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

central nervous system anomalies have been frequently associated with this type of disorder(5). The present study sought to evaluate the effects of the whirler or waltzing-type disorders on metabolic rate and endocrine organ differences between the homozygous recessive mutation and its phenotypically normal heterozygote. In part, the investigation was stimulated by the suggestive physical "similarity" of the circling phenomena in the waltzing mice to the spinning behavior noted in childhood schizophrenics, and the desire to determine relationships between the excitable and nervous behavior patterns of the whirlers and their endocrine structure.

Materials and methods. The original breeding stock of homozygous whirler and normal heterozygous whirler mice was obtained from the Jackson Laboratory, Bar Harbor, Maine. All animals were subsequently bred and raised in air-conditioned quarters with the temperature maintained at 73°F. The mice were fed a diet of Purina Laboratory Chow. Genetically, the main distinction between the mutant whirler and heterozygous mice was that the former were homozygous for the recessive gene (wi wi) and displayed the typical waltzing-syndrome pattern. The latter mice, phenotypically normal in gross locomotor and hearing ability had the (wi +) genotype. In addition, the whirler and heterozygous mice possessed the non-agouti and brown coat color and hair pigment characteristics.

When the breeding units were able to supply sufficient numbers for experimentation, sister homozygous and heterozygous females were selected and weaned at 4 weeks of age for subsequent body weight, metabolic rate and endocrine organ weight studies. To avoid isolation(6) as well as crowding(7) effects, the females were limited to populations of 2 mice per cage. Body weights were measured weekly and oxygen consumption and metabolism rates were measured in the homozygous whirler (Group A) and heterozygous whirler (Group B) female mice at 12 weeks of age.

The oxygen consumption method utilized was similar in theory to the constant pressure, closed system, metabolism apparatus outlined by Shackell(8). To rule out bias effects and differences caused by possible excessive and spontaneous circling, locomotor activity of the homozygous whirler mice, O_2 consumption determinations were made under free activity and semi-restrained conditions. When restrained, the individual mice of both strains were placed in a perforated steel cylinder closed at both ends, having a diameter of 2 inches and a length of 4 inches. Under normal conditions, when permitted to move at random, whirler females have been found to average 407 clockwise and counterclockwise circles in a 10-minute period (unpublished data). Although the restraining cylinder provided adequate room for limited stretching movements, it effectively inhibited the spontaneous turning and rapid circling movements periodically displayed by the whirler mice, as well as the routine exploratory and locomotor activities of the normal animals. The procedure used during the O_2 consumption runs consisted of an initial 30-minute rest and acclimatization period within the metabolism chamber followed by a 30-minute O_2 consumption reading. A second 20-minute acclimatization period preceded the second O_2 consumption study which was then under restrained or unrestrained conditions, depending upon the nature of the first determination. To avoid and nullify any bias effects on metabolism rates due to the length of time within the metabolism chamber, half of the initial first runs were made under either free or semi-restrained conditions.

Representative numbers of homozygous and heterozygous whirler mice were sacrificed by etherization for endocrine and associated organ weight studies at 3½ months of age (liver, thymus, adrenals, ovaries, uterus, thyroids and pituitary).

Results. Analysis of the body weight means by standard t-test procedures(9) demonstrated consistent marked and/or significant decreases in body weights of the whirler females compared to the heterozygous mice.

Table I presents the free and semi-restrained oxygen consumption findings in terms of ml O_2/hr/100 g body weight. The data indicated that the metabolism rates of the whirler mice were consistently and significantly higher under both free activity and semi-restrained conditions. It is evident, how-

TABLE I. Metabolic Rates of Free and Restrained Homozygous and Normal Heterozygous Female Whirler Mice (Age, 12 Weeks).

	n	ml O_2/hr/100 g body wt	
		Free	Restrained
Group A:			
Homozygous whirler	11	419.86	338.06
± S.E.		±21.54	±25.07
Group B:			
Heterozygous whirler	24	313.17	274.19
± S.E.		±11.59	±14.45
% Diff. between Groups A & B		+34.1	+23.3
P value		<.001	.03

ever, that whereas the restraint exercised by the steel cylinder caused a 12.4% diminution in the metabolism rates of the normal heterozygotes, the inhibition induced in the whirler mice was more pronounced as indicated by the 19.5% decrease between the free *vs* restrained metabolism rates of the whirlers.

