tion steps prior to being placed on a linear sucrose gradient. The mean density of the sporozoites isolated from the peak fraction was 1.157 g/cm^3 and the number of sporozoites recovered per mosquito was estimated to be 45,000. The technique can readily be extended to the isolation of sporozoites of other plasmodial species and produces a greater yield than is possible by conventional dissection methods.

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Vitamin D Binding Globulin in the Rat: Specificity for the Vitamins D* (33783)

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An *in vivo* study of serum protein binding of vitamin D_4-^3H in rats has shown that there exists a specific vitamin D binding globulin (1). In that study there was noted an increasing association of radioactivity with this protein with time. This was associated with the conversion of 60% of the vitamin to the major biologically active metabolite. This increasing association with time could have been due to new protein synthesis or to a more specific binding of metabolite than of parent vitamin. To study this further, the following experiments were performed, comparing the *in vivo* serum protein binding of vitamin D_3-^3H and vitamin D_2-^{14}C in the rat.

Materials and Methods. Tritiated vitamin D. Specifically labeled vitamin D_3-1,2-^3H and randomly labeled vitamin D_2-^{14}C were prepared by methods previously described (2, 3). All vitamin preparations were stored in benzene at 4° until use. No preparation was used which was not 97% chromatographically pure.

Animals. Male weanling rats obtained from Holtzman or Sprague-Dawley companies were fed vitamin D-deficient diet No. 11 as described by Guroff *et al.* (4) for 4–8 weeks.

Experimental procedure. Five to 100 IU of vitamins D_2-^{14}C or D_3-^3H in 0.05–0.1 ml of absolute ethanol were injected intravenously via the jugular vein. Blood samples were obtained from the tail vein at 5, 90, 150, and/or 240 min after injection. Serum was obtained by centrifugation at 2000 rpm within 30 min of drawing. Three 0.1-ml samples of each serum were subjected to disc electrophoresis within 5 hr of drawing, as previously described (1).

Radioactivity within each gel was located by combustion of the separated proteins and subsequent trapping of the ^3H_2O or $^{14}CO_2$, as previously described (5). Radioactivity measurements were performed in a Packard Tricarb liquid-scintillation counter model 3003 equipped with external standardization.

Gels were sliced in the patterns shown in Fig. 1. The radioactivity of the blank (dpm/mm) was multiplied by the total millimeters of each segment and subtracted from the radioactivity found in each segment. A mean value ± SD was calculated for each

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segment from the triplicate gels thus analyzed. The amount of radioactivity associated with each segment was then expressed as a percentage of the total of the mean radioactivity in all segments.

