ferences, no significant difference could be detected. Tree-shrews have an S.M.R. in the lower range of that of rats of comparable weight and maturity tested under identical conditions(3).

The oxygen consumption (ml/g/hr) of 3 species of *Sorex*, true shrews, has been investigated by Pearson(1). *Sorex vagrans vagrans* was reported as 7.4-8.6, *S. Trowbridgii montereyensis* as 7.2, and *S. pacificus sonomae* as 5.5-6.1. These values are approximately 8 times those here reported for tree-shrews. It may be noted that should these data be computed on a surface area basis, lesser magnitudes of differences might well result.

Summary. The oxygen consumption of 32 Philippine tree-shrews was 0.87 ± 0.02 cc/g body wt/hr (mean and standard error). Expressed as Standard Metabolic Rate, this represented 29.7 ± 0.8 Cal/sq m body surface/hr, a value corresponding to that of laboratory rats of comparable weight and maturity. No sex difference was observed.

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Isotopic Determination of Vitamin B_{12} Binding Capacity and Concentration.* (27282)

N. GROSSOWICZ, D. SULITZEANU AND D. MERZBACH
Dept. of Bacteriology, The Hebrew University-Hadassah Medical School, Jerusalem, and Dept. of Bacteriology, Rambam Government Hospital, Haifa, Israel

The bulk of Vit. B_{12} in serum is normally found in a bound form, the binding substance being a specific protein carrier(1). This carrier protein is normally in an unsaturated state as it is able to bind exogenously added Vit. B_{12} *in vivo*(2,3) or *in vitro*(4-6). If the amount of added vitamin is large, the binding sites become saturated and the excess remains unbound(7).

Various methods for measuring the additional binding capacity (ABC) of serum have been described, utilizing either microbiological or radiometric technics for estimating the protein-bound vitamin. Estimation of ABC by microbiological assays is rather cumbersome, while the radiometric methods, which require exhaustive dialysis to remove the unbound vitamin (*e.g.*, ref. 6), are time consuming.

This communication describes a simple and rapid method for determining the ABC of serum by using Co^{60} Vit. B_{12}; it has been briefly reported earlier(8). A modification of the ABC method for quantitative determination of Vit. B_{12} in biological material is also given in the present paper.

Experimental. In order to measure ABC, untreated serum (fresh or frozen) is incubated with $Co^{60}B_{12}$ and the excess of unbound radioactive vitamin is removed by adsorption on charcoal(9). Radioactivity of the charcoal is determined directly in a well-type scintillation counter and ABC is calculated by subtracting the radioactivity found in the charcoal from total radioactivity added (8).

To determine Vit. B_{12} content of biological samples, the vitamin has first to be released from the carrier protein. The treated samples (containing free unlabeled vitamin) are incubated with serum of known ABC ("standard serum") in the presence of a fixed amount of $Co^{60}B_{12}$ and the unbound vitamin determined by measuring the radioactivity of the charcoal. The incubated reaction mixture containing both the "standard serum"

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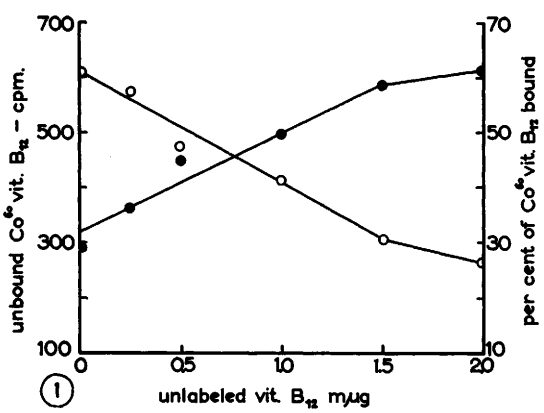

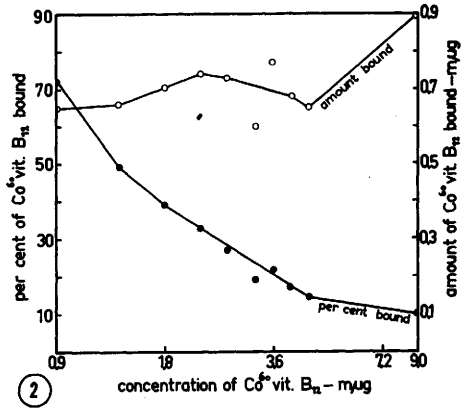

FIG. 1. Effect of dilution with unlabeled vit. B_{12} on binding of radiovitamin to serum proteins. Unlabeled vit. B_{12} in increasing amounts, is incubated with 1 μg of $Co^{60}B_{12}$ and 1 ml of "standard serum" and charcoal radioactivity (unbound vitamin) measured (solid circles). The calculated percentage of bound vitamin is given (curve with open circles).

