Effect of Phenylalanine Deficient Diet on Bone Marrow and Amino Acid Metabolism.* (29808)

F. COCKBURN, J. D. SHERMAN, D. INGALL AND R. KLEIN
Departments of Pediatrics, Boston University School of Medicine and Boston City Hospital, Boston, Mass. and Framingham Union Hospital, Framingham, Mass.

Sherman, Greenfield and Ingall have reported the occurrence of anemia with maturation arrest and vacuolization of the erythrocyte precursors in the bone marrow of a premature infant being treated for phenylketonuria(1). These signs appeared whenever the serum phenylalanine levels dropped below the normal range, and disappeared when phenylalanine feedings returned the serum concentrations to or above normal levels. Morphologically similar bone marrow findings have been reported in chloramphenicol toxicity, acute alcoholism, and erythremic myelosis (Di Guglielmo's syndrome)(2-7).

The present study is an investigation of the effects of this phenylalanine-deficient diet in infants without phenylketonuria and with no evidence of any metabolic abnormality. Specifically, an attempt was made to correlate changes in amino acid metabolism with bone marrow changes. Previous reports of normal infants receiving diets deficient in phenylalanine make no mention of these bone marrow vacuolizations or anemia(8). There is one report of a megaloblastic type of erythroid maturation in a child with phenylketonuria on a phenylalanine-deficient diet, but bone marrow vacuolizations in the erythroid series were not reported(9).

Methods. Subjects. Six healthy premature infants between 12 and 39 days old (FO, SP, JO, HA, HY, HU) were studied while they were in the hospital waiting to attain routine discharge weight. Their weights at the time of the studies were between 1.6 and 2.4 kg. One 2.3 kg newborn infant (RU) with an extensive myelomeningocele was also studied, as were 3 infants, 2 full term newborns weighing 3.2 kg (SU) and 4.5 kg (FR), and the third, a 4-month-old infant weighing 7.5 kg (OB). These 3 subjects were in the hospital awaiting placement in foster homes.

Diets. Diet I was a proprietary cow's milk preparation modified by the manufacturer to simulate human milk.† It was reconstituted to contain 1.5% protein, 7.0% carbohydrate, and 3.7% fat. It contained adequate supplements of vitamins and minerals.

Diet III was a proprietary casein hydrolysate preparation from which the phenylalanine had been removed and to which certain amino acids had been added.‡ Supplements of vitamins and minerals had been added as they had to Diet I. Fat and carbohydrate had been added so that the fat content of the phenylalanine-deficient feeding mixture was 2.7%, carbohydrate 8.5%, and nitrogen equivalent to protein 2.25%. The manufacturer's analysis records that each quart of the feeding mixture contains:

Vit A	1500 U.S.P. units
" D	400 " "
" E	5 international units
" C	30 mg
Thiamine HCl	0.46 "
Folic acid	0.05 "
Biotin	0.03 "
Riboflavin	1.8 mg
Niacinamide	4.0 "
Pyridoxine HCl	0.5 "
Calcium pantothenate	3.2 "
Vit B_{12}	4.5 μg
Choline Cl	150.0 mg
Ferrous sulfate	15.0 "

Diet II was the same proprietary phenylalanine-deficient diet complemented by L-phenylalanine added in amounts of 100 mg per kg of body weight per day.

Design of dietary sequences. Eight of the first 9 studies involved 48-hour periods of ingestion of the deficient diet. In the ninth,

* These observations were made on patients in the Pediatric Clinical Research Center, Boston City Hospital, Boston, Mass. This Center is supported by Grant FR-103 from Nat. Inst. Health. These studies were in part supported by grants from Boston Medical Foundation and H. P. Hood Foundation. Dr. Cockburn's fellowship was supported by a grant from Huntington Hartford Foundation.

† Enfamil®, Mead Johnson Co., Evansville, Ind.

‡ Lofenalac®, kindly supplied by Mead Johnson Co., Evansville, Ind.

FR ingested the deficient diet for 96 hours. Three infants (OB, SP, FO) were studied in the sequence of control Diet I, control Diet II, deficient Diet III, and then Diet II. Two of these infants (SP_2, FO_2) as well as 4 others (HA, HY, JO, FR) were given the deficient diet immediately after Diet I.

Studies were carried out with SU, RU, and FR as subjects in which the infants ingested Diet II for 6 to 14 days and then Diet III for 4 to 5 days.

