natant injected as recently as one day before tumor grafting would have a sufficient period to initiate the processes (which may require some time for their development) leading to abrogation of resistance in the host. In this sense, the conditioning process resembles the time relationship observed in the preparation of animals to produce immune sera. The extremely long period over which the effects of conditioning persist is of interest in this connection. It is hardly likely that tissue antigens would survive in the host over so long a time (at least 45 weeks in these experiments). If an immune reaction is involved in conditioning (and at present this is a moot point), the inoculation of the live tumor graft may elicit an anamnestic response.

Summary. In previous studies, it has been shown that in many instances the normal resistance of mice of an inbred strain to homografts of a tumor can be abrogated by injecting the host with an extract of tumor tissue. It is here shown that such an injection must be given prior to tumor inoculation, or at most one day after inoculation, to obtain loss of resistance. The effects of such a procedure persist for long periods, as shown by progressive growth of tumor homografts in 80-100% of mice grafted approximately 5 months after receiving injections of tumor extract, and in at least 50% of the mice grafted up to 10 months after treatment with extract.

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Vitamin B_{12} Activity of Plasma and Whole Blood from Various Animals.* (21026)

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Numerous reports have recently appeared concerning the vit. B_{12} activity of whole blood (1-4) and blood plasma or serum(5-8) in a variety of animal species. With the exception of the study by Yamamoto *et al.*(9) which indicated that dog erythrocytes contain no microbiological B_{12} activity, information concerning the distribution of vit. B_{12} between the erythrocytes and plasma of blood is lacking.

It seemed of interest, therefore, to study the distribution of vit. B_{12} activity between the cellular fraction and plasma of blood from a variety of animals including mammals, birds and reptiles. The results of this investigation are presented in this report.

Materials and methods. Blood was obtained from the human, rabbit, dog, calf, chicken and alligator in oxalated or heparinized containers. The samples were divided into two portions and one of the portions was

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TABLE I. Total Vit. B_{12} Activity of Whole Blood and Plasma from Various Animals.

			Vit. B_{12} activity (μg/ml)		
Animal	Sample	No. of determinations	Before alkaline treatment	After alkaline treatment	Activity destroyed
Human	WB*	6	.38 ± .05†	.12 ± .01	.26
	P	5	.16 ± .03	0‡	.16
Dog	WB	7	.33 ± .04	.11 ± .01	.22
	P	11	.29 ± .04	.13 ± .01	.16
Calf	WB	9	.31 ± .03	.10 ± .01	.21
	P	9	.10 ± .01	0	.10
Rabbit	WB	6	43.3 ±5.8	2.68 ± .35	40.6
	P	6	31.3 ±7.9	.50 ± .17	30.8
Chicken	WB	6	8.59 ± .65	2.06 ± .24	6.53
	P	7	1.19 ± .40	.13 ± .05	1.06
Alligator	WB	3	7.14 ±1.51	5.48 ±1.46	1.66
	P	8	.06 ± .01	.04 ± .01	.02

* WB = Whole blood. P = Plasma.
† Stand. error.
‡ Values less than 0.01 μg/ml cannot be detected.

used to provide plasma. Hematocrits were determined in the usual manner using conventional Wintrobe hematocrit tubes. The plasma samples were assayed for vit. B_{12}§ activity before and after alkaline hydrolysis by the procedure of Rosenthal and Sarett(8) using *Lactobacillus leichmanii* 4797 as the test organism. Whole blood vit. B_{12} activity was determined with a slight modification of the above procedure. Although the greater bulk of whole blood proteins was removed by heating a 1:6 dilution of blood at pH 5.1, some acid soluble protein remained. This residual protein was found to precipitate readily when a suitable aliquot of the initial acid extract was adjusted to pH 6.8 ± 0.1, and diluted with distilled water to a final concentration of 1:10. The precipitate was then removed by centrifugation and the clear supernatant assayed for vit. B_{12}. In order to release desoxyribosides and vit. B_{12} from nucleoprotein(3), whole blood and plasma from the chicken and alligator was diluted 1:4 with distilled water, layered with toluene and autolyzed at 37°C for 36-44 hours. Since it was found in preliminary experiments that the vit. B_{12} activity of mammalian blood was not increased significantly by autolytic procedures, autolysis of the samples was omitted.

§ In this report, the term vit. B_{12} is restricted to denote material which is microbiologically active for *L. leichmanii* 4797 and is destroyed by alkaline treatment. The term vit. B_{12} activity denotes all substances which are microbiologically active for this organism including the desoxyribosides of guanine, thymine, hypoxanthine and others.

Results. The vit. B_{12} activity of whole blood and plasma from the various animal species before and after alkaline hydrolysis, is shown in Table I. With the exception of the rabbit, the vit. B_{12} activity of whole blood for the mammalian species is similar before and after alkaline hydrolysis. It is of special interest to note the high vitamin content of rabbit blood which contains more than 100 times the vitamin content of any other mammal studied. The whole-blood values for the human, calf and dog, are slightly lower than those previously reported by other workers (1), while the values for the rabbit are higher. The differences reported here may be due to dietary and genetic factors, as Anthony *et al.* (4) have shown in cattle. These workers found variations in blood vit. B_{12} activity not only between different strains of cattle but between groups of cattle of the same strain when fed different rations.

The mammalian plasma vit. B_{12} activity is lower than that of the corresponding whole blood, and again, rabbit plasma contains much more vit. B_{12} than does plasma from the other mammals. The values for the vit. B_{12} activity of human and rabbit plasma are in good agreement with the activity found by Ross

TABLE II. Vitamin B_{12} Activity of Blood and Plasma from the Chicken and Alligator.