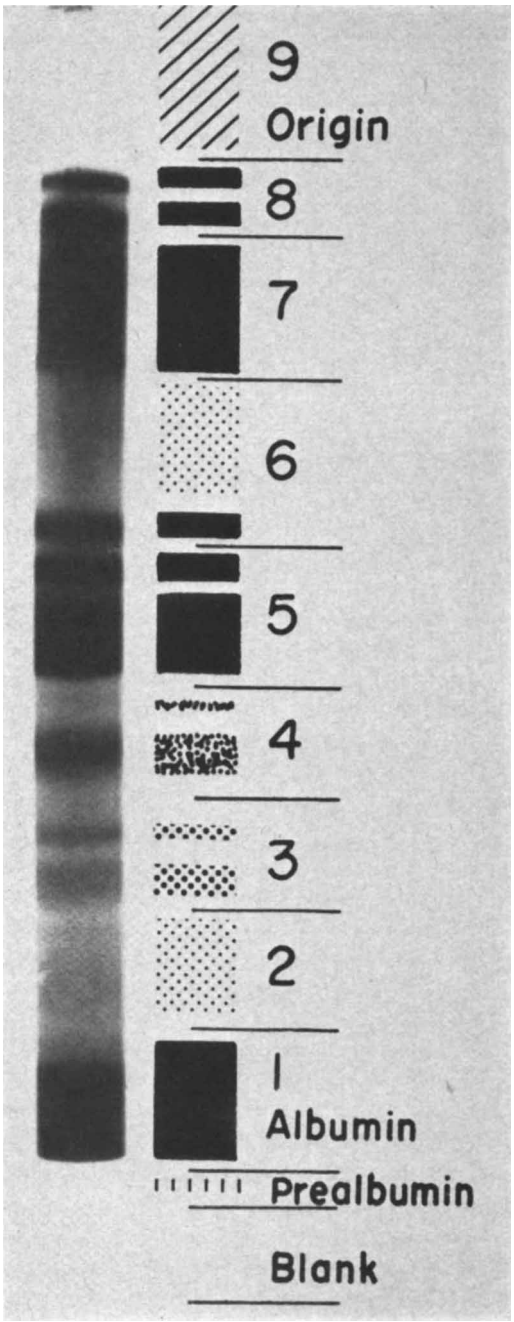

FIG. 1. Polyacrylamide "disc" electrophoresis of rat serum with adjacent sketch showing slicing pattern used.

Results. Figure 1 depicts a typical rat serum protein separation with a diagrammatic representation of the cutting pattern used. The protein segment no. 3 has been shown to be the prime vitamin D binding globulin (1). It has also been shown that segments 5, 6, 8, and 9, containing high and low density lipoproteins, are associated with a decreasing amount of radioactivity with time.

Fifteen min after injection of vitamin D_3 more than 80% of the radioactivity was associated with the alpha globulin segment no. 3 (Table I). In contrast, vitamin D_2 is distributed about equally between this segment and the lipoprotein segments at 15 min. With both vitamin D_2 and D_3 the association of radioactivity with vitamin D binding globulin increases with time, whereas the radioactivity found with the lipoprotein fractions decreases. This latter observation was more definitely seen in the original study with vitamin D_4 (1).

Table II compares the results obtained with each of the vitamins. The similarity of the protein binding of vitamins D_2 and D_4 is in striking contrast to that seen with vitamin D_3. Our interpretation of these results is that the vitamin D binding globulin of the rat has a very high specificity for vitamin D_3. It is of interest that all three vitamins are to some extent associated with the lipoprotein fractions of rat serum and that this decreases with time.

Discussion. The vitamins D differ from each other only in the side chain. Vitamins D_2 and D_4 have an additional methyl group at the 24 position. Vitamin D_2 also has a double bond at the 22–23 position. Vitamin D_3 has neither. The results of this study demonstrate that the molecular interaction between the vitamin and its binding globulin is strongly affected by the composition of the side chain of the molecule. In this regard, a major biologically active metabolite of vitamin D_3 has recently been identified as 25-hydroxycholecalciferol (6). It has been shown that this metabolite probably is bound exclusively by the vitamin D binding globulin.

TABLE I. Distribution of Radioactivity among Serum Proteins after Injection of Vitamin D_3-1,2-^3H or Vitamin D_2-^{14}C i.v. to Vitamin D-Deficient Rats.[a]

Protein segment no.	(min):	Vitamin D_3-1,2-^3H 15	90	150	Vitamin D_2-1-^{14}C 15	90	150
Pre		0.8 ± 0.6	0.1 ± 0.1	0.1 ± 0.2	2.6 ± 2.9	1.6 ± 1.2	0.3 ± 0.3
1		1.1 ± 0.4	1.2 ± 0.9	0.3 ± 0.3	0.5 ± 0.6	0.7 ± 0.7	2.0 ± 0.4
2		1.1 ± 0.2	1.0 ± 0.7	0.3 ± 0.3	8.3 ± 2.3	9.8 ± 1.7	3.9 ± 3.9
3		81.7 ± 4.4	90.0 ± 1.1	92.1 ± 4.8	42.8 ± 1.3	54.2 ± 1.1	66.3 ±16.4
4		3.1 ± 1.0	3.7 ± 0.7	2.9 ± 0.5	7.8 ± 1.8	5.1 ± 1.1	2.9 ± 1.8
5[b]		4.3 ± 0.8	1.2 ± 0.5	0.9 ± 0.3	10.1 ± 3.4	8.6 ± 2.4	8.2 ± 1.6
6[b]		0.9 ± 0.4	0.4 ± 0.3	0.3 ± 0.3	9.9 ± 2.5	8.6 ± 0.4	4.9 ± 4.1
7		2.1 ± 0.4	0.8 ± 0.6	0.3 ± 0.4	3.4 ± 0.5	0.4 ± 0.4	2.9 ± 2.9
8[c]		3.2 ± 0.5	0.9 ± 0.5	1.2 ± 0.4	8.1 ± 1.5	5.2 ± 0.1	3.8 ± 1.6
9[c]		1.7 ± 0.6	0.7 ± 0.6	1.5 ± 1.7	6.4 ± 1.4	5.7 ± 0.9	4.8 ± 0.6

