The elevation of corticosterone levels in the animals receiving shock might provide the answer to the increased pressor response. Elevation of cortical steroids with environmental stimuli has been reported(10), while increase in reactivity to renin with large doses of exogenous steroids has been noted(11).

The significance of these experiments lies in the production of an altered physiologic response by a prior behavioral stimulus. The possible role of this type of change in the development of psychosomatic disease states has been discussed by Engel(12). We have reported alterations in metabolic responses after behavioral stimuli(13,14), and the present data provide evidence of change in reactivity by such stimuli in still another area.

Summary. It has been demonstrated that angiotensin hyperresponsivity can be induced in the normotensive animal by a behavioral stimulus. This hyperresponsivity is limited to the period immediately following application of the stimulus. It is not associated with a change in baseline pressure and does not appear to be mediated through the autonomic nervous system, but it is accompanied by increased levels of plasma corticosterone.

The authors wish to acknowledge the technical assistance of Miss Eileen Tyrrell and Mrs. Thelma Klaniecki.

1. Shapiro, A. P., Ann. Int. Med., 1960, v53, 64.
2. Shapiro, A. P., Moutsos, S. E., Krifcher, E. J., J. Clin. Invest., 1963, v42, 978.
3. Gaskell, J. R., Jones, R. K., Shapiro, A. P., Proc. Soc. Exp. Biol. and Med., 1964, v115, 179.
4. Cohen, G., Goldenberg, M. J., Neurochem., 1957, v2, 58.
5. Fister, H. J., Manual of standardized procedure for spectrophotometric chemistry. Standard Scientific Supply Corp., New York, 1950, p43a.1.
6. Guillemin, R., Clayton, G. N., Smith, J. D., Lipscomb, H. S., Endocrinol., 1958, v63, 349.
7. Edwards, A. I., Statistical Methods for the Behavioral Sciences, Rinehart & Co., New York, 1954, p315.
8. Shapiro, A. P., Melhado, J., Psychosom. Med., 1958, v20, 303.
9. von Euler, U. S., Clin. Pharm. and Ther., 1964, v5, 398.
10. Mason, J. W., Harwood, C. T., Rosenthal, N. R., Am. J. Physiol., 1957, v190, 429.
11. Masson, G. M. C., Corcoran, A. C., Page, I. H., Endocrinology, 1957, v61, 409.
12. Engel, G. L., Psychosom. Med., 1961, v22, 18.
13. Sapira, J. D., Lipman, R. L., Shapiro, A. P., ibid., 1965, v27, 165.
14. Sapira, J. D., Tyrrell, E. M., Klaniecki, T., Shapiro, A. P., Enzymologia, 1966, v30, 97.

Received April 25, 1966. P.S.E.B.M., 1966, v123.

Function of Transcobalamin II: A B_{12} Binding Protein in Human Plasma.* (31400)

CHARLES A. HALL AND ALEXANDER E. FINKLER
Hematology and Radioisotope Research Laboratories, Veterans Administration Hospital and Department of Medicine, Albany Medical College of Union University, Albany, N. Y.

Early studies in vitamin B_{12} binding by plasma showed that more than one protein could participate(1). B_{12} naturally present was bound to an alpha globulin while part of that which was added to plasma *in vitro* was bound to a protein with the mobility of a beta globulin. Subsequently these observations were confirmed by several investigators and by a variety of techniques of protein separation. The significance of the beta globulin in binding has been the subject of much controversy but a physiologic role for this binder usually has been considered unlikely since no one has shown that it bound B_{12} which had been taken into plasma by natural means.

In 1963(2) we found that when vit B_{12} was either injected or taken by mouth it was initially bound to a protein which we subsequently called transcobalamin II (TC II) (3). By conventional electrophoresis, the mobility of TC II is close to that of the beta

* Supported in part by NIH Grant AMO-2808.

TABLE I.