Table II presents the final body weights and relative organ weights of the mutant whirler and normal heterozygous mice sacrificed at 3½ months of age. The significant differences between the body weights of the 2 types necessitated that the statistical analyses and evaluations be based primarily on relative organ weights/100 g body weights. The data revealed significant increases in the relative adrenal weights accompanied conmice. On the other had, the 21.6% increase in the thymus weights. Although the marked decreases in ovarian weights were not statistically significant (P = 0.09), the relative uterine weights were significantly lighter in the homogygous whirlers. It is of interest that on an absolute weight basis both the ovarian and uterine weights were significantly smaller in the whirler mice. The findings further revealed that the relative liver weights were significantly heavier in the homozygous mice. On the other hand, the 21.6% increase in the relative pituitary weights and the 17.2% decrease in the relative thyroid weights were not statistically significant.

Discussion. It is evident that the various body weight, behavioral, metabolic and organ weight data indicate significant and pronounced endocrine abnormalities in the homozygous whirler mice as compared to their

TABLE II. Final Body Weights and Relative Organ Weights of Female Homozygous and Normal Heterozygous Whirler Mice (Age 3½ Mo).

	n	Final body wt, g	Relative organ wt in g or mg/100 g body wt						
			Liver, g	Thymus, mg	Adrenal, mg	Ovaries, mg	Uterus, mg	Thyroid, mg	Pituitary, mg
Group A: Homozygous whirler	11	18.9 ±.8*	5.7113 ±.1554	78.9 ±10.9	24.0 ±2.2	18.0 ± 1.5	84.7 ± 7.0	7.7 ±.8	6.2 ±.9
Group B: Heterozygous whirler	20	21.7 ±.5	5.2277 ±.1366	123.4 ± 9.4	17.5 ± .7	22.1 ± 1.5	125.8 ±12.6	9.3 ±.6	5.1 ±.5
% Diff. between Groups A & B		—12.9	+9.3	—36.1	+37.1	—18.6	—32.7	—17.2	+21.6
P value		<.01	.04	<.01	<.01	.09	.03	.14	.25

* ± S.E.

phenotypically normal sister heterozygotes. The evidence of significantly greater ozygen consumption rates, whether free or semi-restrained, agrees with the observation of rapid, excessive locomotor hyperactivity in the whirler mice. The association of significant increases in the relative adrenal weights with the corresponding significant decreases in the relative thymic weights presents definitive evidence of heightened adrenocortical function in the whirlers. The relative pituitary weight increase, although not statistically definitive by itself, presents somewhat accessory evidence suggestive of stimulated pituitary-adrenal activity in the homozygous mice.

Evaluation of the lighter ovarian and uterine weights in the 3½ month whirler mice would indicate reduced gonadotrophin and hypogonadal function in the recessive mutations. Preliminary fertility and fecundity studies of the whirler *vs* heterozygous mice have also indicated the occurrence of smaller numbers of surviving offspring at gestation and weaning from the whirler *vs* heterozygous mothers (unpublished data). It should be noted that the present alterations in body weight, adrenal, ovarian and uterine weights do resemble changes induced in animals in forced exercise cages(10).

Paradoxically, the significant increase in the O_2 consumption rates of the whirler mice was not accompanied by an increase in the relative thyroid weights. Further I^{131} uptake and release as well as histological studies are needed to determine the level of thyroidal function in the whirlers and to ascertain which endocrine organ (thyroid *vs* adrenal) was exerting the dominant effect on the increased metabolic activity of the whirlers. Both Crile(11) and Brody(12) have cited evidence reporting the influences of adrenal and thyroid hormones on metabolism rates.

The observation of significantly heavier relative liver weights in the whirler mice may represent a compensating mechanism for greater total metabolic function in the mutants. The high bursts of energy expended and needed to support the prolonged, spontaneous running and circling activities would require the ready availability of a large depot of stored glycogen. The capacity and ability of the mice to run for periods as long as 30 minutes or longer would also suggest significant differences in the metabolic processes and enzyme levels of the skeletal muscles of the whirler *vs* normal, heterozygous mice. Subsequent studies with female whirler mice of the same age revealed significantly greater food consumption (unpublished data).

In conclusion, while it is dangerous to extrapolate or relate the excitable emotional behavior and endocrine findings of the neurological mutants to psychotics, the present similarities in the erratic circling behavior, increased adrenocortical and lowered gonadal function parallel findings in certain schizophrenics. An increased output of adrenocorticosteroids has been reported in schizophrenic patients(13,14). It is of interest that Sackler *et al*(15) have associated the possibility of increased adrenocortical activity with or without hypogonadism and thyroidal malfunction to certain schizophrenias.