FIG. 2. Effect of $Co^{60}B_{12}$ concentration on additional binding capacity (ABC). To determine the range of vit. B_{12} concentration at which ABC remains constant, increasing amounts of $Co^{60}B_{12}$ are incubated with 1 ml of the serum selected as "standard." The charcoal radioactivity (unbound vitamin) is determined. Values of bound vitamin (T – N) are expressed as % of total radioactivity (T). See expression(1). Upper line (open circles) shows range of vit. B_{12} concentration at which fluctuation in ABC is minimal and is, therefore, regarded as constant.

and the unknown sample will bind less Co^{60}-B_{12} than the control containing "standard serum" alone, because of the dilution of radioactivity by the unlabeled vitamin in the sample (Fig. 1). Thus, the Vit. B_{12} content of the unknown sample is determined by the increase of the radioactivity in the charcoal over that found in the control containing "standard serum" only. The percentage of binding (B%) is calculated from the following:

$$B\ (\%) = \frac{(T-N)\ 100}{T} \quad \ldots \quad (1)$$

where T = total radioactivity (cpm) and N = radioactivity in the charcoal (cpm).

The amount of unlabeled vitamin in the unknown specimens should be such as to allow 30-60% of total radioactivity to be adsorbed on the charcoal, as these values are on the straight line portion of the curve (Fig. 1).

Methods. a) *Determination of ABC*: 0.7 μg of $Co^{60}B_{12}$ (specific activity 1.08 μc/μg; Merck & Co. Inc.) in saline buffered with phosphate (M/300, pH 7.0) was added together with KCN (1.0 μg $\sim$ 0.015 μmole) to 0.25 ml of serum and the volume made up to 1 ml with buffered saline. The reaction mixture was incubated for 30 to 60 minutes at 37°C, after which one ml of a 10% suspension of charcoal W/V (Norit "A", Amend Drug and Chemical Co., New York) was added. The reaction mixture was shaken in a mechanical shaker for 15 minutes at 37°C, 5 ml of physiological saline was then added (to wash out any B_{12} not adsorbed on the charcoal) and centrifuged at 2,000-3,000 rpm for 5 minutes. The supernate was discarded and radioactivity of the charcoal measured. ABC is expressed in $\mu\mu$g Vit. B_{12}/ml of serum according to the following formula:

$$ABC = (T-N)\ F \cdot 4 \quad \ldots \quad (2)$$

where T = total radioactivity (cpm), N = radioactivity in the charcoal (cpm), and F = the conversion factor of cpm obtained in $\mu\mu$g B_{12} (the count is dependent on the sensitivity of the scintillator used). The values are multiplied by 4 to express them per ml rather than per 0.25 ml of serum.

b) *Quantitative determination of Vit. B_{12} with ABC system. Preparation of sample. Serum.* To 2 ml of serum are added 0.4 ml of KCN (0.1%, 8 ml of acetate buffer (M/5, pH 4.5) and water to make up to 16 ml. The diluted sample (dilution of serum 1:8) is au-

toclaved for 30 minutes at 15 lb/sq inch(10). The supernate (pH 4.5) is concentrated to dryness in an evaporating dish by heating at *ca.* 90°C and is redissolved in 1-2 ml of water. *Urine.* An aliquot (20 ml) is concentrated up to 10-fold by heating at 90°C (in an evaporating dish) after acidifying the sample with glacial acetic acid (to bring the pH to 4.5) and adding 0.5 ml of acetate buffer (M/5, pH 4.5) and 0.2 ml of KCN (1%). The concentrated sample (urine) is taken up in 2 ml of water, and the pH adjusted to 7.0 with sodium hydroxide (5N). If high B_{12} urine values are anticipated (*e.g.*, liver disease, etc.) concentration of the urine is unnecessary.

Other biological samples may be treated similarly. The samples are prepared to contain between 200-2,000 $\mu\mu$g Vit. B_{12}, which is the suitable range for the assay.

