tween individuals of the same species, is hindered by the number of variables which may influence rate of lymph flow and lymphocyte content of lymph collected from any region of the body. The right duct lymph flow in 21 dogs(6) of an average weight of 21 kg was found to be 0.1 ml/hr/kg and in 10 cats (8) of an average weight of 3.4 kg it was 0.15 ml/hr/kg. The white cell content of the right duct lymph of the cat has been reported to be 2,310 cells/mm^3, whereas the thoracic duct lymph in the same animal contained 4,120 cells/mm^3. Right duct lymph flows of 0.46, 0.35, and 0.24 ml/hr in each of 3 rabbits with cell counts of 39,500, 39,800, and 43,600 cells/mm^3 have been reported(3).

Summary. 1. Lymph and lymphocyte outputs from thoracic and right lymphatic ducts of male rats have been measured. 2. Rate of flow and lymphocyte output of the thoracic duct was 1.8 ml/hr/kg and 42.9 million lymphocytes/hr/kg; right lymphatic duct values were 0.3 ml/hr/kg and 5.7 million lymphocytes/hr/kg. 3. Utilizing these data a Daily Replacement Factor for blood lymphocytes of 4.6 was obtained for the anesthetized rat. 4. These results have been compared with similar findings reported for other species.

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Co^{60}Vitamin B_{12} Binding Capacity of Normal Human Saliva.*† (24402)

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Microbiological or radioisotope-dialysis estimates of vit B_{12} binding capacity of normal human saliva have given comparable results (1,2,3). Preliminary to further investigation, this capacity was reevaluated using Co^{60}vit B_{12} and exhaustive dialysis and its relationship to age, sex and multiple factors in saliva was studied.

Methods. Co^{60}vit B_{12} having a specific activity of 0.823 mc/mg was used. Saliva stimulated by chewing gum was collected from 103 healthy adolescents and adults 1-4 hours after breakfast. Specimens were centrifuged to remove cells and debris. To 1 ml aliquots of clear supernatant in Visking bags varying amounts of Co^{60}vit B_{12} ranging from 1 to 100 mμg were added. After 2 hours incubation at room temperature, each bag was subjected to exhaustive dialysis in cold running tap water for 48 hours. Each sample was placed in a 4 ml vial then filled with concentrated sulfuric acid dissolving the bag, and allowing complete mixing within the vial. Standards were prepared in similar vials and included entire range of Co^{60}vit B_{12} added to the bags. Counting was done in a well-type scintillation counter. "Bound" vit B_{12} was measured in terms of residual radioactivity. Refractive index was determined with Bausch and Lomb Dipping Refractometer. Specific gravity was measured with an hydrometer. Optical density at 280 mμ was obtained with Beckman Recording Spectrophotometer D.K. Leukocyte counts were made on undiluted saliva with Neubauer hemocytometer. Studies for ABO blood group types and for secretion in saliva of ABO substances were per-

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† Co^{60}vit B_{12} was provided by Merck and Co., Rahway, N. J.

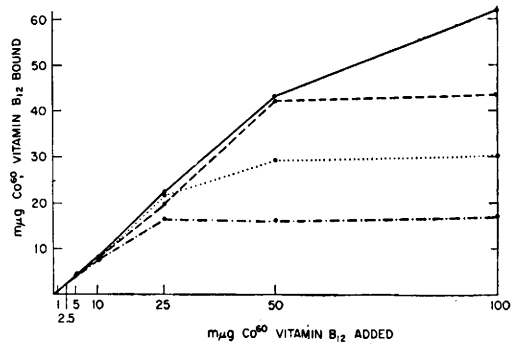

FIG. 1. Co^{60} vit. B_{12} binding capacity of saliva of 4 normal subjects.

formed in the usual fashion, using appropriate sera.

Results. Fig. 1 shows 4 representative assays. In all instances the curve has 2 parts: a rapidly ascending essentially straight line followed by a plateau. Binding capacity is represented by the point of flexion, or, because of the plateau form, the amount bound when 100 mμg Co^{60}vit B_{12} were added. Specimens binding over 50 mμg vit B_{12} per ml had binding capacities estimated perforce at the 100 mμg level. Additions of larger amounts of Co^{60}vit B_{12} were not studied.

Salivary binding capacity varied from 13 to 80 mμg/ml with a mean value of 31 ± 10.2 mμg/ml, and a median value of 28 mμg/ml (Fig. 2). The median 50% of values fell between 22 and 36 mμg/ml.

Co^{60}vit B_{12} binding capacity of saliva tended to be constant for each subject when tested at hourly intervals for 4 hours (Fig. 3), and also at varying intervals of days or weeks (Table I).

An attempt was made to relate Co^{60}vit B_{12} binding capacity of 50 samples of saliva to

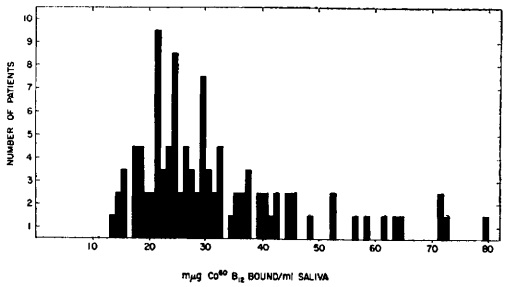

FIG. 2. Range and distribution of Co^{60} vit. B_{12} binding capacity of normal human saliva in 103 subjects.

salivary specific gravity, leukocyte count, refractive index, optical density at 280 mμ, blood groups and secretor factors. No correlation was found. There is a suggestion that subjects less than 30 years of age tend to have higher vit B_{12} binding capacity than those in the older age group (Fig. 4).

