tion of oleic acid did not stimulate esterification of cholesterol. The significance of these findings is discussed.

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Vitamin B_{12} Unsaturated Binding Capacity of Sera from Various Animals.* (27144)

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The native vitamin B_{12} content of serum varies widely among animal species(1-4) and may range from 0.04 mμg/ml in alligator serum to 31 mμg/ml in rabbit serum. Other reports(5-7) have shown that the native vitamin is non-dialyzable and is bound to serum proteins. However, serum is apparently unsaturated and will bind additional cyanocobalamin added *in vitro.* This phenomenon has been termed the serum "unsaturated B_{12} binding capacity" or UBBC by Miller and Sullivan(8).

Gregory and Holdsworth(2) have recently reported that the UBBC of chicken serum is 1100 mμg/ml as compared with that of human serum (2 mμg/ml). Furthermore, it has been shown that the UBBC of serum from patients with chronic myelogenous leukemia is also elevated(8-10). These results suggest a relationship between high concentrations of nucleated blood cells and the UBBC of serum, and it was of interest to determine the UBBC of serum from a wide variety of animal species in order to test this possibility.

Materials and methods. Sera from many animal species were maintained frozen until analyzed by an exhaustive dialysis procedure previously described by Rosenthal *et al.*(10). In brief, 0.3 ml of sample was placed in visking tubing containing 7 ml of Co^{60} cyanocobalamin ($Co^{60}B_{12}$) or Co^{60} dimethylbenzimidazolyl cobamide coenzyme (DMBC) in M/10 phosphate buffer at pH 7.4. Samples with very high UBBC were diluted in saline. In all cases, a 2-3-fold excess of radioactive material was available for binding to serum pro-

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TABLE I. Unsaturated Cyanocobalamin Binding Capacity of Serum from Various Animals.

Species	$Co^{60}B_{12}$ bound, $m\mu g/ml \pm \%$ S.E.*
Mammalia	
Man	1.57 ± 31.2 (8)
Rat	3.16 ± 7.2 (4)
Guinea pig	2.72 ± 9.9 (4)
Horse	4.60 ± 3.7 (2)
Cow	3.83 ± 57.1 (3)
Sheep	6.39 ± 2.0 (2)
Pig	9.49 ± 41.6 (3)
Cat	18.6 ± 13.6 (4)
Rabbit	24.4 ± 30.7 (7)
Whale *(Megaptera nodosa)*	20.0 (1)
Fur seal *(Callorhinus ursinus)*	4.55 ± 5.9 (4)
Aves	
Chicken	176 ± 7.3 (10)
Pigeon	42.3 ± 26.4 (3)
Guinea hen	28.5 ± 12.1 (4)
Duck	86.3 ± 18.3 (4)
Reptilia	
Alligator *(Alligator mississipiensis)*	.94 ± 23.4 (5)
Turtle *(Pseudemys troostii)*	18.1 ± 31.2 (3)
Amphibia	
Frog *(Rana pipiens)*	107 ± 8.3 (7)
Mud puppy *(Necturus maculosus)*	101 ± 8.5 (5)
Pisces	
Pacific cod *(Gadus macrocephalus)*	.76 ± 38.1 (4)
Herring *(Clupea harengus)*	7.52 ± 33.7 (4)
Menhaden *(Brevoortia tyransus)*	50.1 ± 37.3 (4)
Shad *(Alosa sapidissima)*	205 ± 27.5 (4)
Rockfish† *(Sebastodes* sp.*)*	74.5 ± 35.7 (2)
Sea perch *(Damalichthys vacca)*	75.4 ± 17.6 (6)
Albacore *(Germo alalunga)*	50.3 ± 27.0 (7)
Carp *(Cyrinus carpio)*	80.7 ± 24.6 (7)
Halibut *(Hippoglossus stenolepis)*	301 ± 41.1 (7)
Skate† *(Raja* sp.*)*	163 ± 23.9 (7)
Dogfish *(Squalus acanthias)*	250 ± 12.0 (7)
Brown trout *(Salvelinus alpinus)*	108 ± 7.2 (4)
Brook " (" *fontinalis)*	108 ± 10.1 (5)
Chum salmon *(Oncorhynchus keta)*	487 ± 19.8 (6)
Red " *(Oncorhynchus nerka)*	383 ± 22.7 (7)
Silver " *(Oncorhynchus kisutch)*	2015 ± 52.1 (2)
Chinook " *(Oncorhynchus tschawytascha)*	3277 ± 24.7 (6)
Pink " *(Oncorhynchus gorbuscha)*	6956 ± 17.4 (6)

TABLE I (continued)

Species	$Co^{60}B_{12}$ bound, $m\mu g/ml \pm \%$ S.E.*
Crustacea	
Lobster *(Homarus americanus)*	.38 ± 10.0 (4)
Giant crab *(Paralithodes camtschatica)*	.29 ± 17.2 (7)
Xiphosura	
King crab *(Xiphosura polyphemus)*	169 ± 24.2 (4)

* % S.E. = (Stand. error/mean value) × 100.