Form of radioactive B_{12}	Route of administration	Amt of B_{12} given	S.A., $\mu c/\mu g$	Time plasma taken
Cyanocobalamin ($Co^{57}CNB_{12}$)	*In vitro*	1.0 pg/ml	120.0	
Hydroxocobalamin ($Co^{57}OHB_{12}$)	"	25.0 "	8.7	
$Co^{57}CNB_{12}$	Oral*	1.4 μg	11.0	8 hr
$Co^{57}OHB_{12}$	" *	2.0 "	5.8	8 "

* Fasting; followed by light breakfast.

globulins but was separated from them by our system of fractionation. Doubts that TC II is a naturally functioning substance are in part due to the use in these studies of relatively large amounts of a perhaps unnatural form of B_{12}, cyanocobalamin, which was in crystalline form. The present study was designed to investigate further the physiologic function of TC II by determining whether TC II would bind B_{12}; (1) when a truly tracer amount was added to plasma, (2) when several forms of B_{12} were used, (3) when natural conditions of intake were reproduced.

Material and methods. Basic to all parts of the study was incorporation of Co^{57} vit B_{12} into plasma followed by separation of the plasma proteins by a system of DEAE-cellulose anion exchange column chromatography described previously(3). Plasma was taken from normal subjects for *in vitro* studies and the *in vivo* studies were performed in hospital patients who had illnesses that involved neither nutrition, the blood nor vit B_{12} metabolism. Usually 5-20 ml of plasma were separated in each study. The radioactivity in each fraction from the column was measured by scintillation spectrometry. All details of the techniques have been given previously(3,4).

The conditions of each experiment are outlined in Table I. A fifth study was performed in an attempt to reproduce the natural intake of B_{12}. Two one-month-old chicks were placed on a synthetic, vit B_{12} free diet for 12 days. They were then given a 22-day period of 4 nanograms (ng) each of $Co^{57}CNB_{12}$ (specific activity 150 $\mu c/\mu g$) daily. The Co^{57} CNB_{12} was well mixed with the food in advance and the diet was stored in the cold until used. The chicks were then given the diet alone, without B_{12} in any form, for 13 days and sacrificed. The total radioactivity of the 2 livers was measured, and total μg of B_{12} was estimated by *Euglena gracilis* assay of the hepatic B_{12} of a control chicken on the same regimen but receiving the B_{12} as equivalent amounts of non-radioactive B_{12}. The livers were lightly boiled, seasoned, made into a paste and fed to a control subject with a light meal of crackers and chicken broth. The subject received 0.5 μg of B_{12} and 2.4 μc of Co^{57} in 41 g of liver. Fifty-four ml of plasma were collected at 8 hours and fractionated by the usual technique but using a larger column.

Only single studies were made in each experiment. The reproducibility and reliability of the basic techniques had been demonstrated previously(3,4). The sole purpose of the study was to show that the B_{12} would bind to TC II and there was no attempt to show the relative amounts bound to various plasma binders.

Results. Fig. 1a shows a typical chromatogram taken from previous studies(4) to illustrate the positions of the various B_{12} binders. The first 80 fractions which contain gamma and beta globulins are not illustrated since B_{12} is not found in these fractions when small amounts of B_{12} are added. Transcobalamin II (TC II) routinely comes off the columns before albumin, and transcobalamin I (TC I) is found in the alpha globulin region. Endogenous vit B_{12} is bound to TC I. The protein in each fraction was measured in each experiment of the present study but it is not shown in the Figures.

The binding patterns after very small amounts of *in vitro* $Co^{57}CNB_{12}$ and Co^{57} OHB_{12} are shown in Fig. 1b and 1c. The different ordinates in Fig. 1a-c reflect the different amounts of B_{12} added. After oral intake of either form of the vitamin, TC II

