

animal. In our experiments doses several times the lethal dose elicited no responses which suggested release of significant amounts of histamine.

Summary. 1) In anesthetized rabbits, lethal doses of compound 48/80 produced marked depression of respiration with ensuing blood pressure changes that could be altered by administering artificial respiration. Blood pressure and electrocardiographic records in animals artificially respired soon after receiving doses several times the lethal dose revealed no evidence of adverse effects on the vascular system or on cardiac rate and rhythm. 2) Under similar conditions, however, a lethal dose of histamine produced a prompt and profound fall in blood pressure, marked changes in cardiac rate and rhythm and death despite artificial respiration. The effect appears to be primarily on the cardiovascular system. Respiratory effects are prob-

ably secondary to this main action. 3) The lethal action of 48/80 in rabbits is apparently not mediated by release of endogenous histamine since large doses do not elicit histamine-like responses.

1. Paton, W. D. M., *Brit. J. Pharmacol.*, 1951, v6, 499.
2. Dews, P. B., Wnuck, A. L., Fanelli, R. V., Light, A. E., Tornabeni, J. A., Norton, S., Ellis, C. H., DeBeer, E. J., *J. Pharmacol. Exp. Therap.*, 1953, v107, 1.
3. Giscafre, L., DeEspanes, E. M., *Arch. Internat. Pharmacodyn.*, 1948, v76, 25.
4. Dale, H. H., *Ann. N. Y. Acad. Sci.*, 1950, v50, 1017.
5. Rocha E. Silva, M., *Histamine, Its Role in Anaphylaxis and Allergy*. Charles C. Thomas, Springfield, Ill. 1955.
6. Schachter, M., *Brit. J. Pharmacol.*, 1953, v8, 412.

Received January 5, 1959. P.S.E.B.M., 1959, v100.

Co⁶⁰ Vit. B₁₂ Binding Capacity of Normal Human Cerebrospinal Fluid.*† (24715)

LEO M. MEYER, ROBERT W. BERTCHER AND CLAIRE MULZAC
South Nassau Communities Hospital, Oceanside, N. Y.

As extension of continuing investigations in this laboratory on Co⁶⁰ Vit. B₁₂ combining capacity of serum and saline(1,2,3), the following study was made on ability of normal human cerebrospinal fluid to bind radiovitamin. Utilizing microbiologic assay methods, Sobotka, Christoff and Baker recently reported Vit. B₁₂ content of cerebrospinal fluid from normal subjects to range from 0-30 μg/ml(4). These authors found elevation of Vit. B₁₂ in the cerebrospinal fluid and serum of about one-third of patients with multiple sclerosis.

Material and methods. Cerebrospinal fluid was obtained from 22 normal subjects with no apparent neurologic disorder, who were sub-

jected to spinal puncture for injecting an anesthetic agent. The Co⁶⁰ Vit. B₁₂ had a specific activity of 0.823 mc/mg. To successive 1 ml aliquots of cerebrospinal fluid in Visking bags were added 1, 2, 5, 5, 10, 25, 50 and 100 mμg of radiovitamin. After incubation at room temperature for 2 hours, the mixture was subjected to exhaustive dialysis in cold, running tap water for 48 hours. All specimens were set up in duplicate, one to serve as a standard. Standards and dialyzed samples were placed each in 4 ml vials, filled with concentrated sulfuric acid to dissolve the casing, and radioactivity measured in a well-type scintillation counter. "Bound" Vit. B₁₂ was calculated as activity retained in the bag after dialysis. Similar studies were made in 5 instances in which 1 ml of normal saline was substituted for cerebrospinal fluid, Co⁶⁰ Vit. B₁₂ added, and dialysis performed as de-

* This study was supported in part by grant from Nat. Cancer Inst., N.I.H., U.S.P.H.S.

† Co⁶⁰ vit. B₁₂ was provided by Merck and Co., Rahway, N. J.

TABLE I. Co^{60} Vit. B_{12} Binding Capacity of Normal Human Cerebrospinal Fluid.

m μg Co^{60} B_{12} added/ml spinal fluid	1	2.5	5	10	25	50	100
Mean m μg Co^{60} B_{12} bound	.47	.57	.71	1.0	1.6	2.4	4.2

scribed above. Protein content of 10 samples of cerebrospinal fluid was determined by the method of Looney and Walsh(5).

