Electrophoretic Studies on Serum and Plasma of Control and Vitamin Deficient Monkeys.* (19401)

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In connection with the study of various vitamin deficiencies in rhesus monkeys, we investigated the serum proteins of these animals, both by electrophoretic and by chemical means. It was believed that fundamental work of this nature employing highly purified diets with controlled intakes of the vitamins might be of value in interpreting abnormalities observed in the electrophoretic patterns of humans.

Electrophoretic analysis of plasma or serum of monkeys has been investigated in a few animals by Deutsch and Goodloe(1), and by Moore(2). Monkey plasma was shown by Deutsch *et al.* to contain, in addition to albumin and fibrinogen, three alpha globulin components and a component with a mobility greater than that of albumin which they have designated as "f". This component has been observed frequently in the patterns to be described in this paper.

Analyses have been carried out on one or more samples of serum or plasma from a large series of animals, including controls and monkeys subjected to various vitamin deficient regimens.

Experimental. The rhesus monkeys were, in most instances, young animals weighing 1.5 to 3.0 kg when they were purchased. In Table I are listed the composition of the basal diets and the supplements employed. The methods of handling and feeding the animals have been described(3). Deficiency was induced by omission of the appropriate vitamin from the supplement. Blood was usually drawn in the morning before the day's food ration was introduced into the cage. When plasma was desired, Wintrobe oxalate mixture was employed as anticoagulant. The serum or plasma was separated by centrifugation and diluted with 2 to 3 volumes of sodium citrate-veronal buffer(1) (pH 8.6, u = 0.1), depending upon the protein concentration. The diluted samples were dialyzed according to the method of Reiner and Fenichel(4) for a period of 2 hours at room temperature with one change of buffer at the end of the first hour. The electrophoretic technic of Longsworth(5) was followed using a microcell of 2 ml capacity whose use has already been described by Ross *et al.*(6), and by Cohen and associates(7). Electrophoresis was carried out at 2 to 3°, generally with a constant current of 7 to 8 ma. and a field strength of 9 volts per cm, for periods varying from 3000 to 6780 sec., depending on the time required to give maximum development of the pattern. The patterns were photographed by the scanning method of Longsworth(8), and the areas of the peaks were estimated by the method of Tiselius and Kabat(9). Because of their

TABLE I. Composition of Basal Diets.

Constituents	Diet* (S)	(HP)	(MF)	(AP)
Casein	18	36	18	—
α protein of soy beans	—	—	—	18
Powdered sucrose	73	55	59	73
Corn oil	2	2	2	2
Hydrogenated cotton seed oil (Crisco)	—	—	6	—
Salts (Hawk & Oser)	4	4	4	4
Methionine	—	—	—	.3

* (S) Standard; (HP) High protein; (MF) Moderate fat; (AP) α protein.

Supplements: A daily vitamin tablet containing: Niacin 5 mg, riboflavin 1 mg, thiamine chloride .5 mg, calcium pantothenate 3 mg, choline dihydrogen citrate 100 mg, Paba 100 mg, inositol 100 mg, ascorbic acid 25 mg, pteroyl glutamic acid 110 γ, biotin 10 γ. Pyridoxine HCl was administered daily or twice weekly in doses equivalent to 1 mg daily. In addition each animal received by mouth once a week 10 drops of vitamin A and D concentrate (100000 I.U. vit. A; 10000 I.U. vit. D) and 5 drops mixed natural tocopherols. Choline deficient and control animals on diet AP received 3.5 γ vit. B_{12} *per os* twice weekly. Choline deficient animals received no methionine.

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greater sharpness, the ascending patterns were used for all calculations. Total protein, albumin and globulin were determined chemically by the biuret method as modified by Wolfson *et al.*(10) with the exception that the globulin was precipitated by 26% Na_2SO_4 (11).