Discussion. Beerstecher(1), using *E. coli* as a test organism and expressing results in terms of units of apoerythein, found a mean binding capacity of 55.5 mμg/ml saliva for vit B_{12}. Grasbeck(2) reported values of 42 and 45 mμg/ml in 2 subjects using *L. leichmanni* and radioactive vit B_{12} in microbial absorption inhibition method. Schilling(3), using a technic similar to ours, found a value similar to those noted above in 1 specimen. Our results overlap with, but show an average binding capacity somewhat lower than, those

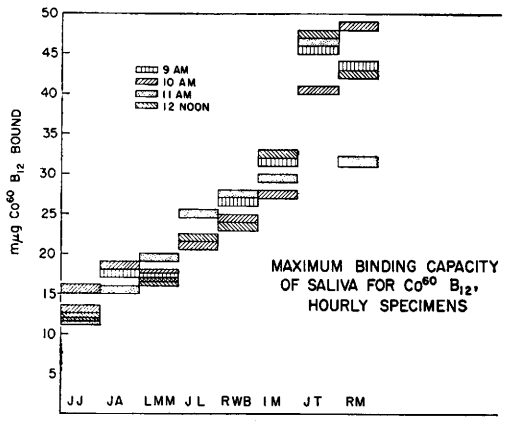

FIG. 3. Binding capacity of saliva for Co^{60} vit. B_{12} at hourly intervals in 8 subjects.

previously reported. Beerstecher collected saliva without stimulation, while the other 2 investigators make no note on this point.

Previous studies from this laboratory(4) suggest more than one vit B_{12} binding substance in the serum of normal individuals. Present studies indicate (Fig. 1) that saliva contains a single binding factor. This fits with Grasbeck's(2) finding by electrophoresis of a single vit B_{12} binding substance in saliva.

Although salivary binding capacity for vit B_{12} varied within the group, relative constancy of the binding factor in each subject's saliva from hour to hour and day to day is worthy of note.

TABLE I. $Co^{60}B_{12}$ Binding Capacity of Saliva of Individual Subjects at Intervals of One or More Days.

	mμg $Co^{60}B_{12}$/ml saliva					
Day	LM	RB	KW	IM	JL	RR
1	17	32	16	19	63	43
2	15	43	15	25	58	42

Gyorgy *et al.*(5), reporting on Bifidus Factor, found a frequent correlation between this substance, blood group secretor factor and Castle's intrinsic factor. If salivary binding of vit B_{12} were a result of intrinsic factor activity, a correlation between this binding capacity and secretor factors might have been anticipated. However, the present study showed no such relationship. Grasbeck and others(5,6), moreover, have demonstrated the absence of salivary intrinsic factor activity.

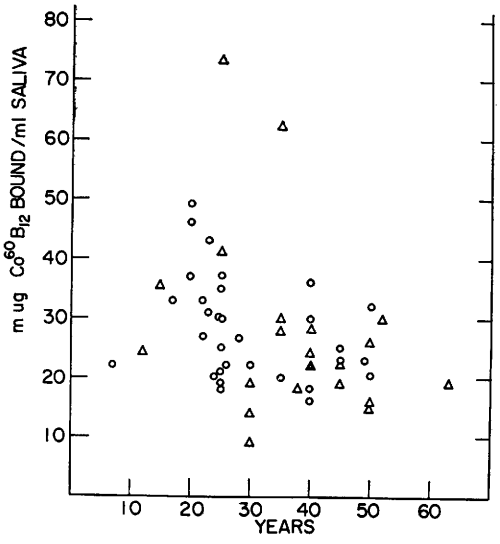

FIG. 4. Correlation of Co^{60} vit. B_{12} binding capacity of saliva with sex and age. ○ = ♀. △ = ♂.

Summary and conclusions. 1) Vit B_{12} binding capacity of normal human saliva has been estimated using Co^{60}vit B_{12} and exhaustive dialysis. It varies from 13 to 80 mμg/ml, with a mean of 31 ± 10.2 mμg/ml. 2) These values appear constant for the individual over a period of hours to days. 3) No correlation was found between vit B_{12} binding capacity and salivary specific gravity, refractive index, optical density at 280 mμ, leukocyte count, ABO blood groups or secretion of ABO substances in saliva.

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Arterial Oxygen Pressure in Dogs Breathing Oxygen at 2,5 Atmospheres Pressure.* (24403)

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In normal men breathing oxygen tensions of 200-760 mm Hg the oxygen pressure in arterial blood is some 30-40 mm Hg lower than in alveolar gas(1). This "A-a" O_2 difference results primarily from admixture of small quantities of systemic venous blood with fully oxygenated pulmonary capillary blood (1,2). There are no theoretical reasons for supposing that A-a difference should increase with further increase in inspired oxygen tensions. However, indirect measurements reported by Lambertsen, *et al.*(3) indicate that this may be the case. In normal men breath-

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