In various ancillary studies, supplements of other amino acids were added to the daily diets as follow:

200 mg of L-methionine to Diet I for 3 days (RU);

150 mg of L-valine and 1.0 g of L-valine to Diet II for 2 days each (FO, JO);

150 mg of L-alanine and 1.0 g of L-alanine to Diet II for 2 days each (SP, HU);

1.0 g of L-alanine to Diet III for 4 days and for 2 days (HU, JO);

0.5 g of L-valine to Diet III for 2 days (JO).

Studies were also carried out in HY when he ingested only 10% glucose in water for 24 hours.

The feeding mixtures were offered to the infants 4 to 8 times a day in *ad lib* volumes. The volume of intake was estimated from the weights of the bottles before and after a feeding. During each study, the infants received no other nutritive materials.

Clinical and laboratory procedures. Infants were weighed at the beginning of each 24-hour period. Urinary collections were made with constant urinary drainage, using external devices. Venous blood specimens for serum amino acid measurements and bone marrow material for examination were obtained at the end of diet periods. The last few amino acid studies, as noted below, utilized plasma from heparinized venous blood. Bone marrow was aspirated from the iliac crests and the tibias. Bone marrow specimens were examined by J.D.S. without his being aware of the patient's clinical or experimental state. All bone marrow specimens were stained with Wright-Giemsa stain and some also with Prussian Blue.

Microhematocrit and reticulocyte counts were performed by standard methods every 2 days during the period of study. Free amino acids in serum or plasma were measured by the Technicon Amino Acid Analyzer adaptation of the technique of Moore and Stein(10). Measurements were made of proline, glycine, alanine, valine, methionine, isoleucine, leucine, tyrosine, phenylalanine, lysine, histidine, and arginine. Total urinary free amino acids excreted by FO, FR, SU, RU, HU were determined by ion exchange on Dowex-50 columns using the ninhydrin end point of Moore and Stein, and the individual amino acids excreted by all patients (except JO) were identified by 2 dimensional paper chromatography using the butanol-acetic acid-water system and the phenol-ammonia-water system(11-13). Estimates of change in individual amino acid excretion were made from the area and intensity of color of the spots on one dimensional paper chromatography. Urinary creatinines were measured, and all aliquots applied to paper represented a volume containing 4 μg of creatinine(14).

Results. Concentrations of individual amino acids in the diets are recorded in Table I. Infants took the diets well. Average intake was 245 ml per kg per day, and no significant difference was noticed among the various diets. There were no changes in weight, hematocrit, or reticulocyte count related to dietary changes. All the infants thrived save for the child with the myelomeningocele whose study had to be interrupted when meningitis due to *P. aeruginosa* supervened. Mild diarrhea was frequently noted when the children received either Diet II or III, both of which contained the casein hydrolysate preparation.

The serum amino acid concentrations for the first 9 studies are shown in Table II. The weight and age of the infants are also indicated. It may be seen that the initial ingestion of the casein hydrolysate mixture in either Diet II or Diet III was accompanied by an increased serum concentration of the measured amino acids except for phenylalanine, of course, and tyrosine which varied unpredictably. Urinary amino acid excretion increased within 8 hours of beginning these diets and continued throughout the period, paralleling the increase in serum concentrations. The increase in total amino acid excretion was accounted for largely by obvious increases

TABLE I. Concentrations of Individual Amino Acids in Diets I and III. (Modified cow's milk feeding mixture and phenylalanine-deficient casein hydrolysate feeding mixture.)