No. of samples analyzed in parentheses.

† Rockfish represent avg values for 1 sample each of *S. auriculatus* and *S. caurinus*. Skates represent avg values for 5 samples of *R. ocellata* and 2 samples of *R. rhina*.

teins. The buffer, prepared daily, contained 0.4 μg $Co^{60}B_{12}$ or DMBC per liter of solution. After 30 minutes, to permit binding to occur, the bags were placed in tubes containing 50 ml non-radioactive buffer and refrigerated in the dark at 4°C for 72 hrs. The external buffer was discarded and replaced with fresh buffer each 3-4 hours between 7:30 A. M. and 11 P. M. At termination of the experiment, the bags were blotted and 5 ml was removed for determination of radioactivity. Saline controls in place of serum indicated complete removal of added radioactivity. Radioactivity measurements were made in a well type scintillation detector. A total of 10,000 counts yielded a probable error of less than 5%. Assays with DMBC were performed under a red photographic safe light due to the lability of the material. Studies with $Co^{60}B_{12}$ were performed under fluorescent lighting.

Results and discussion. The UBBC ranges from values of less than 1 $m\mu g/ml$ in serum from lobsters, giant crab and alligators to values of about 7000 $m\mu g/ml$ in the serum of pink salmon (Table I). Almost all of the animal species with nucleated red cells such as the birds, reptiles, amphibia and fishes have UBBC values considerably higher than those of the mammals. Although this fact appears to be consistent for the majority of sera studied, exceptions occur for the alligator, pacific cod and herring in which the UBBC is within the range or lower than that found in the mammals. Nonetheless, it appears valid to conclude that the UBBC of most animal sera is associated with the presence of high concentrations of nucleated erythrocytes in peripheral blood. It is not now possible definitively to assume the existence of a causal relationship between presence of nucleated cells in blood and the UBBC of serum. Clarification of this relationship must

TABLE II. Comparison of Cyanocobalamin and 5,6-Dimethylbenzimidazolyl Cobamide Coenzyme Binding Capacities of Animal Sera.

Species	No. of determinations	Coenzyme/Cyanocobalamin
Man	6	1.20 ± .19*
Rabbit	6	.95 ± .09
Chicken	6	.90 ± .05
Frog	6	.93 ± .06
Pink salmon	6	.98 ± .10
Shark	6	.96 ± .05
Lobster	5	2.21 ± .41
King crab	4	1.07 ± .01

* Avg values ± stand. dev.

await isolation of the Vit. B_{12} serum binding protein and its source of production in the body.

The UBBC values of Table I for the rat, cow, pig, rabbit and man are in excellent agreement with the values obtained for these species by Gregory and Holdsworth(2) using an ultrafiltration procedure. However, Gregory and Holdsworth obtained UBBC values for chicken sera of 1100 mμg/ml, a value considerably higher than 176 mμg/ml obtained in the present study. An explanation for this divergence of results is not now available but may be due to variations between different strains of birds.

Of special interest is the UBBC of hemolymph from animal species lacking erythrocytes. The lobster and giant crab have very low UBBC values of about 0.3 mμg/ml as compared with 169 mμg/ml for horseshoe (king) crab hemolymph. Because these animals are classified in separate classes, it is interesting to speculate that determinations of the UBBC may be useful for studies of animal systematics.

The UBBC of $Co^{60}B_{12}$ and DMBC of sera from representative animal species are essentially the same with the exception of lobster serum (Table II). The discrepancy for lobster serum appears to be due to experimental error since the UBBC for $Co^{60}B_{12}$ (0.15 mμg/ml) and for DMBC (0.31 mμg/ml) approaches the analytical limit of our assay procedure where errors of 50% or more may occur.

The similarity between the binding of these 2 forms of vitamin suggest that the groups capable of combining with serum proteins are the same. Furthermore, substitution of the cobalt linked moiety apparently has little effect on the binding. Although these data suggest that the cobalt atom itself may not be involved in this binding, it does not entirely rule out such a possibility. It is conceivable that serum may catalyze the removal of cobalt linked substituent groups, thus making the cobalt atom available for binding. It is well known that the cyano group is readily replaceable by hydroxyl ions and that the labile coenzyme is readily converted to hydroxocobalamin(11). These and other factors concerning the binding of Vit. B_{12} and its analogues to serum protein must await further clarification.

Summary. The vitamin B_{12} unsaturated binding capacity (UBBC) of sera from various animals ranged from 0.29 mμg/ml for king crab serum to 6956 mμg/ml for chinook salmon serum. Although exceptions occur, the UBBC of sera from animals with nucleated erythrocytes is higher than that of mammalian sera. The binding capacity for Co^{60} – dimethylbenzimidazolyl cobamide cyanide (cyanocobalamin) and Co^{60} dimethylbenzimidazolyl cobamide coenzyme (DMBC) was the same, suggesting that cobalt ligands may not be involved in binding.

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