		Vit. B_{12} activity (μg/ml)			
		Before autolysis		After autolysis	
Animal	Sample*	Before alkaline treatment	After alkaline treatment	Before alkaline treatment	After alkaline treatment
Chicken	WB	3.16 (6)†	4.18 (6)	8.59 (6)	2.06 (7)
	P	.98 (2)	.09 (6)	1.19 (7)	.13 (7)
Alligator	WB	2.77 (4)	2.95 (4)	7.14 (3)	5.48 (3)
	P	.04 (7)	.02 (7)	.06 (8)	.04 (8)

* WB = Whole blood. P = Plasma.
† The number in parentheses indicates number of determinations performed.

(5) who used *Euglena* as the test organism.

In mammalian whole blood, some of the vit. B_{12} activity is resistant to alkaline hydrolysis and is presumably due to desoxyribosides(3,10). The blood of rabbits contains a larger quantity of the alkaline stable material than does that of the other mammals. This amount, however, represents less than 6% of the total vit. B_{12} activity of rabbit blood and approximately 30% of the activity in the blood of the other mammals. In contrast to whole blood, plasma from the human and calf contains practically none of the alkaline stable material and only small amounts of these substances are present in the plasma of the dog and rabbit.

Whole blood from the chicken and alligator (after autolysis) contains more vit. B_{12} activity than any of the mammals with the exception of the rabbit. The alkaline stable material comprises 24 and 77% of the total vitamin activity of the chicken and alligator blood respectively. Plasma from these animals contains considerably less vit. B_{12} activity than does whole blood. The plasma vitamin activity of the chicken is greater than that of any of the mammals with the exception of the rabbit, while the plasma vitamin activity of the alligator is barely detectable. The residual plasma alkaline stable material in the chicken and alligator is similar to that found in the human, calf and dog.

The apparent vit. B_{12} activity of whole blood from either the alligator or the chicken was increased considerably when the blood was subjected to autolytic procedures (Table II). Similar analysis of the plasma from these animals shows that autolysis releases very little additional vit. B_{12} activity. The vit. B_{12} content of blood is, therefore, presumably associated with the nuclei of the cellular fraction of blood (primarily the erythrocyte fraction) and becomes available to the test organism only after the enzymatic action of autolysis.

It was not possible to determine the proportion of vit. B_{12} present in whole blood prior to autolysis since alkaline treatment to destroy the vitamin invariably released greater amounts of vit. B_{12} activity than was present in the original sample. The additional alkaline stable material is presumably due to liberation of desoxyribosides from the alkaline hydrolysis of nucleoproteins.

The distribution of vit. B_{12} between the plasma and the erythrocytes (Table III) was calculated from the hematocrit and the data for whole blood and plasma vit. B_{12}. For the mammals (with the exception of the calf) the vitamin is almost equally distributed between the erythrocytes and plasma within the limits of experimental error. In contrast to the mammalian species, the vit. B_{12} content of the blood from the chicken or alligator is largely present in the cellular fraction. This activity may be associated with the high nucleic acid content of the nucleated erythrocyte of these animals.

It is apparent from these data that vit. B_{12} is intimately associated with the cellular fraction of blood derived from animals containing nucleated red cells. In those animals in which a nucleated erythrocyte is not a normal constituent of the blood, the vitamin is almost equally distributed between the cells and the plasma. The slightly greater concentration of vit. B_{12} in the cellular fraction of blood from mammals may be due to the amount of

TABLE III. Distribution of Vitamin B_{12} in the Blood of Various Animals.

Animal	Hematocrit (%)	Whole blood vit. B_{12} (μg/ml)	Plasma vit. B_{12} (%)	Erythrocyte vit. B_{12} (%)
Human	38	.26	38.4	61.6
Dog	45	.22	40.9	59.1
Calf	40	.21	28.6	71.4
Rabbit	36	40.6	48.5	51.5
Chicken	29	6.53	11.6	88.4
Alligator	21	1.66	1.2	98.8

vitamin contained in the leukocytes and young forms of red cells. This is especially true of the calf since it is known that immature mammals may contain large numbers of immature, nucleated erythrocytes(10).

Summary. Vit. B_{12} activity of whole blood and plasma from the human, dog, calf, rabbit, chicken and alligator was studied before and after alkaline hydrolysis. The data show a wide variation of blood and plasma vit. B_{12} content between the different species. In mammals, the vitamin is almost equally distributed between the erythrocytes and plasma. In chicken and alligator, the major portion of the vitamin activity is associated with the nucleated erythrocyte.

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Pharmacological Studies with Rescinnamine, a New Alkaloid Isolated from *Rauwolfia serpentina.* (21027)

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The favorable clinical results obtained with preparations of *Rauwolfia serpentina* have created considerable interest in the active principles of this root and their mechanism of action. While at least 8 alkaloids had been found and investigated by various Indian and European workers, the only highly potent alkaloid found was reserpine, first isolated and studied by Müller, Schlittler, and Bein (1,2). In a recent communication from our Laboratories(3) it was stated: "Reserpine is the most potent single alkaloid so far examined. Available evidence suggests that other active alkaloid(s) are present in *Rauwolfia serpentina.*"

Since the known compounds account for only about 50% of the alkaloids in Rauwiloid®,* it was logical to look for additional active principles in the amorphous portion of this preparation. From it Klohs and coworkers(4) succeeded in isolating and identifying a new crystalline alkaloid, the trimethoxycinnamic ester of methylreserpate (M.P. 238-9°, $[\alpha]^{24}_{D}$ −97 ± 2, c, 1.0 in $CHCl_3$). As will be shown in this communication, rescinnamine has all the typical pharmacologic properties of Rauwiloid® and reserpine. The new al-

* The alseroxylon fraction of *Rauwolfia serpentina*, is a concentrate of the active alkaloids of the root, free from non-alkaloidal matter.