Results. Table I shows mean values for Co^{60} Vit. B_{12} combining capacity of cerebrospinal fluid from 22 human subjects with no clinical evidence of neurologic disorder. Mean CSF protein value was 34.8 mg %. Table II summarizes mean results of dialysis in 5

TABLE II. Co^{60} Vit. B_{12} in Saline Retained after Dialysis.

m μg Co^{60} B_{12} added/ml saline	1	2.5	5	10	25	50	100
Mean m μg Co^{60} B_{12} retained	.07	.09	.12	.15	.25	.5	1.0

cases in which saline was substituted for cerebrospinal fluid. Fig. 1 depicts mean spinal fluid binding capacity minus "background" of mean saline values described in Table II. For comparative purposes mean Co^{60} Vit. B_{12} binding capacity of normal human serum is shown in Fig. 1.

Discussion. Previous studies suggest a 2-phase Co^{60} Vit. B_{12} binding capacity of normal human serum, with an increase of first component in chronic myelocytic leukemia, and some patients with myeloid metaplasia and polycythemia.(1,2). This phenome-

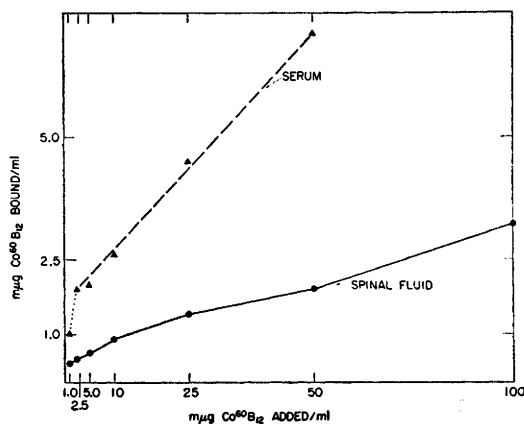


FIG. 1. Comparison of Co^{60} vit. B_{12} combining capacity of normal human serum and cerebrospinal fluid (saline values deducted).

non appears to be lacking in normal cerebrospinal fluid (Fig. 1). Furthermore, slope of the line describing spinal fluid binding capacity does not parallel either of the serum components. Serum binding capacity at the 1 m μg level is 2.5 times that of the spinal fluid and more than 3.5 times higher at the 50 m μg point.

Pitney found serum Vit. B_{12} bound to alpha globulin *in vivo* and to beta globulin when added *in vitro*(6). Pert and Kutt have recently investigated the distribution of protein fractions in cerebrospinal fluid using starch gel electrophoresis(7). Electrophoretic patterns of normal serum and concentrated spinal fluid show alpha and beta globulin peaks that are essentially the same. Consequently small amounts of protein in spinal fluid are probably not responsible for B_{12} binding in the spinal fluid unless they are in some fashion altered and have a strikingly increased number of binding sites or are potentiated by some other substance or substances present. However many other materials have the capacity to bind Vit. B_{12} and their presence or absence in spinal fluid have not been demonstrated(8).

Conclusions. 1. Normal human cerebrospinal fluid has the capacity to bind Co^{60} Vit. B_{12} . 2. This capacity to bind Vit. B_{12} increases as concentration of radiovitamin is increased. 3. Relationship of Co^{60} B_{12} binding capacity to protein content is higher for normal cerebrospinal fluid than for normal human serum.

1. Bertcher, R. W., Meyer, L. M., PROC. SOC. EXP. BIOL. AND MED., 1957, v94, 169.
2. Meyer, L. M., Bertcher, R. W., Cronkite, E. P., *ibid.*, 1957, v96, 360.
3. Bertcher, R. W., Meyer, L. M., Miller, I. F., *ibid.*, 1958, v99, 513.
4. Sobotka, H., Christoff, N., Baker, H., *ibid.*, 1958, v98, 934.
5. Looney, J. M., Walsh, A. I., *J. Biol. Chem.*, 1939, v127, 117.
6. Pitney, W. R., Beard, M. F., Van Loon, E. J., *ibid.*, 1954, v207, 143.
7. Pert, J. H., Kutt, H., PROC. SOC. EXP. BIOL. AND MED., 1958, v99, 181.
8. Grasback, R., *Acta Med. Scand.*, 1956, suppl. 314, v154, 3.

Received January 6, 1959. P.S.E.B.M., 1959, v100.