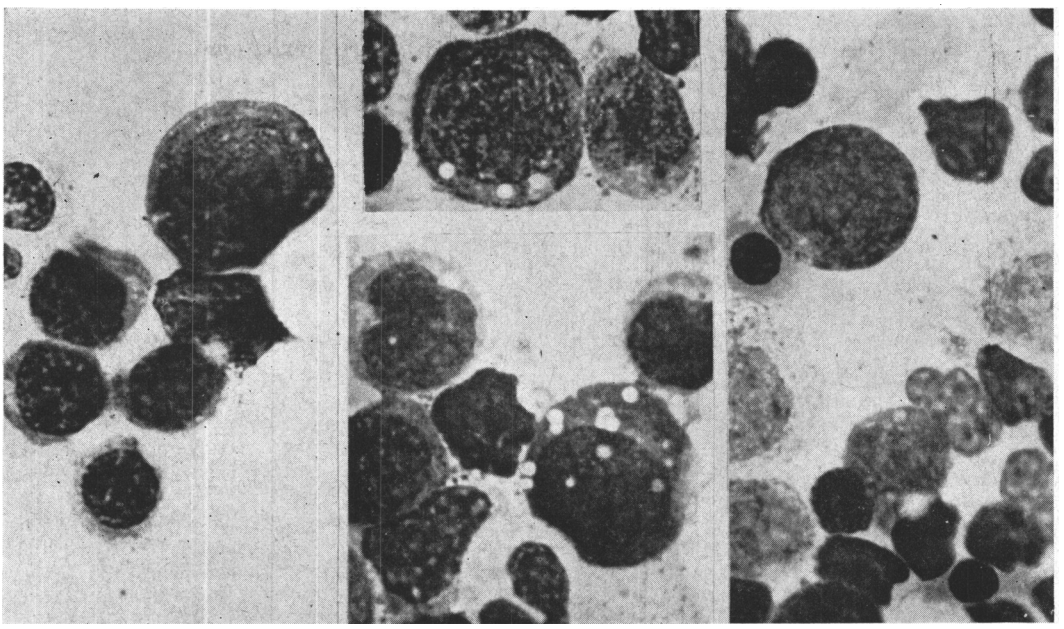
Amino acid	Diet I Modified cow's milk* (mg/100 ml formula)	Diet III Phenylalanine deficient† (mg/100 ml formula)
Glutamic acid	202	280
Proline	89	98
Glycine	20	33
Alanine	43	105
Valine	38	179
Methionine	16	71
Isoleucine	26	99
Leucine	87	277
Tyrosine	50‡	110
Phenylalanine	36	10
Lysine	67	415
Histidine	22	51
Threonine (Manufacturer's analyses)	78	132
Tryptophane (Manufacturer's analyses)	15	32

* Enfamil®.
† Lofenalac®.
‡ Acid hydrolysis of Diet I resulted in marked decomposition of tyrosine and the value given was extrapolated to zero time. No correction has been made for decomposition of glutamic acid and proline(15).

in urinary valine, alanine, glycine, methionine, and histidine. No further increase in either serum or urinary amino acids was seen in these studies when the patient went from Diet II to Diet III. Indeed, there were some decreases in serum concentrations.

At the end of the period of dietary deficiency, serum phenylalanine concentrations had fallen significantly in each patient. Final values were less in the smaller children (4-9 μmols/l *vs.* 18 and 22 μmols/l). Phenylalanine concentrations after 48 hours of Diet III were slightly lower in SP and RO when Diet III was preceded by Diet I (6 and 4 micromoles per liter) than when Diet III was preceded by Diet II (9 and 8 micromoles per liter).

Two days on Diet III (phenylalanine-deficient) were sufficient to produce significant bone marrow erythroid vacuolizations in the 5 premature subjects when this diet was preceded by Diet I (modified cow's milk). The number of erythroid precursors with vacuolizations and the number of vacuoles in each immature red cell varied from subject to subject, with the bone marrows of SP_2 and FO_2 being the least involved. The Wright-Giemsa stained bone marrow specimens revealed similar patterns regardless of the site from which the marrow was aspirated. In general, the cellularity of the specimens was considered normal throughout the period of study. The most striking findings were the presence of vacuolizations in the erythroid elements, most numerous in the erythrogones and normoblasts A and less numerous in normoblasts B. The vacuoles were cytoplasmic, round, and of variable size and number in a given cell. They were never seen in the more mature erythroid precursors (normoblasts B) when absent in the erythrogones and normoblasts A. The maturation of the erythroid series was always normoblastic; megaloblasts were not seen, and there was no evidence of a maturation arrest. Maturation of the myeloid series was also normal without maturation arrest, and the megakaryocytes were present in normal numbers and appeared to be making platelets. Myeloblasts, promyelocytes, myelocytes, and metamyelocytes also contained cytoplasmic vacuoles. These vacuoles were round and of variable size within a cell. In general, they were slightly larger and less numerous in any given cell than were the vacuoles in the erythroid precursors. The largest vacuoles were noted in the eosinophilic myelocytes and metamyelocytes. Vacuoles were not found in the megakaryocytes, reticuloendothelial cells, and lymphocytes. (The Prussian Blue stains for iron satisfactorily demonstrated the presence of this element.) Fig. 1 depicts the typical cytoplasmic ery-



