cobalt from possible breakdown of the vit. B_{12} molecule is suggested by the different binding capacity of serum for $Co^{60}Cl_2$.

Pitney(2) found vit. B_{12} bound to an alpha globulin *in vivo.* However, when the vitamin was added to serum *in vitro,* some travelled electrophoretically with beta globulin. This fraction of vitamin, unlike that bound to alpha globulin, was directly available to the test organism, and was consequently termed "free." The discrepancy in binding capacities assayed by the different methods of measurements may, therefore, occur because the amount of vit. B_{12} made available to a micro-organism by heating serum does not represent the total vitamin bound to the serum proteins. It is possible that though the vitamin is bound to an alpha globulin at usual body levels, this mechanism is quickly saturated and nonspecific binding to other serum protein fractions takes place at higher concentrations.

Summary. A method is presented for estimating binding capacity of serum for radioactive vit. B_{12}. This capacity was found to increase as the concentration of the vitamin was raised. "Bound" vit. B_{12} measured in this way is not synonymous with that considered bound because it is unavailable to certain microorganisms.

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Effect of Reserpine (Serpasil®)* on Oxygen Consumption of Euthyroid, Hypothyroid, and Hyperthyroid Guinea Pigs. (22889)

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Experimental observations regarding the influence of reserpine on thyroid function are contradictory. Although reserpine completely antagonized increase in oxygen consumption produced by exogenous thyroxine in rats(1), it produced neither significant change in metabolic rate(2) nor consistent alteration in 24-hour I^{131} uptake of thyroid gland of euthyroid rats(3). However, reserpine exhibits a significant direct antithyroid effect *in vitro,* an effect which consisted chiefly of inhibition of organic binding of I^{131}(4). Patients placed on prolonged reserpine therapy showed no change in thyroid status(3) or metabolic rate(5), and Ford, *et al.*(6) were unable to demonstrate any change in basal metabolic rate or I^{131} uptake of the thyroid gland in euthyroid patients after chronic treatment with total alkaloid extract of *Rauwolfia serpentina.* Moncke(7), however, found that chronic reserpine therapy over a period of months produced "normalization" of basal metabolic rate in a group of 15 hyperthyroid patients and was more effective than methyl thiouracil in combating cardiac arrythmias associated with the disease. When reserpine was used in combination with methyl thiouracil, smaller doses of both drugs were needed. Most studies have been concerned chiefly with the effect of reserpine in a single thyroid state.

To resolve the apparent dissimilarities in response to this alkaloid, experiments were conducted to compare its effects on oxygen consumption of hypothyroid, euthyroid, and hyperthyroid guinea pigs.

Materials and methods. A modification of

* Serpasil used was generously supplied by Ciba Pharmaceutical Products Inc., Summit, N. J.

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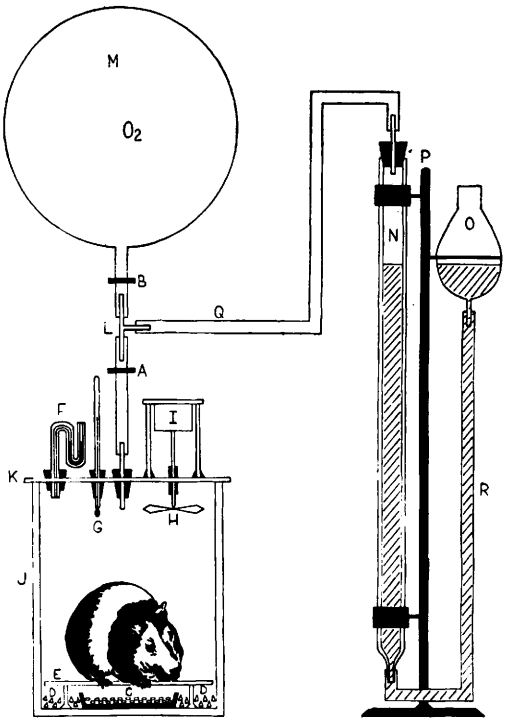

FIG. 1. Metabolimeter. A and B, clamps; C, anhydrous calcium chloride; D, soda lime; E, wire mesh platform; F, capillary tube manometer; G, thermometer; H, fan; I, motor; J, glass museum jar; K, brass plate cover; L, glass "T" joint; M, rubber bladder; N, calibrated burette; O, leveling bulb; P, ring stand; Q, rubber tubing; R, water.