Assay. Two-fold dilutions of the concentrated sample are mixed with a fixed amount of $Co^{60}B_{12}$ (1 μg = 1.08 μc) and 1 ml of "standard serum", and incubated (as described for ABC determination) after adjusting the final volume to 2.0-2.5 ml. The percentage of $Co^{60}B_{12}$ is calculated from expression(1), converted into $\mu\mu$g (from Fig. 1), and multiplied by the dilution factor; values of the different dilutions are averaged.

Results. Table I summarizes ABC values obtained in healthy subjects and in various physiological and pathological conditions. With the amount of radiovitamin employed (approx. 3,000 $\mu\mu$g B_{12}/ml) the ABC in normals was found to be 808 ± 300 $\mu\mu$g B_{12}/ml which is in good agreement with results obtained by other methods(5,6,11). ABC of normal average values was found in various diseases not related to the hematopoietic system. In pregnancy ABC is significantly increased (1558 ± 381) as compared to normals. However, ABC of fetal blood is normal. The difference in ABC between mother and newborn is preserved at delivery as shown in 9 paired samples of maternal and fetal blood(8). The ABC in various liver diseases is slightly elevated.

TABLE I. Co^{60} Vit. B_{12} Additional Binding Capacity (ABC) of Serum.

Subjects	No. of cases	ABC in $\mu\mu$g/ml Range	Avg ± S.D.
Healthy subjects	70	58-1800	808 ± 300
Pregnant women	49	630-2180	1558 ± 381
Newborns (fetal blood)	77	220-2060	865 ± 437
Maternal & fetal blood (paired samples)			
(a) Mother	9	480-1975	1377
(b) Newborn	9	170- 716	499
Liver diseases*	34	91-1890	966 ± 436
Lymphatic leukemia	6	650- 800	724 ± 47
Myeloid leukemia and metaplasia	14	650-2100	1605 ± 474
Polycythemia vera	2	1700-2035	1870
Diseases not related to hematopoietic system	55	162-2100	835 ± 406

* Tumors, cirrhosis, infectious hepatitis.

Lymphatic leukemia cases show normal ABC values (724 ± 47), while those in myelogenous leukemia and myeloid metaplasia are definitely elevated (1605 ± 474), as are the values in polycythemia vera. Again the ABC values in this group of diseases are in agreement with those obtained with other methods(1,2,11-13). Differential diagnosis based on increased values of ABC alone should be made with caution due to the elevation found in normal pregnancy.

Table II shows Vit. B_{12} concentrations in urine and serum obtained with the ABC system. Repeated estimation of the same urine specimens gave minimal variations. Comparative microbiological estimations carried out on some sample showed good agreement with the radiometric ones.

Discussion. Several investigators have found the binding capacity of serum to increase with the rise of vitamin (or radiovitamin) B_{12} concentration(5,6). However, over a defined range of B_{12} concentrations (between 900-3,500 $\mu\mu$g per 1 ml serum) the ABC of serum remained fairly constant (mean variation ± 3.6%, Fig. 2). Reproducible and comparable results can, therefore, be obtained in this range of $Co^{60}B_{12}$. In the ABC determinations 2,800 $\mu\mu$g of $Co^{60}B_{12}$ are used per ml of serum. The method described here enables rapid determination of ABC, as it employs charcoal adsorption instead of exhaustive dialysis(6) to remove the unbound radiovitamin. It was shown(9) that while unbound B_{12} is quantitatively ad-

TABLE II. Radiometric Assay of Vit. B_{12} with the ABC System.

Samples	No. of cases	Vit. B_{12}, $\mu\mu$g/ml Radiometric method	Vit. B_{12}, $\mu\mu$g/ml Microbiological method
Urine			
Healthy subjects	2	200	
Vit. B_{12} added to urine specimen	1	4000	5000
Vit. B_{12} (.5 mg) given orally	5	100–1000*	
Vit. B_{12} (.5 mg) given intramuscularly	5	1000–1,500,000*	
Infectious hepatitis	11	250–16,000*	100–12,000
Serum			
Healthy subjects	2	500–600	
Infectious hepatitis	3	2500–4000	2000–4800
Liver cirrhosis	1	1500	1600
Tumor of liver	1	2000	3200
Myeloid leukemia	1	2500	3500
Macrocytic anemia (after treatment with B_{12})	1	1000	1200

* Samples determined repeatedly. Repeated estimations of the same urine specimens gave minimal variations. However, different samples varied in Vit. B_{12} activity.

sorbed on activated charcoal, protein bound B_{12} resists adsorption.