Results. Data on the nutritional status, diet, duration on the regimen along with the comparative results of chemical and electrophoretic analyses of the serum or plasma proteins of a part of the monkeys studied are given in Tables II and III. Table II summarizes data on control and Table III on vitamin-deficient monkeys. A serum pattern of a control and of a vit. B_6-deficient monkey are given in Fig. 1 and 2. The electrophoretic values of the individual components are given as relative concentrations which are expressed in percent of the total area. The electrophoretic mobilities of the plasma or serum components of a group of control and deficient monkeys are given in Table IV. The relative concentrations of the various components of serum or plasma vary considerably in individual control animals. However, the values are similar to those observed by Deutsch and Goodloe(1). The component "f" described by the latter has been found in the patterns of the majority of the monkeys studied and its presence or absence is indicated by (+) or (—) in the tables. It is possible that this component occurs in all monkey patterns but may have been obscured in a number of instances. An average value of 1% of the total proteins has been obtained for this fraction. This value is approximately twice that reported by Deutsch *et al.*

In the case of control monkeys maintained on diet S, the average electrophoretic mobilities of the gamma globulin and Ø are higher, and those of the remaining components are lower than the corresponding values reported by Deutsch and Goodloe(1). Since measurements of mobility are not in general extremely precise, no great significance is attached to these differences. The mobility of the "f" component was not calculated.

Effect of vit. B_6 deficiency. With the exception of one animal (No. 16), all monkeys

TABLE II. Chemical and Electrophoretic Data* on Serum and Plasma Proteins of Control Monkeys.

Serum (S) or Plasma (P)	No. of animals	Diet	Chemical: Total protein, g %	Chemical: Albumin, g %	Chemical: Globulin, g %	Chemical: A/G	Electrophoretic, % of total area: Albumin	α1 and 2	α3	β	Ø	γ	A/G	f
P†	5	S	6.7 ± .2	3.9 ± .15	2.8 ± .15	1.4	49 ± 1.65	9.5 ± 1	7 ± .9	15 ± 1.5	11 ± .3	9.5 ± .6	.95	Present in 4 animals
S†	6	S	7.45 ± .2	4.4 ± .3	3.3 ± .2	1.3	57 ± 1.05	7.5 ± .6	8 ± 1.4	15 ± 1.35	—	13.5 ± 1	1.3	
S†	2	HP	6.7	4.35	2.35	1.85	56.7	13	9.25	12.25	—	11.25	1.3	Present in 2 animals
P	1	HP	6.6	4.1	2.5	1.6	47	11	16	8	9.5	8.5	.9	?
S	1(#31)	AP	6.8	4.6	2.2	2.1	66	6	9	8.5	—	10.5	1.95	?
S	1(#38)	MF(‡	7.9	4.85	3.05	1.6	50.5	8.5	15	10	—	14.5	1	
S	1(#21)	SC‡	7.45	4.1	3.3	1.2	48	9.5	17	9	—	16.5	.9	

* Since α-1 and α-2 were not sharply separated in a few instances, the sum of the two are given throughout.
† Represent average values. ± standard error of mean. ‡ 1% cholesterol added to diets S and MF.

TABLE III. Chemical and Electrophoretic Data* on Serum or Plasma Proteins of Vitamin Deficient Monkeys.

