($10^{-4}$) virus, growth seemed to be slighter than in the controls 2 days after inoculation. However, one day later, virus growth in all 45 tubes was the same as in the controls. In a second experiment, to control more closely the possible retarding effect of mucoprotein on the infection, virus inocula decreasing to the limit of infectiveness (from $10^{-4}$ to $10^{-8}$) were used, with a fixed dose, 100 $\mu$g/tube, of mucoprotein; in one series of tubes the mucoprotein was kept in contact with the cells for 24 hours, then washed (before inoculation), in another, it was present for the duration of the experiment. The mucoprotein induced no measurable effect on virus development and infectivity in this experiment including 2 series of 5 replicate tubes for each of the 5 virus dilutions used. In another experiment of the same size, previous exposure of the cells for 4 days to 100 $\mu$g mucoprotein or even its continued presence after inoculation did not influence growth and infectivity. Nor did mixing $10^{-4}$ to $10^{-8}$ inocula with 100 $\mu$g mucoprotein for one hour at room temperature change virus growth. In neutralization test, using mice for test of virulence, up to 2500 $\mu$g/ml mucoprotein had no direct effect on the virus: all 50 mice inoculated died at the same time as the controls. (In the same test, the minimal neutralizing dose of gammaglobulin, 625 $\mu$g/ml, gave 100% survival.)

*Conclusions.* It appears that certain mucoproteins exert a protective action against experimental poliomyelitis infection of mice and, interestingly, by oral administration also. This action does not seem to be related to virus neutralization, nor to an interference of virus uptake by the cell, or to virus multiplication and infectiveness, according to the results of tissue culture experiments. Excluding the direct action of mucoprotein on the virus, on its cellular uptake and multiplication, the hypothesis of a "nonspecific" effect, whereby treatment would increase in some manner the nonspecific resistance of the organism, becomes suggestive. The lack of dose-effect relationship is favorable to such an hypothesis, though it is quite possible that the dose range was above the minimal required to elicit protective action. The acid mucoprotein (C) and the $B_{12}$ binding substance (B) appeared to be less effective than the blood group substance (A) in the single experiment in which they were compared. However, our data are not sufficient for quantitative comparison between different types of preparations. Whether the action noted is an integral property of this and perhaps other types of mucoproteins, or related to a single, more simple component or subunit, such as a mucopolysaccharide, requires further investigation.

*Summary.* Mucoproteins obtained from hog gastric mucosa exert a protective effect in experimental poliomyelitis (Type II) infection of mice, while they have no action on the virus in tissue cultures. The protective action is attributed to some mechanism increasing nonspecific resistance of the host.

1. Cresseri, A., *Boll. Soc. Ital. Biol. Sperim.*, 1954, v30, 718.

2. Bodian, D., *Virology*, 1956, v4, 575.

Received January 29, 1960. P.S.E.B.M., 1960, v103.

## Transfer of Radioiodinated Human Serum Albumin (RISA®) from Cerebrospinal Fluid to Blood Plasma.* (25643)

C. A. VAN WART, J. R. DUPONT, L. KRAINTZ (Introduced by H. E. Hoff)
*Department of Physiology, Baylor University College of Medicine, Houston, Texas*

There have been many conflicting reports regarding the site of reabsorption of cerebrospinal fluid. Cerebrospinal fluid protein plays a significant role in association with many pathological states of the CNS; however, little is known about the dynamics involved. Behring(1), Sweet(2), and others(3) have described transfer of various ions and non-

* Supported by grant from Nat. Inst. of Neurol. Dis. and Blindness USPHS.

electrolytes between blood stream and cerebrospinal fluid. Apparently each plasma constituent has its own rate of exchange. Until quite recently, nearly all work on CSF dynamics had been done with dyes and colloids, or particulate matter only(4). Use of radioactive tagged serum albumin offered a promising approach to the problem of protein transfer between CSF and plasma. Fishman (5) obtained an equilibrium time on dogs of 18-20 hours for albumin by administering radioiodinated serum albumin and monitoring cisternal fluid and plasma. Sweet's data with 2 patients gave quite varied rates of clearance from the CSF for intrathecally administered radio-albumin.

Our purpose was to examine plasma to determine appearance time and equilibrium time for intracisternal administered radioiodinated serum albumin in dogs. Concentration of CSF-protein is somewhat lower in the dog than in the human, but all other considerations indicate that the dog is an ideal test animal for this purpose. A control study was also done to demonstrate whether colloidal gold would pass from CSF to plasma under similar conditions.

*Methods.* The dogs were anesthetized with a combination of morphine sulfate and sodium pentobarbital. Doses of 25 $\mu$c of RISA (Abbott) containing approximately 0.5 mg of albumin were prepared in ½ ml volume after passing it through an anion exchange resin to eliminate ionic iodide. A series of 12 mongrel dogs averaging 13.5 kg received ½ ml of the above preparation into the cisterna magna *via* a 3 inch 22 gauge spinal needle. Blood samples were taken at varying time intervals up to 24 hours from the whole blood. Radioactivity was assayed in a well type scintillation gamma counter. All samples were counted for at least 5000 counts and corrected for background. The nature of the plasma radioactivity was determined by scanning paper electrophoresis strips of the samples. To compare data obtained from different animals, radioactivity of the plasma was calculated as percent of equilibrium. One hundred percent equilibrium was defined as amount of radioactivity which would have been present in the total circulating plasma had the radioactivity been administered intravenously. Circulating plasma volumes of the dogs were determined by the dye dilution technic using Evans Blue. In addition, 3 dogs were given ½ ml of Au-198 (Aurcoloid, Abbott) intrathecally. A clear tap was demonstrated in all instances before injection. These dogs were scanned for radioactivity utilizing the same technics as for thyroid gammagrams. The blood of these dogs was also radioassayed.