Summary. Body weight, metabolic rate and endocrine organ weight differences were determined between sister female whirler mice and their phenotypically normal heterozygotes. The whirlers, a recessive waltzing mouse mutation, show syndromes of rapid, circling, locomotor activity, headshaking and deafness. O_2 consumption rates were obtained at 12 weeks of age. Two weeks afterward, both types were autopsied for endocrine and associated organ weight studies. The findings indicated markedly or significantly lower body weights in the homozygous whirler mice and significantly higher O_2 consumption rates. The adrenal, thymic, ovarian and uterine findings presented evidence of increased adrenocortical activity and decreased gonadal function in the whirler mice. Similarities between the behavioral and endocrine characteristics of the excitable whirler mice to human psychoses are noted.

1. Freudenberger, C. B., Am. J. Anat., 1932, v50, 293.

2. Chai, C. K., Amin, A., Reineke, E. P., Am. J. Physiol., 1957, v188, 499.

3. Elliott, T. R., Tuckett, I., J. Physiol., 1906, v34, 332.

4. Lane, P. W., J. Hered., 1963, v54, 263.

5. Grüneberg, H., The Genetics of the Mouse,

Martinus Nijhoff, The Hague, 1952.

6. Weltman, A. S., Sackler, A. M., Sparber, S. B., Opert, S., Fed. Proc., 1962, v21, 184.

7. Christian, J. J., Proc. Nat. Acad. Sci. U. S., 1961, v47, 428.

8. Shackell, L. F., J. Pharmacol. Exp. Therap., 1937, v60, 117.

9. Snedecor, G. W., Statistical Methods, Iowa State College Press, Ames, 1949.

10. Conner, M. H., Shaffner, C. S., Endocrinology, 1954, v55, 45.

11. Crile, G., A Neuro-endocrine Formula for the Civilized Man, The Education Record Supplement, Am. Council on Education, Washington, 1941.

12. Brody, S., Bioenergetics and Growth, Reinhold Pub., New York, 1945.

13. Altschule, M. D., New Engl. J. Med., 1957, v257, 919.

14. Sackler, M. D., Sackler, R. R., Sackler, A. M., Van Ophuijsen, J. H. W., J. Clin. Psychopath., 1950, v11, 15.

15. Sackler, M. D., Sackler, R. R., Co Tui, Marti-Ibanez, F., LaBurt, H. A., Sackler, A. M., Quart. Rev. Psychiat. & Neurol., 1952, v7, 59.

Received May 3, 1966. P.S.E.B.M., 1966, v123.

The Need for Calcium in Adrenomedullary Secretion Evoked by Biogenic Amines, Polypeptides, and Muscarinic Agents. (31402)

A. M. POISNER* AND W. W. DOUGLAS (Introduced by A. Gilman)
Department of Pharmacology, Albert Einstein College of Medicine, New York City

The stimulant effect of acetylcholine and various nicotinic agents on adrenal medullary secretion is critically dependent on the presence of calcium in the extracellular environment, and it has been suggested that these secretogogues act by promoting an influx of calcium ions into the medullary chromaffin cells(1,2). This hypothesis has been supported by the demonstration of increased calcium^{45} uptake in the medulla exposed to acetylcholine(3), and by studies of the effect of various alkaline metals on medullary secretion(4,5,6).

Since acetylcholine and nicotine-like drugs have many pharmacological properties in common, it is not unexpected that their actions on the adrenal medulla should share a requirement for calcium. However many other substances capable of evoking adrenomedullary secretion have widely different chemical structures and pharmacological properties: these include the polypeptides, bradykinin and angiotensin(7,8,9), the biogenic amines, histamine(10,11,12) and 5-hydroxytryptamine(13), and the muscarinic agents, muscarine, pilocarpine and methacholine(14,15). In the present experiments we have found that each of the secretogogues requires calcium for its stimulant effect on adrenal chromaffin cells. A preliminary account of some of the results has been published(16).

Methods. The experiments were carried out on cats' adrenal glands, acutely denervated and perfused *in situ* through their arteries with Locke's solution or Ca-free Locke's solution by the method previously described (1). In preparing the Ca-free perfusion medium the normal content of $CaCl_2$ (2.2 mM) was omitted; no precautions were taken to remove the traces of calcium present in the other reagents and no chelating agent was added. In each experiment an adrenal gland was perfused first with Ca-free Locke's solution for about 20 minutes and the drug to be tested was introduced for the last 10 to 90 seconds of this period. Then perfusion was switched to Locke's solution and after a further 20 minutes the drug was retested. The perfusates escaping from the adrenal vein during perfusion with the drug-containing solutions, and in the corresponding control periods immediately before each test, were collected in chilled vessels, and were acidified and frozen until they were assayed fluorimetrically for catecholamines (adrenaline plus noradrenaline) by the tri-hydroxy indole method(17). Secretory responses were measured as increments in catecholamine out-

* Career Development Awardee USPHS.