Animal No.	Deficiency	Diet	Deficiency, mo	Serum (S) or plasma (P)	Chemical: Total protein, g %	Chemical: Albumin, g %	Chemical: Globulin, g %	Chemical: A/G	Electrophoretic: % of total area: Albumin	α 1 and α 2	α 3	β	Ø	γ	f	A/G	Liver damage
16	B6	S	28	S	6.6	4.15	2.45	1.7	55	15	5	8	—	17	?	1.2	
16	"	S	32	S	6.95	3.75	3.2	1.15	48.5	7.5	10	12	—	22	+	.95	
54	"	S	8½	S	4.1	2.2	1.9	1.2	44	5	3	21	—	27	?	.8	+
56	"	S	11	S	6.4	1.9	4.5	.4	25	3	5	17	—	49	+	.35	++
56	"	S	11½	S	4.7	1.3	3.4	.4	19	5	4	20	—	52	+	.2	++
35	Low B6	MF	a	S	6.7	3.4	3.3	1	52	13	9.5	7.5	—	18	+	1.1	
35	"	MF	b	P	6.7	1.8	4.9	.35	36	9.5	14	8	18.5	14	+	.55	++
35	"	MF	c	S	6.1	2.9	3.2	.9	47	8.5	15	11	—	19.5	?	.9	++
36	"	MF–C†	d	S	7.2	3.5	3.7	.95	42.5	7.5	17	9.5	—	23.5	+	.75	
37	"	MF–C†	e	S	7.5	3.8	3.7	1	42.5	9	29.5	12	—	12	+	.75	
37	"	MF–C†	f	S	6	2.2	3.7	.6	25.5	9	28.5	20	—	22	?	.35	+
41	"	S	g	S	7.45	4.05	3.4	1.2	48	10	8	13	—	21	+	.9	
41	"	S	h	S	6.95	4.1	2.85	1.45	52	11	4.5	15.5	—	17	?	1.1	
42	"	S	i	S	6.65	4.15	2.5	1.65	59	10.5	8	10.5	—	12	+	1.45	
42	"	S	j	S	6.25	3.55	2.7	1.3	48	12.5	7	14.5	—	18	+	.9	+
43	"	S	k	S	6.9	3.65	3.25	1.1	42	7.5	12	12.5	—	26	?	.7	
22	"	S	l	S	7.8	3.75	4.05	.9	51	5	9	13.5	—	20.5	—	1.1	
25	"	S–C†	m	S	7.5	3.9	3.7	1.05	52.5	9	12	13	—	14	+	1.1	
25	"	S–C†	n	S	7.1	4	3.1	1.3	50	8.5	11.5	12.5	—	17.5	+	1	
26	Choline	AP	31½	S	6.15	3.5	2.65	1.3	45	12	6	16.5	—	20	—	.8	
27	"	AP	34¼	S	7.05	3.4	3.65	.95	46.5	19.5	11	15.5	—	7.5	+	.85	
28	Niacin	AP	17½	S	7.45	4.6	2.85	1.6	58.5	7	5	9.5	—	20	+	1.4	
28	"	AP	19	S	8	4.8	3.2	1.5	53.5	8.5	4	10.5	—	23.5	+	1.15	
29	"	AP	13	S	8.15	5.2	3	1.75	57	7	8	11	—	16	+	1.3	
59	Asc. acid	S	o	P	7.4	3.4	4	.85	47.5	9.5	15	4.5	13.5	10	—	.9	
60	"	S	p	P	7.1	3.8	3.3	1.15	40	14	7.5	13.5	10.5	15	—	.65	
61	"	S	q	P	7.7	3.9	3.8	1	48.5	8.5	8.5	10.5	13	10.5	+	.95	
62	"	S	r	P	7	3.3	3.7	.9	36	13.5	9	15	13.5	13	?	.55	

* Since α-1 and α-2 were not sharply separated in a few instances, the sum of the two are given throughout.
† See reference ‡ Table II.

a No B6 2 mo; 25 γ 5¼ mo
b 12
c 14
d 4.5
e 4.5
f 15
g 15

h No B6 2 mo; 25 γ 17 mo; followed by 1 mg 64 days
i 30 γ 15¼ mo
j 17¾
k 15¼

l 250 γ B6 9¼ mo; 125 γ 9¾ mo; 60 γ 15½ mo
m 24¾
n 25¾
o No ascorbic acid 2¼ mo; 1 mg 20 days
p 2¾ 11
q 2¼ 19
r 21

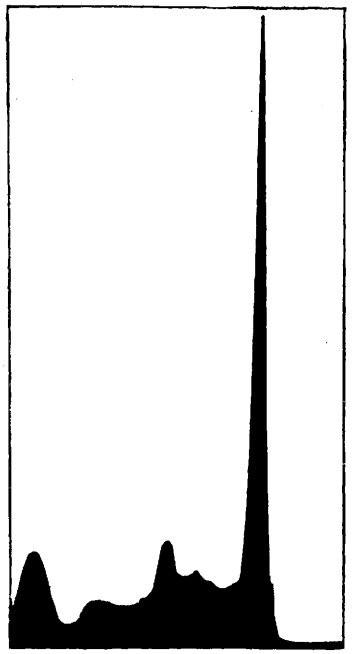

FIG. 1. Ascending pattern—control serum.