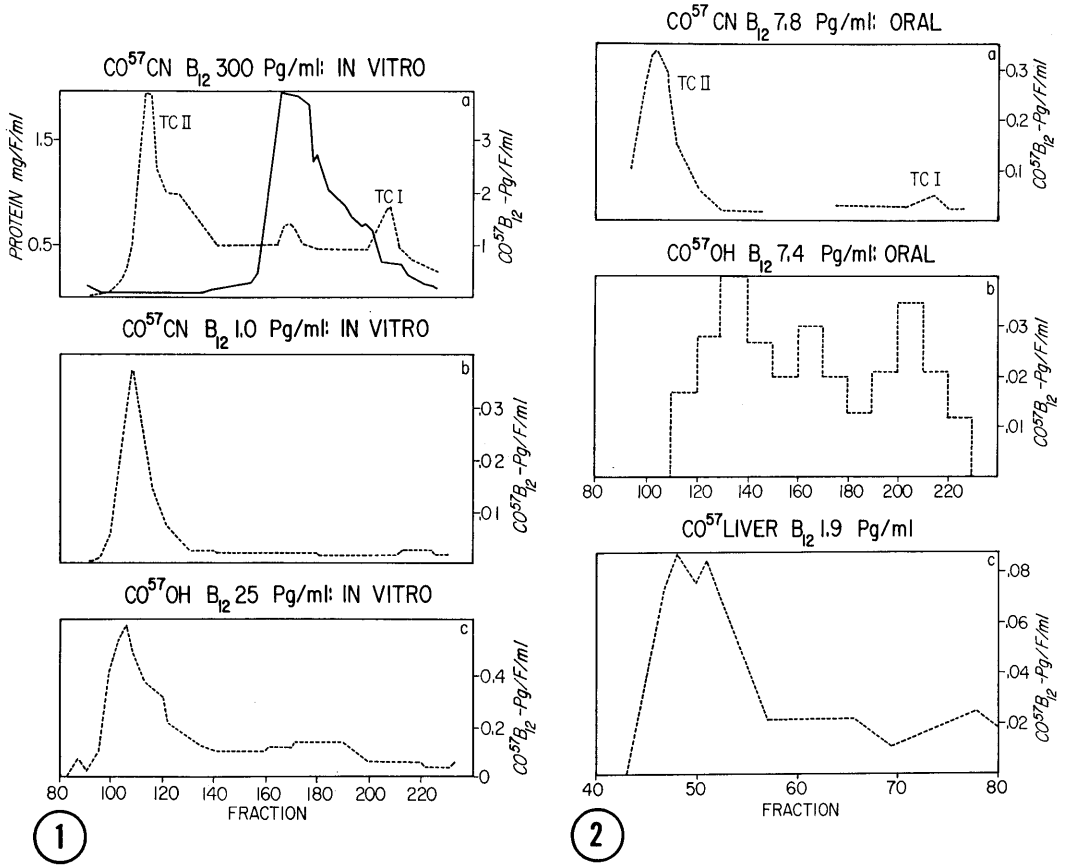

FIG. 1. Results of DEAE-column chromatography of $Co^{57}B_{12}$ bound to plasma *in vitro*. All sections show the same regions of the chromatograms. The figure of pg/ml above the chromatogram in each case is the rise in B_{12} per ml of plasma due to the added radioactive B_{12}. The pg/F/ml in the ordinates is the $Co^{57}B_{12}$ per fraction related to 1 ml of the plasma that was applied to the column. ——Protein, ... Co^{57}.

(a) The basic chromatogram of routine *in vitro* $Co^{57}B_{12}$ binding studies in this laboratory.

(b) After 1.0 pg Co^{57}/ml cyanocobalamin added *in vitro*.

(c) After 25 pg Co^{57}/ml of hydroxocobalamin added *in vitro*.

FIG. 2. (a) Chromatogram of plasma taken 8 hr after Co^{57} cyanocobalamin by mouth. 7.8 pg/ml is the rise in plasma B_{12} at 8 hr due to intake of $Co^{57}B_{12}$.

(b) After Co^{57} hydoxocobalamin by mouth. Low counting rates made it necessary to pool and concentrate the fractions in units of 10.

(c) After Co^{57} liver B_{12}. The illustration is constructed so that the 80 fraction system used here corresponds to the 220-230 fraction system used in the other experiments.

also bound a large part of the recently absorbed B_{12} (Fig. 2a, 2b). It should be noted that when B_{12} is given by mouth, it is difficult to build up a high TC II-B_{12} peak since the intake of B_{12} into the plasma is slow and the TC II-B_{12} is removed rapidly(3). The difference in ordinates used in Fig. 2a and 2b suggests that the $Co^{57}OHB_{12}$ was poorly absorbed but the similar $Co^{57}B_{12}$ concentrations in the plasma after the oral intake of the CN and OH forms suggest an equal degree of absorption. There was greater spreading of the $Co^{57}B_{12}$ in the chromatogram of the OH form and the spread was increased by the need to pool fractions. The absolute difference between the position of TC II in Fig. 2a and b is not unusual in this type of chromatography. The relative position of the various substances is, however, consistent. When the B_{12} was in the form of Co^{57} liver B_{12}, Fig. 2c, TC II was still the dominant binder.