A B C

FIG. 1. Representative bone marrow specimens obtained from 2 premature infants. A. Photomicrograph of normal bone marrow obtained from infant at the end of 2-day period of modified milk diet, Diet I. B. Two photomicrographs of bone marrows obtained at the end of 2-day period of phenylalanine diet, Diet III. Typical cytoplasmic vacuoles are seen in both erythroid and myeloid precursors. Upper picture is from baby whose marrow is also seen in A and C. C. Photomicrograph of marrow after 2 more days of the deficient diet plus 100 mg/kg/day of phenylalanine, Diet II. All abnormalities have disappeared.

throid and myeloid vacuolizations produced by feeding Diet III for 2 days after the infant had been receiving Diet I.

Erythroid vacuolization was not seen when FO and SP received Diet II for 2 to 4 days and then Diet III for 2 days. Nor were erythroid vacuoles seen in the bone marrow of the 2 full term infants FR and SU and the older child OB after they had ingested the deficient diet for 2 to 4 days in various experiments. Definite vacuoles morphologically indistinguishable from those in Fig. 1 did appear in the cytoplasm of the myeloblasts, myelocytes, and metamyelocytes under these experimental conditions in FO, SP, OB, and FR.

Bone marrow and blood examinations were also carried out after only 24 hours on Diet III following Diet I in infant HA. The findings were the same as seen in all 5 premature infants after 48 hours of deficiency although they were less marked. Two days of Diet II following the deficient diet sufficed to revert the bone marrow to normal morphologic appearance in all cases although the serum phenylalanine concentrations had not fully returned to control values in each instance.

Three infants (SU, RU, FR) were fed Diet II for 6 to 14 days and then Diet III for 4 to 5 days in order to show more clearly the effect of the ingested load of amino acids upon serum and urinary concentrations and differentiate it from the dietary deficient of phenylalanine *per se*. Serum and urine concentrations of amino acids for these 3 infants are given in Table III. The increases in amino acid concentrations in serum and urine which were found in all infants receiving Diet II occurred in these studies. When the diets fed these infants were changed from Diet II to Diet III, no significant effect on amino acid concentrations of the phenylalanine deficiency was demonstrable. Vacuoles in bone marrow red cell progenitors of RU were seen after 5 days of Diet III but not after 2 days in this sequence. SU developed no marrow changes, and FR developed only vacuoles in the myeloid cells.

II. Effect of 48 Hour Dietary Periods on Morphologic Appearance of Bone Marrow and Concentration of Amino Acids in = modified cow's milk feeding mixture. Diet II = Diet III plus 100 mg per kg per day of L-phenylalanine. Diet III = phen casein hydrolysate feeding mixture.

ect	Wt, kg	Day	Diet	No. of vacuoles in bone marrow		Amino acids, micromoles/L									
				Erythroid series	Myeloid series	Proline	Glycine	Alanine	Valine	Methionine	Isoleucine	Leucine	Tyrosine	Phenyl-alanine	Lysine
	1.6	2	I	0	0	211	270	435	191	29	55	145	105	55	144
ys	1.7	4	III	++++	++++	278	306	786	347	294	63	177	38	6	365
	1.7	8	I	0	0	256	246	615	219	16	71	131	109	44	114
	2.3	2	I	0	0	130	243	261	163	27		121	161	51	129
ys	2.4	4	III	++++	++++	295	219	482	484	207	99	76	106	6	533
	2.4	6	II	0	0	248	278	646	339	105	54	127	75	27	301
‡	1.7	2	I	0	0	263	171	426	199	27	53	118	194	54	143
ys	1.6	4	III	++	++	188	269	390	215	86	47	129	95		297
	1.6	6	I	0	0		324	365	134	41	51	57	315	57	196
	2.3	2	I	0	0	169	159	297	169	25	49		73	38	84
ys	2.4	4	III	+	+	287	186	530	417	224	87		91	4	366
	2.1	2	I	0	0	140	163	228	137	27	33	91	80	41	91
ys	2.2	4	III	+	+	294	174	439	391	190	77	255	97	6	339
*	1.7	2	I	0	0	151	187	296	167	32	40	93	103	34	122
ys	1.8	6	II	0	0	212	224	553	371	144	49	156	30	35	310
	1.9	8	III	0	++	221	177	433	303	122	51	168	36	8	289
	1.9	10	II	0	+	177	173	528	256	84	27	94	37	25	89
†	1.8	2	I	0	0	178	183	296	201	30	47	120	117	41	124
ys	1.8	4	II	0	0	308	293	657	432	253	68	220	51	46	309
	1.9	6	III	0	++	239	200	458	314	204	47	146	27	9	234
	1.8	8	II	0	+	275	233	498	250	100	24	95	33	25	129
	4.5	2	I	0	0		216	201	154		50	94	65	38	153
ys	4.5	6	III	0	++		396	584	508	116	78	253	90	22	553
	4.6	8	I	0	0		361	567	339	85	85	237	50	72	538
	7.6	2	I	0	0	173	163	410	158	18	41	99	47	53	94
os	7.5	4	II	0	0	404	106	443	440	42	102	207	104	92	185
	7.5	6	III	0	++	265	218	583	367	55	55	144	47	18	224
	7.5	8	II	0	+	190	226	480	309	44	55	136	43	52	137