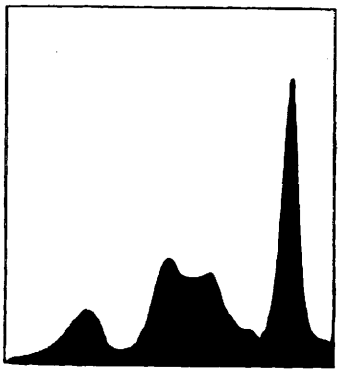

FIG. 2. Ascending pattern—vitamin B_6 deficient serum.

on diet S which were deprived of vitamin B_6 showed marked lowering of the albumin, but an increase in the gamma globulin concentration was observed in all. Lowered albumin and increased total globulin are also evident in the chemical data. Gamma globulin values as high as 52% have been observed. Since gamma globulin has been shown to be related to liver damage(12-15) and a number of these monkeys were found to have had marked liver injury, this was not entirely unanticipated. Deficient animals which have come to autopsy and in which liver damage was evident are indicated in the last column of Table III. A few animals also exhibited a significant increase in beta globulin. Significant increases in the gamma-globulin component were also evident in the majority of animals receiving a lowered intake of the vitamin. The mobilities of the serum components do not appear to be influenced by either partial or total deprivation of vit. B_6.

Effect of cholesterol feeding. Cholesterol feeding to control monkeys results in a significant increase in the alpha-3 component (Nos. 38 and 21). One animal receiving the high protein diet (HP) also exhibited an increase in this component. Increase in the beta component of serum of the rabbit as a result of cholesterol feeding has been reported by Fishberg *et al.*(16). Two animals (Nos. 36 and 37) subjected to a low intake of pyridoxine while on a moderate fat diet containing cholesterol showed, in addition to the increase in the alpha-3 component, a lowering of the albumin and an increase in the gamma globulin.

Low choline diet. Only 2 animals (Nos. 26 and 27) were followed on this diet (AP). In comparison with the one control animal (No. 31) on this diet, both exhibited significant decreases in albumin and increases in the beta-globulin components. In addition, monkey 26 was found to have an increased concentration of the gamma component, while No. 27 also showed a significant rise in the alpha-1 plus alpha-2 components.

Low niacin-low B_{12} diet. Although 2 animals were maintained on a diet lacking in both niacin and vit. B_{12} for a period of over 21 months these monkeys did not exhibit any obvious manifestation of either deficiency. These animals (Nos. 28 and 29) are listed in Table III merely as niacin deficient. In comparison with the serum of their control animal (No. 31) the sera of the 2 monkeys maintained on this diet were observed to have a lowered albumin and an increased concentration of gamma globulin.

Ascorbic acid deficiency. No significant alterations were observed in the serum protein patterns of 4 monkeys (Nos. 59 to 62) who

TABLE IV. Electrophoretic Mobilities of Serum or Plasma Proteins of Control and Vitamin Deficient Monkeys.

Animal No.	Deficiency (D) and control (C)	Duration, mo	Diet	Serum or plasma	Mobilities (CM^2/volt sec. $\times 10^{-5}$) Albumin	$\alpha 1$	$\alpha 2$	$\alpha 3$	β	Ø	γ
Avg (18, 40, 53, 34)	C		S	S	5.29	4.67	4.27	3.66	3		1.72
51	C		HP	S	5.1	4.5	4.05	3.5	2.9		1.5
21	C		S+chol.	S	6.2	5.4	5.05	4.25	3.7		2.5
38	C		MF+chol.	S	5.3	4.4	4.2	3.8	3.4		2.2
16	D-B6	32	S	S	5.2	4.5	4.3	3.6	3.1		1.75
50	D-B6	14	S	S	4.9	4.05	3.65	2.95	—		2.6
41	a		S	S	5.5	4.65	4.2	3.6	3.15		1.4
42	b		S	S	5	—	3.9	3.1	2.6		1.05
42	c		S	S	5.7	—	4.65	4	3.5		2.1
43	d		S	S	5.2	4.6	4.1	3.6	3		1.55
22	e		S	S	5.1	4.35	3.85	3.4	3		1.35
25	f		S+chol.	S	6.2	5.4	4.85	4.3	3.8		2.15
25	g		S+chol.	S	5.1	4.3	4.05	3.4	3		1.45
37	h		MF+chol.	S	5.9	—	5.15	4.5	3.5		1.75
26	D-choline	31½	AP	S	5.5	4.75	4.4	3.9	3.5		2.06
28	D-niacin	17½	AP	S	5.4	4.6	4.4	3.7	3.35		1.9
31	C		AP	S	5.15	4.25	4.1	3.4	2.75		1.5
57	C		S	P	4.9	4.3	3.85	3.7	2.8	2	1.35
58	C		S	P	5.25	4.55	4.1	3.6	3	2.35	2
Avg (57, 58)					5.07	4.42	3.97	3.65	2.9	2.17	1.67
59	i		S	P	5.9	5.25	4.7	4	3.35	—	2.3
60	j		S	P	5.7	5.05	4.55	3.9	3.5	2.6	1.8
61	k		S	P	6.25	—	5.35	4.45	4.15	3.25	2.35
62	l		S	P	6.15	5.6	5.3	4.45	4.05	3.3	2.75
		Avg			6	5.3	4.9	4.2	3.76	3.05	2.3