Discussion. The amount of natural B_{12} in plasma is generally accepted to be of the order of 400-500 pg/ml. The increases in plasma B_{12} due to that added *in vitro* in the present study ranged from 1-25 pg/ml or about 0.2-6.0% of the normal plasma B_{12}. TC II bound almost all of the added B_{12} and it seems safe to assume that the binding was not caused by an overload of B_{12}.

TC II was the dominant binder of the recently added B_{12} regardless of the form of the added B_{12} and after the natural route of intake of B_{12}. The binding studies of B_{12} after administration in liver were most important since the normal human intake of B_{12} was reproduced to the degree permitted by present knowledge. Although the B_{12} present in meat, the human dietary source of B_{12}, may originally be in the form of coenzyme B_{12}, it is unlikely to remain in this form en route to the table. The animal is killed and the meat is aged, transported, stored, butchered, and cooked before it is eaten. It seems therefore likely that the B_{12} of food is in the form of a biologically active degradation product of the coenzyme B_{12} in animal tissues. In the present study the chickens took in the B_{12} in small amounts daily, mixed with their food. The 2-week period between the last intake of B_{12} and the removal of the liver was necessary since it is known that this period of time is required for body equilibration of added B_{12}(5). The experiment differed somewhat from the natural human intake of B_{12} since the chicken liver was not stored or aged prior to use but it was cooked and given with other food. The binding to TC II after this type of intake into the body is strong evidence of a physiological function of TC II.

The fact that TC II-B_{12} cannot be detected by bioassay of plasma B_{12} is not at all surprising. The 0.5 μg of liver B_{12} used here raised the plasma B_{12} by 1.9 pg/ml. This amount of B_{12} in plasma cannot be measured by bioassay and 10 times this amount would be difficult to detect reliably. If TC II functions only transiently in the early phases of B_{12} transport, as now seems likely(3), it can be detected only when radioactive B_{12} is added to plasma *in vitro* or in blood samples taken shortly after the intake of radioactive B_{12}.

Summary. 1. Transcobalamin II (TC II), a plasma vitamin B_{12} binding protein, took up cyanocobalamin and hydroxocobalamin when added to plasma in amounts of 1.0 & 25 pg/ml of plasma. 2. It took up B_{12} when either form of B_{12} or liver B_{12} was given by mouth. 3. TC II appears to have a physiological function in plasma transport of B_{12} when small amounts of B_{12} are taken into the body in a natural fashion.

1. Pitney, W. R., Beard, M. D., Van Loon, E. J., J. Biol. Chem., 1954, v207, 143.

2. Hall, C. A., Finkler, A. E., Biochim. Biophys. Acta, 1963, v78, 233.

3. ———, J. Lab. Clin. Med., 1965, v65, 459.

4. ———, Blood, 1966, v27, 611.

5. Newman, G. E., O'Brien, J. R. P., Spray, G. H., Witts, L. J., Vitamin B_{12} und Intrinsic Faktor, Ferdinand Enke, Stuttgart, 1962, 424.

Received May 2, 1966. P.S.E.B.M., 1966, v123.

Metabolic and Endocrine Function in Whirler Mice. (31401)

A. S. WELTMAN AND A. M. SACKLER (Introduced by R. H. Cheney)
Laboratories for Therapeutic Research, Research Institute of Brooklyn College of Pharmacy, Long Island University, Brooklyn, N. Y.

The effects of strain and genetic differences on the endocrine organs have been reported in rats(1), mice(2), and guinea pigs(3). The whirlers(4) represent a recessive behavior mouse mutation located in the VIII linkage group and show syndromes of rapid, circling locomotor activity, head-shaking and deafness. In general, these and similar waltzing-type neurological mutants are extremely excitable, restless and nervous(5). Labyrinthine and