Cyanocobalamin and aquocobalamin (hydroxocobalamin) have different affinities for the carrier protein(14). Addition of cyanide to the ABC system assures complete conversion of the vitamin to the cyanocobalamin form and thus contributes to a reproducible estimation of the binding capacity. If the ABC of hydroxocobalamin is to be measured, proper treatment of the radiovitamin is necessary prior to the assay.

Determination of native Vit. B_{12} in urine and serum by means of ABC is as simple and rapid as estimation of ABC itself. Biological samples usually have to be concentrated prior to testing as values of 200-500 $\mu\mu$g are close to the sensitivity limit of the method. The concentration is carried out at pH 4.5 in the presence of KCN(10), to eliminate possible destruction of the vitamin during heating. The proposed test is more accurate in myeloid leukemia, liver disease and other conditions with elevated B_{12} levels, than *e.g.*, in pernicious anemia. However, addition of small amounts (50-200 $\mu\mu$g) of unlabeled vitamin as a carrier should allow accurate determination of B_{12} in pernicious anemia and in Vit. B_{12} deficiency.

Instead of the method described above which utilizes isotopic dilution for determination of Vit. B_{12} with the ABC system, a "saturation procedure" can be used: the binding sites of the carrier protein are saturated by preincubation with the unlabeled vitamin of the unknown specimen and the system is reincubated with a constant amount of the radiovitamin. The radioactivity of the charcoal is counted as described. Such a modification is plausible, since under the conditions of the assay the vitamin is irreversibly bound to the carrier protein and no exchange between the unlabeled and labeled form occurs(15).

A direct isotopic method for determination of Vit. B_{12} in blood has recently been described(16). The method, which appears to be highly accurate, requires exhaustive dialysis and, therefore, is tedious and time consuming.

Summary. A method for measuring additional binding capacity (ABC) of Vit. B_{12} in serum with the aid of radio-B_{12} is described. Fixed amounts of untreated serum (fresh or frozen) and of $Co^{60}B_{12}$ are incubated for 30-60 minutes at 37°C and the excess of unbound vitamin adsorbed on charcoal; the radioactivity of the charcoal is counted directly in a well-type scintillator. ABC values in various physiological and pathological conditions are given. The ABC assay was also applied to measure Vit. B_{12} concentrations in biological material, by first releasing the vitamin from the carrier protein and incubating it together with a "standard serum" (of known ABC) with $Co^{60}B_{12}$. Vitamin content is determined by the increase in radioactivity in the charcoal over

that found in the control containing "standard serum" only. Both methods are simple, rapid, and particularly suitable for serial determinations. The results obtained with these assays showed good agreement with microbiological and other radiometric methods. Various aspects of the assays are discussed.

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Serum Glutamic-Oxalacetic Transaminase Levels after Exercise in Men.* (27283)

JERRY B. CRITZ† AND ARTHUR W. MERRICK (Introduced by W. O. Read)
Department of Physiology and Pharmacology, University of Missouri Medical Center, Columbia

The report of LaDue, Wróblewski and Karmen(1) stimulated much of the interest in glutamic-oxalacetic transaminase (GOT), and led to the use of the transaminase analysis as a diagnostic test for confirming the existence of a myocardial infarct. Wróblewski and LaDue extended this test to include detection of infectious hepatitis(2,3).

This research led other workers to more widespread and diversified investigation of GOT. Vagotomy in the pigeon resulted in increased GOT activity of heart, muscle and hepatic tissue(4). The metabolic stimulant, 2,4-dinitrophenol, increased GOT activity in muscle and hepatic tissue(5). Barbieri discovered that GOT was most concentrated in those chambers of the heart doing the most work(6). Katsura and Yamamoto reported endocrine tissue had a relatively low transaminase activity(7). These later investigations suggested a relationship might exist between GOT and muscular or metabolic activity. Perhaps increased tissue activity could result in an increased GOT content within that tissue, which could be mobilized from the serum or synthesized in the active tissue itself. This hypothesis was tested in humans by measuring SGOT titers before and after 3 three-minute rounds of boxing(8). A significant increase in SGOT level was observed immediately thereafter. Rats compelled to walk for 16 hours responded with an increased level of SGOT(9). These investigations did not exclude the possibility of tissue damage from the blows struck in boxing and in the exercised animals there was histologic evidence of damage to skeletal muscle. Such damage has been shown to result in elevated serum levels of GOT(10-14). Experiments

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† Present address: Dept. of Physiology and Pharmacology, School of Med., State Univ. of South Dakota, Vermillion.