spite of inhibition of its oxidation by the liver. Since nearly all deaths from hexobarbital alone or combined with these agents occurred within the first half hour, it also appears that malathion and chlorothion did not maintain a sufficiently high concentration of barbiturate over a long enough time to be lethal.

The effect of these anticholinesterases on concentration of hexobarbital in some tissues of these animals, and their effect on duration of action of other barbiturates and other drugs which are oxidatively metabolized by liver are being investigated. In a preliminary test pentobarbital sleeping time in mice was also markedly prolonged by chlorothion. Such findings may form the basis for caution by those whose work cause exposure to this type of insecticide and for whom barbiturates or other drugs oxidized by liver are simultaneously prescribed.

Summary. Organic phosphate insecticides, OMPA, EPN, malathion, chlorothion and phostex, which are rapidly metabolized in liver, markedly increased hexobarbital sleeping time in mice, while BFP and TEPP did not. Malathion and chlorothion did not alter toxicity of hexobarbital. TEPP hastened onset of action of barbital while chlorothion did not. Certain anticholinesterases which are rapidly metabolized by liver may compete for enzymes which are responsible for destruction of hexobarbital.

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Alterations in Rat Serum Proteins in Single Deficiencies of Folic Acid and Vit. B_{12}.* (24140)

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The influence of a deficiency of folic acid (PGA) and Vit. B_{12} on rat serum proteins was reported(1). A lowering of total protein level was attended by reductions in serum concentrations of albumin, α_1-globulin and γ-globulin. An increase in proportion of β-globulin was interpreted as indicative of higher serum level of free Vit. B_{12} in the deficient state. In continuation of this work, the effects due to single deficiencies of Vit. B_{12} and PGA on serum proteins and Vit. B_{12} have been evaluated and are here reported.

Materials and methods. Young male rats (Wistar strain), 50 g weight, were depleted of their Vit. B_{12} and PGA reserves by maintenance on a deficient iodo-casein diet containing succinyl sulphathiazole(1) for 4 weeks. The animals were then divided into 4 comparable groups and were fed the following diets: (a) basal ration, (b) basal ration plus Vit. B_{12}, (c) basal ration plus PGA and (d) basal ration plus Vit. B_{12} and PGA. The basal ration consisted of (g/100 g of diet): Vit.-free casein, 10; succinyl sulphathiazole, 2; arachis oil, 6; shark liver oil, 2; salt mixture (U.S.P. No. 2), 4; sucrose, 10; and maize starch, 66, with vitamin additions as stated previously(1). Additions of Vit. B_{12} and PGA, where made, were at levels of 50 μg and

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TABLE I. Blood and Liver Picture in Deficiencies of Vit. B_{12} and Folic Acid. Results represent mean values for 6 rats ± S.D.

Supplement	Erythrocytes/mm³ (× million)	Hemoglobin, g/100 ml	Leucocytes/mm³ (× 1,000)	Liver PGA, μg/g	Liver vit. B_{12}, mμg/g
PGA + vit. B_{12}	6.2 ± .8	14.8 ± .4	7.1 ± 2.6	7.2 ± 2.1	92.8 ± 20.7
PGA	4.4 ± 1.1	12.3 ± .9	6.2 ± 1.4	5.4 ± 1.7	34.5 ± 15.9
Vit. B_{12}	5.6 ± .9	12.6 ± .7	4.9 ± .9	2.1 ± .3	78.4 ± 13.9
None	3.1 ± 1.4	10.8 ± 1.3	3.6 ± 1.2	2.4 ± 1.5	28.6 ± 17.9

5 mg respectively/kg diet. After a further period of 5 weeks the animals were dissected under anesthesia, and blood obtained from the inferior vena cava. A portion was immediately heparinized for cell count and hemoglobin determinations and the rest allowed to clot at 37°C for 1 hour and later centrifuged at 2°C to obtain the serum. Livers were quickly excised and chilled in cracked ice. Hemoglobin was determined by acid hematin method. Vit. B_{12} in serum was determined as total and free vitamin, by the method of Ross (2) using *Euglena gracilis* as test organism. Assays were in triplicate using 50 μl serum in 4 ml basal medium. Total Vit. B_{12} was determined after liberating bound vitamin by heating mixture at 100°C for 15 minutes. For determination of free vitamin, the mixture was sterilized at 56°C for 30 minutes prior to inoculation of test organism. Growth was measured turbidimetrically after 8 days using Klett-Summerson photo-colorimeter with 660 mμ filter in position. Total serum was determined by biuret method(3). Fractionation of serum proteins was carried out by electrophoresis on Whatman No. 3 paper strips as described previously(1). The separated proteins were stained with bromophenol blue by method of Jencks *et al.*(4). Relative percentages of the separated protein components were evaluated densitometrically. Liver PGA was determined after autolysis of tissue in 0.2 M phosphate buffer of pH 7.6 using *Streptococcus faecalis R* (ATCC 8043) as test organism and assay medium of Mitbander and Sreenivasan(5). Liver Vit. B_{12} was determined after liberation from tissue by overnight incubation under toluene with papain in 0.1 M acetate buffer of pH 4.6. Assays were made with *Lactobacillus leichmannii* (ATCC 7830) by a turbidimetric adaptation of the U.S.P. method(6).