a No B6; 2 mo, 10 γ/kg 15 mo
b 15¼
c 17¾
d 15¼
e 250 γ B6, 9¼ mo; 125 γ B6, 9¾ mo; 60 γ B6, 15½ mo
f 24¾ mo
g Same as f except 125 γ B6 for 25¾ mo
h No B6; 2 mo, 10 γ/kg 15 mo
i D—Ascorbic acid 2¼ mo; 1 mg 20 days
j D— 11
k D— 19
l D— 21

were investigated during the early stages of chronic scurvy, *i.e.*, 2¼ to 2¾ months of complete deficiency followed by a daily supplement of 1 mg of ascorbic acid for 11 to 21 days. There appeared to be some increase in the mobility of the various components in the scorbutic animals (Table IV). However, this requires further study.

Discussion. Numerous publications (12-15) have reported hypoalbuminemia and hyperglobulinemia (primarily of the gamma component) as the characteristic alteration of the serum proteins in chronic liver disease. Since generally one observes various degrees of liver damage ranging from mild fatty infiltration to marked cirrhosis in the vit. B_6 deficient monkey, it is not surprising that hypoalbuminemia and marked hyper-gamma-globulinemia occur. It is quite possible that the hypoalbuminemia is the result of interference with albumin synthesis, since there is considerable evidence which indicates that vit. B_6 plays an important role in metabolism of proteins and amino acids. Previous experiments on malnutrition in humans (17,18) and in animals (19) have been offered as evidence (15) that malnutrition is not responsible for the rise in gamma globulin in chronic liver disease. These observations have dealt mainly with protein underfeeding or depletion. Since vit. B_6 deficiency in the monkey is a rather slowly progressing and debilitating disease and is similar in many respects to a state of chronic malnutrition, the former could be classified as a specific type of chronic malnutrition. On this basis the present work affords evidence that this specific type of malnutrition (vit. B_6 deficiency) may bring about a marked increase in gamma globulin. Some evidence is also reported which indicates that

hypoalbuminemia and hyper-gammaglobulinemia may also occur in other vitamin deficiencies, such as niacin.

Summary. The plasma or serum proteins of rhesus monkeys maintained on various vitamin deficient diets were studied by chemical and by electrophoretic analysis. Partial or complete deficiency of vit. B_6 resulted in hypoalbuminemia and a significant increase in the gamma globulin component. On a niacin deficient diet 2 monkeys developed a lowered albumin and an increase in the gamma globulin. A low choline diet led to a decrease in the albumin and to an increase in the beta globulin. The addition of 1% cholesterol to the diet of control or vit. B_6 deficient monkeys produced a significant increase in the alpha-3 component. The electrophoretic composition of the plasma did not appear to be altered by a state of early chronic scurvy. The mobilities of the plasma components appeared to be increased by an ascorbic acid deficient diet but were unaffected by the other regimens.

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Effects of Purine and Pyrimidine Analogues on Development of *Rana pipiens* (19402)

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Analogues of the purine and pyrimidine constituents of the nucleic acids have been found to have a variety of biological effects which may be ascribed to various types and degrees of interference with the biosynthesis of nucleic acids and related compounds(1).

The desirability of testing such antimetabolites on a variety of biological species and systems has previously been mentioned(1) for such studies contribute both to the comparative biochemistry of nucleic acid metabolism and to the knowledge of the mode of action of the antimetabolites. The present report is concerned primarily with an extension of the antimetabolite studies to the problems of chemical embryology. Although the effects