

gammaglobulinemia in the adrenalectomized state.

The critical question as to the mechanism of the increased sensitivity of these animals to antigenic stimulation remains unanswered. Whether their increased antibody production is a primary effect of absence of adrenal hormones or is a secondary phenomenon reflecting decreased plasma volume, hemoconcentration, or other direct effects of adrenal depletion is not known. Further studies employing more exacting quantitating technics would seem to be required.

Summary. Increased circulating levels of antibody following a single intravenous injection of bovine serum albumin were demonstrated in adrenalectomized rabbits, as compared with normal intact controls. Additionally, there was a delay in appearance of maximum titers of antibody in the test group of animals. In animals previously immunized

with bovine serum albumin, adrenalectomy produced increased gamma globulin concentrations but failed to recall specific anti-BSA antibody into circulation.

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Metabolic Rate of Tree-Shrews (*Urogale everetti*).^{*} (27281)

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The tree-shrews (Tupaiaidae) comprise a family concerning which there has been some taxonomic disagreement, some authorities considering them as belonging to the insectivores, while others believe they are the closest living representative of the primitive, generalized primates. The evidence supporting the latter view makes these animals of special interest, for the availability of a small (rat-sized) primate for laboratory investigations in endocrinology, nutrition, metabolism, and radiobiology would be desirable.

During general observations on a small

colony of Philippine tree-shrews data were obtained on their metabolic rates. It was of interest to compare these rates with the high rates reported for insectivore shrews(1).

Sex and age. Of the 32 animals tested, 20 were females and 12 males. No precise ages are known. They had been captured as immature animals at least 9 months prior to the metabolic rate determinations. Roentgenograms made 1 month before these determinations showed complete epiphyseal union in all long bones in 28 of the 32 animals, although in many the persistence of the "scar" of epiphyseal union suggested its recent completion. In the remaining 4 animals (2 male, 2 female) the majority of epiphyses showed completed union, but the persistence of notches at the tibial tuberosity and of partial epiphyseal plates (chiefly at the distal ends of radius and ulna) suggested that these animals were slightly younger, perhaps in late adolescence.

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TABLE I. Oxygen Consumption of Tree-Shrews.

Group	No.	S.M.R. (Cal/m ² /hr)		cc/g/hr	
		Mean	Range	Mean	Range
Adult males	10	28.1 ± 1.4	23.4-38.0	.80 ± .04	.66-1.08
" females	18	30.1 ± .9	24.7-39.4	.90 ± .03	.73-1.11
" combined	28	29.4 ± .8	23.4-39.4	.86 ± .02	.66-1.11
Adolescents	4	31.8 ± .8	30.1-33.9	.94 ± .02	.91-1.00
Entire group	32	29.7 ± .8	24.7-39.4	.87 ± .02	.66-1.11

Nutrition.[†] The animals were maintained on a fruit-meat-cereal diet. On alternate days they received fruit (bananas or fresh pineapple) or oatmeal porridge in which the fluid was composed of equal portions of water and milk (reconstituted from milk powder). A multivitamin supplement (Stuart Formula) was mixed with the porridge. The meat ration alternated between ground beef (raw or cooked) and live new-born rats (the excess young resulting from litter-limitation in a breeding colony). Raw eggs were fed occasionally. All foods were well-accepted, the oatmeal being the last-consumed. Body weight appeared stabilized over a 5-month period. Males ranged from 227 to 342 g, with a mean of 276 ± 8.0 g. Females ranged from 190 to 314 g, with a mean of 252 ± 6.3 g. The difference was not statistically significant.

Caging and behavior. The animals were kept in pairs in $2\frac{1}{4}$ cu. ft. guinea pig cages. Within each cage a small "nesting-box" with sliding door and top facilitated handling of the animals. Such small retreats have been reported to be preferred by tree-shrews, perhaps because they simulate the body-contact conditions in natural tree-hollow dwellings. Animals showed many signs of contentment under these conditions, although continuing to be both shy when approached (except at feeding-time) and aggressive when handled. Activity was doubtless markedly reduced in the cages provided, as compared with the range of movement allowed in natural ar-

boreal habitats. It is noteworthy that they adapted readily to the small restraining containers used in the metabolimeter, and remained quiescent throughout tests, perhaps again reflecting their preference for body-contact enclosures.

Metabolimetry. Determinations of metabolic rate were made in a closed circuit respiration apparatus like that of Kleiber (3), modified slightly as described by Evans *et al.* (4). The metabolimeter allowed 8 animals to be tested at a time, following the Evans procedure for rats in detail. All animals were in a post-absorptive state after an over-night fast. They were first placed for one hour in the apparatus to adjust to its temperature (held to 30°C within 0.1°C); the volume of oxygen consumed over the following $2\frac{1}{2}$ -hour period was determined for each animal and corrected for temperature and barometric pressure. The oxygen consumption rate could thus be computed either as ml/g/hr or converted to S.M.R. (Standard Metabolic Rate, the term preferred to distinguish from the basal metabolic rate as defined for human beings). S.M.R. is computed in terms of kcal/sq m body surface/hr by (a) converting liters of oxygen per hour into kcal per hour by the factor 4.80, and (b) converting body weight into square meters of body surface. The body size and proportions of the tree-shrews corresponded so closely to those of adult rats that Benedict's formula (5) for the rat was used as a suitable approximation:

$$\text{Area (m}^2\text{)} = \frac{9.0 \times \sqrt[3]{(\text{body wt in g})^2}}{10,000}$$

[†] Except for much-reduced cage space, the nurture of these animals followed recommendations given in personal communications from Lois W. Mednick, and from Dr. J. R. Hendrickson, Dept. of Zoology, Univ. of Malaya. The latter has reported on behavior and nurture of the tree-shrew, *Tupaia glis*. (2)

Table I gives oxygen consumption (means with standard errors, and ranges) in both S.M.R. and cc/g/hr, to facilitate comparison with reports on other animals. Although the data were examined for sex and maturity dif-

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 B₁₂ Binding Capacity and Concentration.* (27282)

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The bulk of Vit. B₁₂ 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. B₁₂ *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 Co⁶⁰ Vit. B₁₂; it has been

briefly reported earlier(8). A modification of the ABC method for quantitative determination of Vit. B₁₂ in biological material is also given in the present paper.

Experimental. In order to measure ABC, untreated serum (fresh or frozen) is incubated with Co⁶⁰B₁₂ 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. B₁₂ 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 Co⁶⁰B₁₂ 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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