Diet	Proline	Glycine	Alanine	Valine	Methionine	Isoleucine	Leucine	Tyrosine	Phenyl-alanine	Lysine	H
I	178 ± 13.3	196 ± 11.2	317 ± 28.5	171 ± 7.2	27 ± 1.11	46 ± 2.6	110 ± 7.0	99 ± 12.1	45 ± 2.7	121 ± 7.4	7
II	308	208	551	414	146	73	194	62	58	268	8
III	258 ± 14.7	238 ± 27.2	520 ± 41.2	332 ± 34.2	166 ± 25.8	67 ± 6.4	169 ± 20.9	70 ± 12.4	10 ± 2.2	356 ± 38.7	9
I-III	<.01	NS	<.01	<.01	<.01	<.01	<.02	NS	<.01	<.01	

) mg per day L-alanine added to Diet II in last period.
mg per day L-valine added to Diet II in last period.

‡ Plasma utilized for amino acid determinations.

TABLE III. Concentrations of Amino Acids in Plasma During Prolonged Periods of Ingestion of the Deficient Diet Adequately Complemented with Phenylalanine and of Subsequent Ingestion of Deficient Diet Alone. Urinary excretions of total free amino acids are also recorded.

Subject	Weight, kg	Day	Diet	Amino acids, micromoles/L												Urine free amino acids millimoles/100 mg cnne
				Proline	Glycine	Alanine	Valine	Methionine	Isoleucine	Leucine	Tyrosine	Phenylalanine	Lysine	Histidine	Arginine	
RU	2.3	2	I													2.6
6 days		4	I													2.9
		5	I													2.8
		7	II	68	139	237	136	42	17	64	18	32	65	49	42	3.4
	2.3	9	II	164	286	298	217	67	34	109	41	31	224	52	60	4.3
		11	II													4.7
		12	III													6.2
		13	III													5.0
	2.6	16	III	170	92	215	188	79	25	77	87	25	152	55	57	
	2.3	20*	II	172	134	322	165	51	17	64	23	29	102	36	90	
SU	3.2	1	I		306	530	274	29	73	149	100	77	259	114		
12 days		2	I													3.9
		3	II													3.9
		4	II		252	424	444	101	79	194	73	20	524	99		4.5
		5	II													5.7
		6	II													6.9
	3.4	7	II	362	437	674	438	79	63	135	79	52	410	147		7.0
		8	III													
		9	III													6.4
		10	III													5.3
	3.5	11	III	307	303	721	507	90	78	235	81	15	408	105		5.5
		12	I													2.7
		13	I													1.4
FR		-8	I		216	201	154		50	94	65	38	153	66		1.3
2 days	4.6	3	I													1.9
		4	II													2.7
		5	II													2.4
		6	II													3.7
	4.9	7	II													4.3
		8	II		432	528	490	90	81	204	104	66	504	121	146	1.9
		9	II													2.9
		10	II		404	406	356	66	78	170	73	67	354	120	76	2.7
	5.0	11	II													1.5
		12	II													4.7
		13	II													3.5
		14	II													6.3
		15	II													9.1
	5.1	16	II		350	400	246	47	51	119	69	87	230	103		6.2
		17	III													3.8
		18	III													5.4
		19	III													4.8
	5.1	20	III		396	500	432	93	39	114	55	8	188	108		5.9
		21	I													4.5
		22	I													4.9
		23	I													3.2
	5.3	24	I													2.9

* Meningitis diagnosed.

