age between lipids and proteins is not understood but it has been generally considered to by a stoichiometric linkage with some stability rather than an adsorption complex. The experiments reported in the present paper show that unesterified cholesterol molecules initially present in plasma lipoproteins interchange with those in red cell lipoproteins during incubation. It appears highly unlikely that the entire lipoprotein molecules interchange since plasma lipoproteins contain both free and esterified cholesterol whereas red cells contain only the free form. Furthermore, red cell lipids occur in the water insoluble stroma in contrast to the highly water soluble plasma lipoproteins. All the free cholesterol molecules of plasma and of red cells are apparently capable of interchange. Although the radioactive cholesterol molecules measured in these experiments were recently synthesized, there is no reason to believe that a plasma lipoprotein molecule containing newly synthesized cholesterol will behave any differently from one containing "older" cholesterol. In the case of red cells, the fact that complete equipartition with free plasma cholesterol is attained both *in vivo* and *in vitro* is strong evidence for the assumption that all the free red cell cholesterol is exchangeable. The time required to reach 50% equipartition can be seen from Fig. 1 or 2 to be about one hour, corresponding to a turnover time of 1.44 hours. Thus, any given sample of plasma will have a minimum of 1.16% of the free cholesterol present leaving each minute and being replaced by an equal amount of red cell cholesterol. For example, in a liter of average blood the total cholesterol exchanged between plasma and red cells would be about 460 mg per hour.

The mechanism of this exchange is not simple diffusion since equalization in the free cholesterol concentrations of the 2 phases does not occur. The simplest assumption is that equilibria exist between cholesterol in each of the various lipoproteins concerned and cholesterol in unbound form. This last might be present in minute concentration, possibly in true aqueous solution, and still be capable of acting as intermediate in a rapid equilibration of lipoprotein cholesterol. Experiments to test this hypothesis are now in progress.

Summary. Plasma and red cells containing C^{14}-labeled cholesterol were obtained by feeding dogs with C^{14}-acetate. The labeled blood components were separated and incubated at 37°C for several hours with their unlabeled counterparts under an atmosphere of oxygen-carbon dioxide. The exchange of cholesterol between the two components was followed in each mixture by measurement of the cholesterol specific activities of each component at various time intervals during the incubation. It was observed that equipartition of red cell and plasma unesterified cholesterol was closely approached in 4 hours and 50% equilibration took place in one hour. Esterified cholesterol of plasma did not take part in the exchange phenomenon under these conditions. The possible significance of these results in relation to the nature of lipoproteins is discussed.

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Complement-Fixing Murine Typhus Antibodies in Vitamin Deficiency States.* II. Pyridoxine, and Nicotinic Acid Deficiencies. (19065)

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The authors have presented data(1) concerning the effects of adequate vit. B complex, 1/10th optimal level vit. B complex, total

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vit. B complex, pantothenic acid and thiamine deficiencies upon complement-fixing antibody production by rats in response to washed murine rickettsiae (*Rickettsia typhi*) as the antigenic stimulus. The present paper deals with the effects of pyridoxine, and nicotinic acid upon complement-fixing antibody production under similar experimental conditions. The purpose of this investigation, and others in progress, is an attempt to find an explanation for the increased susceptibility and mortality that occurs in malnourished populations exposed to typhus fever(2). Wertman and Sarandria(1) reported that pantothenic acid and thiamine deficiencies influenced the antibody response when a relatively small amount of immunizing material was injected. However, the influence was not as apparent when relatively large amounts of the antigenic material were employed. A severe impairment of antibody response has been observed in pyridoxine deficient rats (3,4).

Experimental. Male weanling albino rats of the Sprague-Dawley strain, distributed into groups as indicated in the table, were housed individually in wide-meshed, screen-bottom cages and weighed weekly. The ingredients used in preparing the different diets were obtained from General Biochemicals, Inc. and Nutritional Biochemicals Co. All ingredients were of the highest purity obtainable and each basal diet formulated so that only a minimum amount of the B complex vitamins to be studied were present. The basal diet for the pyridoxine groups was as follows: sucrose, 65.12%; casein (vitamin free), 17.23%; vegetable oil (hydrogenated), 9.55%; salt mixture No. 2, 3.88%; cod liver oil (Squibb), 2.00%; corn oil (Mazola), 2.00%; choline chloride, 0.20%; i-inositol, 0.01%; and paraminobenzoic acid, 0.01%. In preparing the nicotinic acid basal diet, casein was held to a minimum for casein has a high content of tryptophan which is a precursor of nicotinic acid(5). Corn grits were employed because of their low tryptophan content. It was felt necessary to add l-cystine for corn grits contain only a trace of l-cystine. The basal diet for the nicotinic acid groups was as follows: corn grits, 40.00%; casein, 9.00%; salt mixture No. 2, 4.00%; choline chloride, 0.20%; i-inositol, 0.03%; l-cystine, 0.15%; corn oil (fortified vit. E), 2.00%; sucrose, 44.62%; and vit. K, 0.001%. In addition two drops of Haliver oil (3,000 U.S.P. of vit. A and 24 U.S.P. of vit. D) were given by dropper to each rat once a week. The rats in the pyridoxine and nicotinic acid groups received their supplemental vitamins in the form of a daily pill. The pyridoxine deficient group had pyridoxine omitted from the pill and the nicotinic acid deficient group had nicotinic acid omitted. Each pill was compounded to supply the following vit.(6); thiamine, 40γ; riboflavin 60 γ; pyridoxine, 50 γ; pantothenic acid, 150 γ; nicotinic acid, 150 γ; biotin, 1 γ; folic acid, 1 γ.