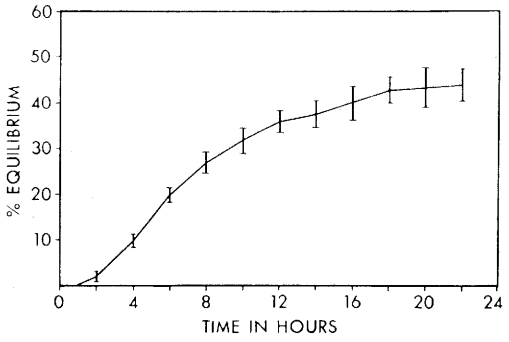


FIG. 1. Appearance of radioactivity in plasma after intrathecal instillation of RISA. One hundred percent equilibrium is defined as level of plasma radioactivity which would be found if total inj. dose was completely transferred from CSF to plasma. Values at each time interval represent mean ± stand. error obtained in 12 dogs.

*Results.* Fig. 1 shows the appearance of radioactivity in plasma after intrathecal instillation. The curve represents the average of 12 dogs. After intrathecal injection, RISA migrates to the plasma. Little radioactivity was found in blood within the first hour, however levels significantly above background appeared at the end of 2 hours. Sensitivity of the determinations at that point was limited by amount of radioactivity administered, plasma volume, time needed for mixing of the injection within the CSF-space, and sensitivity of the counting equipment. There was a fairly rapid increase over the first 16-18 hours, when the curve levels off. Constant values were found between 16-20 hours in these experiments. At equilibrium there was no further increase in radioactivity of the plasma. Paper strip electrophoresis of the plasma samples showed that radioactivity was associated with the albumin fraction.

When Au-198 was injected intrathecally and the blood radioassayed for its appearance,

no values above background could be detected for a 24 hour period. A gammagram at 26 hours indicates that the colloidal gold was localized within the cranial cavity of the dog. Most of the gold appears in the midline with only a slight extension down into the spinal space. This lack of extensive migration within the fluid space is perhaps due to the horizontal position of the dog, or rapid phagocytosis of colloid by CSF-macrophages. No radioactivity was detected in the region of cervical or thoracic lymph-nodes. The spleen and liver were also not above background.

*Discussion.* The methods used offer several advantages. Repeated taps of a small space such as the CSF are avoided, and trauma or leakage around the puncture-needle brought to a minimum. No special care need be taken in collecting the sample or regarding the position of the dogs during experiment. Negligible amounts of protein are injected so as not to upset concentration or dynamics of the fluid.

In the present study the value of 16-20 hours agrees well with the equilibrium time reported by Fishman. Data obtained by Sears *et al.*(6) indicate, however, that over 24 hours approximately 52% of intravenously injected RISA is lost from the plasma; only a fraction of this, in all likelihood, going to the CSF. Similar results were obtained under our experimental conditions. The radio-albumin entering the blood stream from the cerebrospinal fluid is hence distributed in the same manner. After RISA begins to leave the CSF-space it is distributed throughout plasma, lymph, interstitial fluid, and presumably the CSF. The failure of the curve in Fig. 1 to reach more than 45% of the calculated equilibrium is thus clearly explained. The 3-5% remaining is ascribed to experimental error and the rest left in the cerebrospinal fluid.

Under the condition of our experiments Au-198 was not detected outside the CSF. Colloidal dyes have been reported to collect around the arachnoid villi, in the perivascular spaces, or around nerve roots. Wollard(7) noticed that mesothelial cells ingested Trypan blue. This behavior of colloidal particles was taken as evidence that the Pachionean bodies were major factors in CSF absorption. Although the present study cannot confirm this, it does indicate that lymphatic drainage or a route involving the Virchow-Robin's spaces are not of importance in absorption of colloids of the size of colloidal gold. Added support is thus lent to Bowsher's(8) leptomeningovascular pathway.

*Summary.* Passage of albumin from cerebrospinal fluid to blood plasma was determined in anesthetized dogs by injecting radio-iodinated human serum albumin into the cisterna magna and monitoring the plasma radioactivity. An equilibrium time of 16-20 hours was obtained. Radioactive colloidal gold injected intrathecally was not detectable in the general circulation in 24 hours and appeared to be well localized within the cerebral fluid space.

1. Behring, E. A., *J. Neurosurg.,* 1954, v11, 234.
2. Sweet, Brownell, School, Bowsher, Benda, Stickley, *Proc. Assn. Res. Nerv. Ment. Dis.,* 1956, v34, 101.
3. Greenberg, Aird, Boelter, Campbell, Cohn, Murayama, *Am. J. Physiol.,* 1943, v140, 47.
4. Weed, L. H., *J. Med. Res.,* 1914, v31, 51.
5. Fishman, R. A., *Am. J. Physiol.,* 1953, v175, 96.
6. Sears, Allen, Gregerscn, *ibid.,* 1953, v175, 240.
7. Wellard, H. H., *J. Anat.* (London), 1923, v58, 87.
8. Bowsher, D. R., *Anat. Rec.,* 1957, v128, 23.

Received February 1, 1960. P.S.E.B.M., 1960, v103.