Richards and Collison's(8) closed-chamber method was employed for determining oxygen consumption as a measurement of thyroid function (Fig. 1). The chamber, a circular glass museum jar, was 10 inches high and 8 inches diameter. A brass plate was used as cover. Water pump lubricant served to create an air-tight seal between cover and jar. A motor driven fan in upper part of chamber circulated air continuously. The fan shaft was sealed with lubricating oil. This permitted more rapid equilibration of gases and stabilization of temperature within the jar at 28 ± 0.5°C. Within the container the animal rested on wire mesh platform. Carbon dioxide and water vapor were absorbed by soda lime and anhydrous calcium chloride at bottom of jar. Reproducible readings were obtained if animals were allowed 25 minutes to come into thermal equilibrium. During equilibration and readings, the system was charged with 100% oxygen. Pressure changes within the jar were measured by a capillary tube manometer attached to cover. As oxygen was utilized, amounts sufficient to maintain atmospheric pressure within the system were delivered from a reservoir balloon. Oxygen consumed was measured at atmospheric pressure over water in a burette. When accustomed to the apparatus, the animals made only slight and infrequent movements during the experiments. Occasionally they became restless and an increase in consumption of oxygen followed. If movements lasted for some time, the increase persisted for a considerable time after movement ceased. For this reason, observations were made only during periods in which the animals manifested minimal activity. An average oxygen consumption for two 10 minute periods, which did not differ by more than ± 8%, was recorded. In the event of a greater difference, observations were extended until 2 consecutive periods were within limits. Since the observations of Cramer and M'Call (9) indicated that excess metabolism which followed eating of food by rats did not persist for more than 8 hours, our animals were starved for 12 hours prior to observations. The longer period was chosen to obviate variations due to the species of animal and the amount of food consumed. Thirty unselected guinea pigs, weighing 360 to 700 g, were divided into 3 equal groups, A (hypothyroid), B (hyperthyroid), and C (euthyroid), according to Fisher and Yates' tables of random numbers(10). Each animal occupied a separate cage in room in which the temperature was thermostatically maintained at 21°C. Ground Purina Rabbit Pellets, tap water, and supplemental cabbage were provided *ad libitum*. Drugs were thoroughly mixed in the ground food. Group A received 0.1% thiouracil for 5 weeks, Group B 0.1% desiccated thyroid§ for 3 weeks, while Group C served as controls. Thyroid and thiouracil medications were discontinued after signifi-

§ Desiccated thyroid USP Strong (0.3% I), was generously supplied by Parke, Davis and Co., Detroit, Mich.

TABLE I. Oxygen Consumption.