At the end of the 6-week period, each rat was injected intraperitoneally with 0.5 cc (0.0073 mg N)‡ of washed, formalinized suspensions of *Rickettsia typhi* (murine typhus).† One cc inoculations were repeated at 7-day intervals until each rat had received a total of 2.5 cc (0.0365 mg N).‡ During this period, the animals were bled 7 days after each inoculation of murine typhus vaccine to determine if the amount of antigenic stimulus played a role. The complement fixation technic employed in this study was identical to that of Plotz(7), Plotz and Wertman(8), and Wertman(9). Fixation was

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‡ Nitrogen content determined by Biophysics Department, University of Pittsburgh.

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TABLE I. Complement-fixation Titers and Body Weight Change of Rats in Vitamin Deficiency States.

Diet	No.	Complement-fixation titers following		Body wt g	
		1 inj, .0073 mg N	3 inj, .0365 mg N	Init, avg, g	Final avg, g
Nicotinic acid deficient group	41	Neg.	1:640		
	42	1:20	1:128		
	43	"	1:128		
	44	"	1:640	38	75
	45	Neg.	1:128		
	46	1:20	1:128		
Nicotinic acid control group	51	Neg.	1:128		
	52	1:40	1:128		
	53	"	1:128		
	54	"	1:256	38	126
	55	1:10	1:640		
	56	"	1:640		
	57	1:20	1:128		
Pyridoxine deficient group	61	Neg.	1:80		
	62	"	1:320		
	63	"	*		
	64	"	1:320		
	65	"	1:320	35	96
	66	"	1:640		
	67	"	1:160		
	68	"	1:160		
Pyridoxine control group	71	1:40	1:128		
	72	1:20	1:640		
	73	1:40	1:128		
	74	1:10	1:128	40	200
	75	1:40	*		
	76	1:40	1:128		
	77	1:20	1:128		
	78	1:40	1:128		

* Animals died.

allowed to take place for 18 hours at 4 to 6°C. The sensitized cells (0.25 ml sheep cells 3% and 0.25 ml amboceptor containing 3 MHD) were added and the tubes incubated for 30 minutes at 37°C in a waterbath. The tests were read following secondary incubation and only 4+ and 3+ fixations were accepted as end-points. The controls necessary to insure valid results were included in all the titrations and serum dilution tests.

Results. The nicotinic acid and pyridoxine control rats at the end of the 6-week feeding period, all had gained weight, appeared healthy and possessed smooth even coats. The rats fed on the nicotinic acid and pyridoxine deficient diets showed the signs which might be expected to accompany their respective deficiency. The initial average and final average weight of each group are recorded in the table. The nicotinic acid deficient group and the pyridoxine deficient group gained much less weight than their respective control group.

The table demonstrates the end-point titers obtained in the complement fixation test using a washed suspension of murine typhus rickettsiae as the antigen. It appears that nicotinic acid deficiency does not interfere with the production of circulating antibodies when even a relatively small amount of antigenic material was injected. A deficiency of pyridoxine, however, did affect the production of circulating antibodies when a relatively small amount of immunizing material was injected. The animals in this group did not produce detectable complement-fixing antibodies. However, the sera specimens obtained after the administration of 2.5 cc (0.0365 mg N) of vaccine did contain demonstrable concentrations of complement-fixing antibodies. A

quantitative difference between the pyridoxine deficient and pyridoxine control groups existed when relatively large amounts of murine rickettsia were injected. The animals reported in this paper were bled prior to immunization and tested by complement-fixation. All of these sera were found to be negative.

Summary. These studies indicate that nicotinic acid deficiency does not appreciably influence the production of circulating complement-fixing antibody when either relatively small or large amounts of antigenic stimulation were employed. Pyridoxine deficiency, however, influenced unfavorably the antibody response when either small or large amounts of the antigen were injected. The single immunizing injection (0.0073 mg N) did not bring forth circulating antibodies that were demonstrable by complement-fixation.

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Role of Sodium Chloride, Water, Kidneys, and Adrenals in the Production of Congestive Edema.*† (19066)

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Evaluation of the respective roles of the adrenals and kidneys in the genesis of edema is dependent on the availability of an animal form and technic whereby an edematous state can be regularly and simply produced. The experiments reported here provide evidence that such an edema can be produced in the rat by ligation of the inferior vena cava, if the animal is allowed free access to 1% NaCl solution postoperatively. This animal preparation has been employed to study the effects of removal of the adrenals and/or kidneys on the development of edema. It is demonstrated that edema does not develop in the nephrectomized rat. However, removal of the adrenals in the nephrectomized rat does result in the development of an edematous state, suggesting that renal retention of sodium and water is not an obligatory factor in congestive edema formation.

Methods. Unfasted male rats of the Long-Evans strain, maintained on this laboratory's Diet XIV, were subjected under brief ether anesthesia to various operative procedures, using clean surgical technics, employing midline ventral abdominal wall incisions. The inferior vena cava was ligated at the caudal margin of the liver, proximal to the right renal vein, with a single silk suture, taking care to include only the vena cava in the ligature. Bilateral nephrectomy was carried out by ligation of the renal pedicles distal to the entrance of the adrenal veins into the renal veins. Bilateral adrenalectomy was carried out by cautery dissection, the glands being removed en bloc with surrounding fat, together with any accessory adrenal cortical nodules visualized. Various sham operations were performed for control purposes. Care was taken to suture doubly all incisions, in order to avoid wound rupture postoperatively as the result of excessive edema formation. After recovery from the brief period of anesthetization, the animals were given access to either tap water or 1% NaCl in tap water for drinking purposes and were allowed free intake of Diet XIV. The animals were weighed postoperatively twice daily. Maximum gains or losses in body weight within the first 24-36 hour period postoperatively were noted and calculated as % change from the onset body weight. The data are presented in Table I.

Results. Ligation of the inferior vena cava

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