Results. The results of blood and liver analyses for control and deficient groups of animals are summarized in Table I. Table II summarizes data on serum proteins and Vit. B_{12}. Typical electrophoretic profiles for control and deficient groups are reproduced in Fig. 1.

While manifestations of single and combined deficiencies are as may be expected, modifications in serum protein profile induced by Vit. B_{12} deficiency are different from those induced by PGA deficiency. In Vit. B_{12} deficiency, the decrease in total protein is characterized by a drop in albumin and in α_1-globulin fractions. In PGA deficiency, on the other hand, β- and γ-globulins are also affected. Reductions in albumin and in α_1-globulin fractions, however, are significantly greater in Vit. B_{12} deficiency than in PGA deficiency. Reductions in albumin, α_1-globulin

TABLE II. Serum Proteins and Vit. B_{12} in Folic Acid and Vit. B_{12} Deficiency. Results represent mean values for 6 rats ± S.D.

Supplements	Serum proteins: Total protein	Albumin	α_1-globulin	α_2-globulin	β-globulin	γ-globulin	Serum vit. B_{12}: Total	Free
	g/100 ml serum						μμg/ml serum	
PGA + vit. B_{12}	5.65 ± .21	2.30 ± .05	1.05 ± .08	.46 ± .10	.86 ± .08	.97 ± .12	730.6 ±102.4	206.4 ± 98.2
PGA	4.89 ± .34	1.65 ± .11	.88 ± .07	.49 ± .05	.85 ± .05	1.02 ± .07	146.0 ± 39.8	85.2 ± 26.2
Vit. B_{12}	4.70 ± .21	1.93 ± .08	.98 ± .04	.47 ± .08	.63 ± .06	.68 ± .09	614.2 ± 78.8	190.0 ± 44.3
None	4.18 ± .38	1.48 ± .09	.74 ± .05	.52 ± .12	.90 ± .07	.54 ± .14	120.4 ± 32.0	69.8 ± 28.4

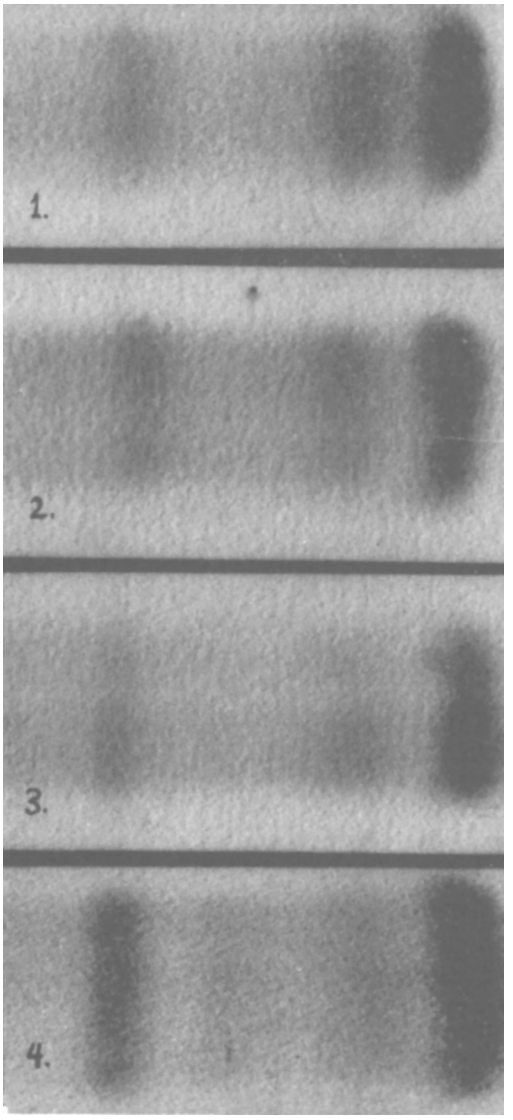

FIG. 1. Alteration in electrophoretic pattern of serum proteins in deficiencies of PGA and vit. B_{12}. Patterns 1-4 represent typical profiles, respectively, of control, vit. B_{12} deficient, PGA deficient, and vit. B_{12} and PGA deficient groups. The bands reading from the starting line are, in order, γ-globulin, β-globulin, α_2-globulin, α_1-globulin and albumin.

and γ-globulin in combined deficiency of the 2 vitamins exceed those in single deficiencies of either PGA or Vit. B_{12}. The relative concentration of β-globulin is markedly increased in Vit. B_{12} deficiency and in combined deficiency of Vit. B_{12} and PGA but not in single deficiency of PGA. There is a similar elevation in ratio of free Vit. B_{12} to total Vit. B_{12} in sera of animals deficient in Vit. B_{12} alone or in Vit. B_{12} and PGA. In PGA-deficient animals the ratio is essentially normal.