	Day	Liters oxygen/(kg body wt)$^{2/3}$ × 0.1/hr — Reserpine treated: Mean ± S.D.	Reserpine treated: Range	Solvent treated: Mean ± S.D.	Solvent treated: Range	% difference of means	Probability of diff. between means
Group A Hypothyroid	0	5.8 ± .58	5.5- 6.5	5.6 ± .56	4.5- 5.9	+ 3.6	>.2
	1	6.2 ± .49	5.7- 6.9	5.9 ± .73	5.0- 6.7	+ 5.1	"
	2	5.9 ± .36	5.5- 6.3	5.9 ± .70	5.0- 6.8	0	
	3	6.0 ± .52	5.8- 6.9	6.2 ± .58	5.3- 6.8	- 3.2	"
	5	5.6 ± .71	4.3- 6.1	5.9 ± .70	5.1- 6.7	- 5.1	"
	7	6.0 ± .58	5.5- 6.7	6.1 ± .75	5.5- 7.2	- 1.7	"
	9	6.2 ± .31	6.0- 6.8	6.7 ± .45	6.1- 6.9	- 7.5	"
	11	6.5 ± .48	5.8- 6.7	7.0 ± .33	6.5- 7.3	- 7.1	"
	12	6.9 ± .44	6.2- 7.3	7.1 ± .48	6.6- 7.6	- 2.8	"
Group B Hyperthyroid	0	9.5 ± .62	8.3-10.0	9.4 ± .58	8.9-10.3	+ 1.1	>.2
	1	8.1 ± .49	7.6- 8.9	8.9 ± .63	8.2- 9.8	- 9.0	<.1 but >.05
	1.17	7.2 ±1.29	4.9- 8.7	9.0 ± .70	7.9- 9.8	-20.0	<.05 but >.02
	2	7.2 ± .84	5.7- 8.2	8.8 ± .82	7.5-10.0	-18.2	*Idem*
	3	6.8 ± .47	6.0- 7.7	8.7 ± .64	8.0- 9.6	-21.8	<.01
	5	5.9 ± .81	5.4- 6.7	8.4 ± .71	7.3- 9.7	-29.8	"
	7	6.0 ±1.15	4.4- 7.3	7.7 ± .60	6.8- 8.5	-22.1	<.05 but >.02
	9	5.9 ± .36	5.4- 6.4	7.2 ± .20	7.0- 7.6	-18.1	<.01
	11	6.3 ± .36	5.8- 6.8	7.3 ± .46	6.9- 8.1	-13.7	"
	12	6.8 ± .22	6.6- 7.1	7.1 ± .32	6.7- 7.5	- 4.2	<.2 but >.1
Group C Euthyroid	0	7.2 ± .81	6.3- 8.0	7.2 ± .33	6.8- 7.8	0	
	1	6.7 ± .87	6.3- 7.8	7.3 ± .48	6.5- 7.8	- 8.2	<.1 but >.05
	2	5.8 ± .87	4.2- 6.6	7.3 ± .64	5.7- 8.1	-20.6	<.05 but >.02
	3	5.5 ± .68	5.1- 6.9	7.3 ± .20	7.1- 7.7	-24.6	<.01
	5	5.3 ± .99	4.6- 6.9	6.9 ± .45	6.3- 7.5	-23.2	<.05 but >.02
	7	5.9 ± .63	4.9- 6.9	7.3 ± .39	6.7- 7.8	-19.2	<.01
	9	6.4 ± .37	6.0- 7.0	7.4 ± .52	7.0- 8.3	-13.5	<.02 but >.01
	11	6.7 ± .47	5.9- 7.0	7.4 ± .66	7.8- 8.6	- 9.5	<.1 but >.05
	12	7.0 ± .19	6.7- 7.5	7.2 ± .50	6.7- 8.0	- 2.8	>.2

cant and stable degrees of hyperthyroidism and hypothyroidism were produced. At this time each group was divided into 2 subgroups composed of equal numbers of reserpine-treated and solvent-treated animals. Reserpine (Serpasil®) was injected intraperitoneally, 0.5 mg/kg on first day, followed by 0.35 mg/kg on second and third days, and 0.1 mg/kg on fourth day. Control animals received corresponding amounts of solvent vehicle in the same manner. Oxygen consumption was determined before starting reserpine therapy and at intervals of 4, 8, and 24 hours after each of the first 3 injections, and on days 5, 7, 9, 11, and 12. All values were corrected to standard temperature and pressure. Oxygen consumption was expressed as liters of oxygen/(kg body weight)$^{2/3}$ x 0.1/hour(11). The animals were autopsied at conclusion of experiments. Results were analyzed statistically for significance of the difference between group means by Student's "t" test(10).

Results. The data related to oxygen consumption have been summarized and their significance computed. Except in one instance, observations which were made at 4 and 8 hours, after each injection to detect acute effects of the drug, did not differ significantly from daily trends and were omitted for brevity (Table I). A graphic comparison of these same data (including 4 and 8 hour observations after each injection) is presented in Fig. 2.

Solvent-treated controls. Treatment with solvent had no effect on oxygen consumption of euthyroid guinea pigs, nor did it alter the gradual (12 day) return of oxygen consumption of hypo or hyperthyroid animals to euthyroid levels after cessation of thiouracil or thyroid treatment.

Hypothyroid animals. No significant differences in oxygen consumption between control and reserpine-treated hypothyroid animals were observed.

Euthyroid animals. Reserpine-treated euthyroid animals exhibited a significant decrease in oxygen consumption below corre-