Three infants (SP, HU, JO) were given supplemental amounts of 150 mg, 1 g, and 1 g of alanine per day respectively and two infants (FO, JO) 150 mg and 0.5 g of valine per day during control periods of Diets I or II and during periods on the deficient diet to determine whether there was any toxic effect on bone marrow. No vacuoles or other toxic effects of the amino acids were demonstrable nor were any seen in RU's bone marrow when

L-methionine, 200 mg per day, was added for three days to Diet I. Serum levels of the administered amino acid were increased after these supplements.

No significant changes in bone marrow specimens or amino acid concentrations in infant HY were demonstrated following a 24-hour period in which he ingested only carbohydrate and water.

Discussion. We were struck by the rapidity with which the premature infants showed evidence of phenylalanine deficiency with depression of serum phenylalanine concentration and with the rapid development of vacuoles in the cytoplasm of the red cell precursors in the bone marrow. The bone marrow lesions were as rapidly reversed when phenylalanine was added to the diet.

In none of these infants have anemia, change in circulating reticulocytes, or untoward symptoms been seen in the short periods in which they received the deficient diet nor in an observation period of several months thereafter.

The severity of the phenylalanine deficiency may have been accentuated by the increased intake of other amino acids in the deficient diet. A load effect was clearly demonstrated with marked increases of serum concentrations of methionine, valine, alanine, and lysine and lesser increases of other amino acids when infants ingested either Diet II or III. This was accompanied by increased urinary excretion of these amino acids. The effect of the increased intake of amino acids was sufficient to obscure any possible effect of phenylalanine deficiency *per se* on amino acid concentrations. Supplemental ingestion of methionine, alanine, and valine produced no apparent bone marrow or hematologic effects. Therefore, we do not believe that the bone marrow changes noted can reasonably be attributed to other than phenylalanine deficiency.

Premature infants RU, FO, and SP did not develop cytoplasmic vacuoles in cells of the erythroid series after 48 hours of the deficient diet when the preceding control diet had been Diet II. RU continued to be fed Diet III for another 3 days and then had erythroid vacuoles in his bone marrow. This inhibition of vacuole formation may have been the result of greater phenylalanine intake with Diet II than with Diet I (125 mg per kg per day *vs.* 90 mg per kg per day). If this were so, it would suggest that cow's milk modified as in Diet I contains less than optimal quantities of phenylalanine for the needs of premature infants. On the other hand, the greater tyrosine content of Diet II might have made the infants more resistant to phenylalanine deficiency since tyrosine can replace about 75% of the daily requirements for phenylalanine of the older individual. The age of these premature infants prevents acceptance of this speculation although the increased tyrosine intake might have been important in the resistance of the full term and older infants to the effects of the phenylalanine-deficient diet.

All subjects except SU developed vacuoles in their white cell precursors after 48 hours of the deficient diet. The presence of myeloid vacuoles in the absence of erythroid vacuoles may reflect the more rapid rate of turnover of white cells. On the other hand, vacuolization of mature granulocytes is seen in a variety of bacterial infections(16). Some support for the hypothesis that longer periods of deficiency are required for development of erythroid vacuoles is derived from the observation that erythroid vacuoles never were seen in these studies in the absence of myeloid vacuoles.

The bone marrow changes may be reversed before the serum phenylalanine concentrations are fully returned to control values when the infants are given phenylalanine supplements. The bone marrow changes were identical with those previously reported in the premature infant with phenylketonuria in association with phenylalanine deficiency. We have since seen the same lesions under the same circumstance in a full term infant with phenylketonuria. They are also morphologically identical with those associated with chloramphenicol administration, erythremic myelosis, and acute alcoholism. The relationship between dietary phenylalanine and prevention of bone marrow changes produced by chloramphenicol will be published elsewhere(17).

Although the concentrations of amino acids tended to be greater when serum rather than plasma was used, the changes noted were comparable. This was not unexpected since it has previously been reported that the con-

centration of total amino acids is greater in serum than in the comparable plasma(18). This same relationship has been found to hold for individual amino acids(19).