Discussion. Vit. B_{12} in human liver tissue exists in loose combination with a protein fraction resembling serum β-globulin in electrophoretic mobility(7). When added to human serum *in vitro,* it is found partly in combination with β-globulin fraction(7-9). In normal human sera, the α-globulin fraction is the primary source of the bound vitamin; free B_{12} found in 3 out of 13 samples corresponded in amount to that found in 3 β-globulin fractions(7). In our work(10) with the rat, serum α_2-globulin is chiefly responsible for binding of vitamin under normal conditions; however, a part may also remain associated with β-globulin when large amounts of the vitamin enter the circulation. Furthermore, a good parallelism has been obtained between quantity of vitamin found associated with α_2-globulin and bound vitamin in whole serum, and between free vitamin concentration and β-globulin-bound vitamin suggesting that, while α_2-globulin may be primarily responsible for immediate binding and storage of vitamin entering the circulation, β-globulin may aid in transporting the vitamin to its functional sites and depots in a manner analogous to iron transport mechanism of serum wherein a specific β_1-globulin is known to function by carrying iron to and from its deposits in tissues(11,12). The recent case report(13) that a patient with unexplained anemia had no β-globulin in circulation is of interest in this connection.

Ludovici and Axelrod(14) have demonstrated that ability of the rat to produce antibodies to human erythrocytes is markedly impaired in a deficiency of folic acid but not in Vit. B_{12} deficiency. The present findings, that serum γ-globulin concentration is decreased in PGA deficiency but is essentially normal in Vit. B_{12} deficiency, may suggest that Vit. B_{12} and PGA play similar roles in γ-globulin and antibody syntheses.

Summary. 1. In Vit. B_{12} deficient rat there is a decrease in serum levels of albumin and α_1-globulin. In PGA deficiency these fractions are affected to a lesser extent but

there is, in addition, a significant drop in levels of β- and γ-globulins. Reductions in serum levels of albumin, α_1-globulin and γ-globulin in combined deficiency of Vit. B_{12} and PGA are greater than those in single deficiencies of either vitamin. 2. In Vit. B_{12} deficiency, an increase in proportion of β-globulin is attended by increase in relative concentration of free Vit. B_{12} in serum. It is suggested that there may occur a preferential synthesis of β-globulin in the deficient state whereby mobilization of the available vitamin may be effected. 3. The observed alterations in serum level of γ-globulin are similar to the known effects of corresponding vitamin deficiencies on antibody synthesizing ability of the animal.

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Some Systemic Chemical Responses to Local Inflammation.* (24141)

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Dunphy and Udupa(1) have recently described chemical alterations in rat skin during wound healing. Using simplified micromethods(2), we studied the effect of inflammation on hexosamine, nitrogen and hydroxyproline concentration of both necrotic and normal skin of rats injected intradermally with croton oil to produce localized injury.

Materials and methods. Wistar strain male rats weighing 200 to 300 g were used. Appropriate areas of shaved skin were removed from animals killed by etherization, dissected clean of adhering fat and muscle and stored in frozen state until analysis. The tissue was minced, and autoclaved 3 hours with 10 ml/g 4N HCl. Subsequent analytical procedures have been described(2). The results are expressed in terms of μmoles of hexosamine or hydroxyproline/mmole nitrogen. To provide controlled areas of necrosis, 0.4 ml croton oil (Magnus, Mabee and Reynard Co. N. Y.) was injected intradermally into a previously shaved area of rat skin. The preparation gave no gross evidence of wound healing for at least 7 days, but produced necrosis throughout an area of about one cm^2 within 3 days. The injury included dermis, epidermis and subcutaneous tissue with no suppuration and minimal sloughing. To demonstrate relative homogeneity of the chemistry of rat skin, 6 rats weighing 250 to 300 g were killed. The following 4 areas of whole rat skin were selected for study; ventral, mid dorsal, right foreleg and left hindleg. Ratio of hexosamine and hydroxyproline to nitrogen and of hexosamine to hydroxyproline for each area of skin was determined in each animal, and mean ratios and

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