[a] Values expressed as percentage of total radioactivity in all segments.
[b] Are or contain high density lipoproteins.
[c] Are or contain low density lipoproteins.

Thus the increasing association of radioactivity with the vitamin D binding globulin in time is probably related to the formation and very specific protein binding of a vitamin D which has an additional hydroxyl group on the side chain.

The postulate has been made that the vitamin D binding globulin plays a significant, if somewhat obscure, role in the metabolism and metabolic functions of the vitamin in the animal body. Of interest in this regard is the observation that vitamin D_4 is only 75% as effective as vitamin D_3 in curing rickets in rats (7). It has also been suggested that vitamin D_2 is not as effective as vitamin D_3 in raising serum calcium in rats (8). It has recently been demonstrated that vitamin D_3 is much more effective than vitamin D_2 in preventing osteodystrophia fibrosa in the monkey (9).

Probably the best known and most widely studied discrimination between the vitamins is the chicken's poor response to any vitamin other than vitamin D_3. One of the other interesting studies in this regard is the recent study by Imrie *et al.* (3) of the time course of appearance and disappearance of radiolabeled vitamins D_2 and D_3 in various organs of the chicken. They reported that vitamin D_2 disappeared from blood, bone, and muscle more rapidly than did vitamin D_3. This was apparently related to a more rapid degradation of the vitamin D_2 molecule.

These observations and those of the present study may be considered together in the following light. It has been postulated

TABLE II. Comparison of Rat Serum Protein Binding of Vitamins D_2, D_3, and D_4.[a]

Protein	Vitamin	(min): 15	90	150
Gamma-globulin	D_2	42.8 ± 1.3	54.2 ± 1.1	66.3 ±16.4
	D_3	81.7 ± 4.4	90.0 ± 1.1	92.1 ± 4.8
	D_4	44.0 ± 0.8		56.9 ± 2.6
HD lipoproteins	D_2	20.0 ± 5.5	17.3 ± 2.0	13.2 ± 2.5
	D_3	5.2 ± 1.1	1.5 ± 0.7	1.3 ± 0.6
	D_4	14.6 ± 1.5		8.4 ± 2.0
LD lipoproteins	D_2	15.1 ± 3.6	10.9 ± 1.0	8.6 ± 2.2
	D_3	4.9 ± 0.6	1.6 ± 0.8	2.7 ± 2.0
	D_4	15.4 ± 0.4		5.0 ± 1.0

[a] Expressed as percentage of radioactivity in the serum.

that the vitamin D binding globulin preserves the vitamin D molecule from metabolic degradation and regulates its metabolic function by releasing it or its metabolically active metabolite exclusively to receptor sites in target organs. Thus, the higher the binding affinity of a vitamin D for this globulin, the greater would be its metabolic effect. This postulate, if borne out, could help explain the findings of Imrie *et al.*, and the discrimination between the vitamins in the other instances cited above. Perhaps future studies will prove this postulate to be a viable one.

Conclusions. The vitamin D binding globulin of the rat exhibits a much greater affinity for vitamin D_3 than for vitamins D_2 and D_4. The addition of a hydroxyl group to the side chain of any of the vitamins increases their affinity for the protein. At physiologic dose levels, lipoproteins would play almost no role in the *in vivo* binding of vitamin D_3 in rat serum. It is suggested that the affinity of a vitamin for the vitamin D binding globulin may play an important role in the biologic effectiveness of the molecule.

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