Summary. Normal premature infants fed a diet deficient in phenylalanine developed changes in their bone marrow within 24 to 48 hours. These marrow changes consisted specifically in cytoplasmic vacuolization of erythroid precursors. Vacuoles also occurred in the myeloid elements. The vacuoles of the red cell precursors were associated with marked depression of serum phenylalanine concentrations to levels of 4 and 6 micromoles per liter. These effects were reversible within 48 hours by the addition of 100 mg per kg of phenylalanine to the diet. Any possible effects of the phenylalanine deficiency on other amino acids was obscured by the increased serum and urine concentrations of these amino acids resulting from increased intake provided by the proprietary casein hydrolysate preparation. Addition of valine, alanine, and methionine to the normal and deficient diets did not produce marrow changes or other toxic effects.

The authors gratefully acknowledge their indebtedness to Miss Meta Bohanan, R.N., and her staff for help in carrying out the observations on the patients and to Drs. H. P. Baker, R. Feigin, F. K. Kennedy, D. Palant, N. Pleva and P. Sexton of the resident staff for major contributions to the care and study of the patients.

1. Sherman, J. D., Greenfield, J. B., Ingall, D., New Eng. J. Med., 1964, v270, 810.

2. McCurdy, P. R., J.A.M.A., 1961, v176, 588.

3. Rosenbach, L. M., Caviles, A. P., Mitus, W J., New Eng. J. Med., 1960, v263, 724.

4. Saidi, P., Wallerstein, R. O., Clin. Research, 1960, v8, 131.

5. Saidi, P., Wallerstein, R. O., Aggeler, P. M., J. Lab. & Clin. Med., 1961, v57, 247.

6. McCurdy, P. R., Pierce, L. E., Rath, C. E., New Eng. J. Med., 1962, v266, 505.

7. Di Guglielmo, L., Le malattie eritremiche ed eritroleucemiche, Rome, Il pensiero scientifico editore, 1962, 110.

8. Holt, L. E., Jr., 30th Ross Pediatric Research Conference, Ross Laboratories, Columbus, Ohio, 1958, 11.

9. Toyston, N. J. W., Parry, T. E., Arch. Dis. Child., 1962, v37, 430.

10. Spackman, D. H., Stein, W. H., Moore, S., Anal. Chem., 1958, v30, 1190.

11. Moore, S., Stein, W. H., J. Biol. Chem., 1954, v211, 907.

12. Smith, I., Chromatographic and Electrophoretic Techniques, William Heineman, London, 1960, v1, 83.

13. Morrison, R. I., Biochem. J., 1953, v53, 474.

14. Peters, J. H., J. Biol. Chem., 1942, v146, 179.

15. Hirs, C. H. W., Stein, W. H., Moore, S, ibid., 1954, v211, 941.

16. Ponder, E., Ponder, R. V. O., J. Lab. and Clin. Med., 1942, v28, 316.

17. Ingall, D., Sherman, J. D., Cockburn, F., Klein, R., 1964, unpublished.

18. MacFayden, D. A., J. Biol. Chem., 1942, v145, 387.

19. Stein, W. H., Moore, S., ibid., 1954, v211, 921.

Received September 2, 1964. P.S.E.B.M., 1965, v118.

Rapidly Labeled Rat Liver Nuclear RNA Particles.* (29809)

KENNETH S. MCCARTY, JOHN LASZLO† AND JOHN R. OVERMAN

Departments of Biochemistry and of Medicine, Duke University Medical Center, Durham, N. C.

The mechanism of nuclear RNA synthesis in regenerating rat liver involves the production of a rapidly labeled RNA copy of DNA. Initial investigations from this laboratory indicate that pulse labeled nuclear RNA could be isolated, purified and sedimented as a clear pellet in the presence of Mg^{++} ions. This pellet contained 60-100% of the radioactivity. Experiments concerning the nature of this pellet comprise the basis of this report.

Materials and methods. 1. Preparation of rat liver nuclei: Wistar rats, 150-250 g, were

* This research was supported in part by USPHS Grants GM-06975-03, HD-0068-06, 2 G-516, Nat. Science Foundation G-13194 and Am. Cancer Soc. Grant A.C.P363.

† Supported in part by USPHS Student Training Grant CSP-8520-C2.