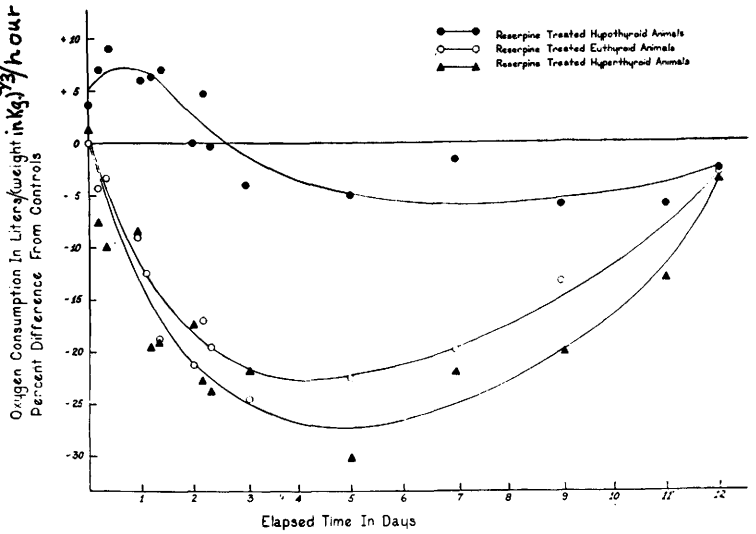

FIG. 2. Effect of reserpine treatment on oxygen consumption of hypothyroid, hyperthyroid and euthyroid guinea pigs.

$$\frac{(\text{Mean of reserpine-treated} - \text{mean of solvent-treated})\ 100}{\text{Mean of solvent-treated}} = \%\ \text{difference.}$$

sponding controls after 48 hours. The greatest effect occurred on the third day, at which time there was no difference between reserpine-treated euthyroid and the hypothyroid controls, which indicates that reserpine treatment had rendered the euthyroid subgroup hypothyroid. Oxygen consumption of reserpine-treated euthyroid subgroup was the same as that of controls by the twelfth day.

Hyperthyroid animals. A significant decrease in oxygen consumption of the reserpine-treated subgroup was observed after 28 hours, and the maximum effect occurred at 5 days. The reserpine-treated hyperthyroid animals were considered to be hypothyroid at this time, since there was no difference between these animals and the solvent-treated hypothyroid subgroup. Oxygen consumption gradually rose to euthyroid values at conclusion of the experiments.

There was a rapid decrease in weight of all 3 reserpine-treated subgroups (Table II) which was most marked in the hyperthyroid animals. The losses were maximum on the fourth day for all 3 subgroups and returned to within control values by the end of the experiment. No significant change in weights of thyroid, liver, spleen, kidney, adrenal, or lung was demonstrated, nor was there any gross evidence of infection.

Discussion. Oxygen consumption was reduced to hypothyroid levels in reserpine-treated hyperthyroid and euthyroid subgroups. No significant effect was obtained in the reserpine-treated hypothyroid subgroup. Since oxygen consumption was not reduced below hypothyroid levels in hyperthyroid and euthyroid animals, it appears that an antagonism for thyroid hormone might be the mechanism for reduction of oxygen consumption.

It seems likely that depression of oxygen consumption observed in reserpine-treated hyperthyroid animals probably was not due to an effect on the thyroid or pituitary since

TABLE II. Changes in Body Weights.

Group	% change in body wt at 4 days* Solvent treated	Reserpine treated	Probability of difference
Hypothyroid	+4.3 ± 2.0	−11.5 ± 6.6	<.01
Euthyroid	+1.1 ± 3.7	−18.4 ± 12.7	<.02 but >.01
Hyperthyroid	−3.1 ± 5.0	−20.9 ± 4.9	<.01

* Includes mean and S.D.

suppression of activity of these glands by exogenous thyroid hormone has been well established(12,13). The failure of reserpine to antagonize the increase of oxygen consumption produced by 2,4 dinitrophenol(1) and depress oxygen consumption of hypothyroid animals makes a generalized depression of metabolism unlikely. These observations strengthen arguments, advanced by Kuschke and Gruner(1), for reserpine as a thyroxine antagonist. Similar responses of reserpine-treated euthyroid and hyperthyroid subgroups favor a similar mechanism of action operating in the 2 groups; however, a central hypothalamo-hypophyseal or a direct thyroid effect(4) are not eliminated. Nevertheless, it is important to consider that, although oxygen consumption and thyroid hormone activity are frequently associated, they are not necessarily related in any given instance.

The failure of reserpine to produce changes in metabolic rate(2,5) and I^{131} uptake by the thyroid(3) needs further clarification.

Changes in body weights which occurred in the reserpine-treated animals were probably due to decrease in food and water intake during drug therapy.

Summary. Oxygen consumption was reduced to hypothyroid levels in reserpine-treated euthyroid and hyperthyroid guinea pigs, but no significant changes were noted in the reserpine-treated hypothyroid animals. The evidence presented and discussed suggests that reserpine antagonizes the effect of thyroid hormone on oxygen consumption.

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