was determined by the analysis of

[2] Fremont-Smith, F., *Arch. Neurol. and Psych.*, 1927, xvii, 317.

[3] Updegraff, H., Greenberg, D. M., and Clark, G. W., *J. Biol. Chem.*, 1926, lxxi, 87.

[4] Hamilton, B., *J. Biol. Chem.*, 1925, lxv, 101.

[5] Van Slyke, D. D., "Factors affecting the distribution of electrolytes, water and gases in the animal body." Harvey Lectures, 1926.

serum ultrafiltrates, the ultrafiltration being carried out according to the procedure described by Greenberg and Gunther.[6] The values obtained for total calcium, diffusible calcium and spinal fluid calcium on the 9 subjects available are given in Table I. It is unfortunate that more subjects were not available to obtain a more extensive series of results and thus a more rigorous test of the relation between the diffusible and spinal fluid calcium. However, since work on the problem has been discontinued for the present, it seems desirable to publish the data obtained rather than put off publication in the hope of obtaining a larger series of results at some future time.

TABLE I.

A Comparison of Diffusible Calcium and Cerebrospinal Fluid Calcium.*

Name	Diagnosis	Total Ca mg. per 100 cc. Serum	Diffusible Ca mg. per 100 cc. Ultrafiltrate	Spinal Fluid Ca mg. per 100 cc.	$R = \frac{\text{Ca S.P.}}{\text{Ca d}}$
Cro.	Post influenza encephalitis	10.5	6.5	5.1	0.78
Cal.	Diagnosis not established. Spinal fluid globulin 3+	8.1	3.7	5.2	1.40
Pit.	Mitral stenosis	10.3	4.9	5.1	1.04
Geo.	Urethral calculus	8.3	4.3	5.0	1.16
Jor.	Acute myocitis	10.6	5.7	5.1	0.89
Sne.	Syphilis	9.6	4.2	5.0	1.19
Ahe.	Syphilis. Spinal fluid globulin 3+		4.8	4.9	1.02
Dav.†	Infant		4.3	4.5	1.04
Eis.	Syphilis	9.6	4.4	4.3	0.98

* The blood and spinal fluid samples were obtained through the courtesy of Drs. Lewis Gunther and Wm. J. Kerr of the Department of Medicine, University of California Medical School.

† Blood obtained by heel puncture.

It is to be noted that all the subjects presented various pathological conditions although none were definitely known to be suffering from a disease in which calcium metabolism is known to be disturbed. The use of pathological cases is an advantage for the experimental test to be made in the present instance. For under such circumstances greater fluctuations of the blood constituents and thus a better chance to detect lags in the maintenance of equilibrium with the spinal fluid are to be expected. The diagnoses of the cases studied are given in the table.

If there is an equilibrium between blood and spinal fluid, differences between the obtained values for diffusible calcium and spinal

[6] Greenberg, D. M., and Gunther, L., *J. Biol. Chem.*, 1930, lxxxv, 491.

fluid calcium are to be expected because of the use of serum rather than plasma and because of the Donnan distribution. However, a rather constant ratio between the 2 calciums should be obtained if this idea is correct. The results obtained show that in 4 of the cases, the difference between diffusible and spinal fluid calcium was not marked, being little more than the probable error of the analytical method. The differences in the other 5 cases are considerable. The variations are indicated by the ratios of spinal fluid calcium to diffusible calcium given in the last column of Table I. The values for spinal fluid calcium obtained are in good agreement with the figures published by Hamilton[4] and others. Of the values for diffusible calcium, the figure for subject "Cal," 3.7 mg. per 100 cc. of ultrafiltrate is markedly lower than the normal level and 6.5 mg. per 100 cc. for "Cro" is high. For subject "Cal" the total calcium is also concomitantly low. The other values are within the normal limits for diffusible calcium.

The results of Table I point to the conclusion that when the blood calcium is at a normal stable level there is an approximate approach to an equilibrium between blood and spinal fluid and the spinal fluid calcium is then a close measure of the diffusible calcium. But on the other hand, when the blood constituents are undergoing marked fluctuations the spinal fluid changes do not keep pace. This hypothesis offers an explanation of the anamolous findings of Cameron and Moorhouse[1] who on parathyroidectomized dogs obtained but little lowering of the spinal fluid calcium although the total calcium dropped greatly. The hypothesis of a near approach to equilibrium in cases of a normal stable level of the blood constituents and the failure to keep pace with marked fluctuations also harmonizes with the approximate agreement to a Donnan membrane distribution found in some instances and the wide departure in others noted by Hamilton.[4]