that increased weight gain in hypophysectomized animals was achieved despite a significantly smaller quantity of food ingested. When weight gains are corrected for amount of food ingested, increases in weight of the handled over unhandled group are greatly magnified. In the normal rat, handling has no influence on amount of food ingested(6). The findings indicate that handling increases efficiency of food utilization (increased intestinal absorption and/or diminished metabolism) and that this effect is not dependent upon presence of pituitary or adrenal hormones.

It is apparent that increased skeletal growth previously reported in the handled normal young rat(6), is abolished by hypophysectomy. This suggests that the effect of handling on skeletal growth requires pituitary hormones, presumably growth hormone and/or thyrotropic hormone.

It is pertinent to consider the contribution of handling to weight gains previously reported in hypophysectomized rats treated with insulin(13) or force-fed(14). Since these studies involved extra handling of experimental animals as compared with control animals, it is conceivable that weight gains observed were due in part to extra handling.

Summary. Handling in young hypophysectomized rats resulted in significantly increased weight gain despite ingestion of significantly less food. No effect on skeletal growth or percentage of carcass protein and fat was observed. Increased weight gain in the handled hypophysectomized rat is due to greater efficiency of intestinal absorption of food and/or diminished metabolism rather than a direct stimulation of growth or increased food ingestion.

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1. Bernstein, L., *Psychol. Bull.,* 1952, v49, 38.
2. ———, *J. Comp. Physiol. Psychol.,* 1957, v50, 162.
3. Weininger, O., *Science,* 1954, v119, 285.
4. ———, *J. Comp. Physiol. Psychol.,* 1956, v49, 1.
5. Weininger, O., McClelland, W. J., Arima, K., *Canad. J. Psychol.,* 1954, v8, 147.
6. Ruegamer, W. R., Bernstein, L., Benjamin, J. D., *Science,* 1954, v120, 184.
7. Ruegamer, W. R., Silverman, F. R., PROC. SOC. EXP. BIOL. AND MED., 1956, v92, 170.
8. Brooker, H., unpublished M.A. thesis, Univ. of Toronto, 1955.
9. McClelland, W. J., *ibid.,*
10. Bernstein, L., Elrick, H., *Metabolism,* 1957, v6, 479.
11. Greenspan, S. F., Li, C. H., Simpson, M. E., Evans, Herbert M., *Endocrinology,* 1949, v45, 455.
12. Edwards, A. L., Rinehart and Co., New York, 1950.
13. Salter, J., Best, C. H., *Brit. M. J.,* 1953, v2, 353.
14. Levin, L., *Am. J. Physiol.,* 1944, v141, 143.

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Pyridoxal Phosphate (B_6-al-PO_4) Levels in Organs, Leukocytes and Blood of Rats with Developing Vit. B_6 Deficiency.* (23918)

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Recently a method has been described for determination of pyridoxal phosphate (B_6-al-PO_4), the coenzymatically available active form of Vit. B_6, in whole blood and isolated leukocytes(1). Using this technic we estimated the B_6-al-PO_4 content in the only readily accessible prototype tissue, *i.e.*, leukocytes in blood of healthy, nonpregnant controls, mothers at term, and in cord blood of their babies(2). It was found that the pyridoxal phosphate levels of circulating leukocytes were significantly lower in pregnant women than in nonpregnant controls. Since cord blood showed the highest levels, it was concluded that the growing fetus can successfully compete with the mother for this impor-

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TABLE I. Pyridoxal Phosphate in Organs and Blood of 15 Control Rats Fed a Complete Synthetic Diet *Ad Lib.*

Organ wt			Concentration							
			Heart, γ/g		Liver, γ/g		Kidney, γ/g		Muscle, γ/g	
Heart	Liver	Kidney	Mean	Range	Mean	Range	Mean	Range	Mean	Range
.60	7.28	1.49	.82 ± .09	.56–1.35	.86 ± .10	.65–1.80	.93 ± .06	.56–1.70	1.15 ± .21	.72–2.1

Total content			Blood					
			Leucocytes, mγ/mill.		Plasma, mγ/cc		Whole blood, mγ/cc	
Heart, γ	Liver, γ	Kidney, γ	Mean	Range	Mean	Range	Mean	Range
.49 ± .03	6.74 ± .48	1.14 ± .12	1.34 ± .13	.76–2.78	116.4 ± 24.14	84.0–245.4	122.6 ± 19.2	73.0–232.5

tant vitamin and that during pregnancy there exists a relative B_6 deficiency. To substantiate this conclusion it was deemed desirable to investigate whether the pyridoxal phosphate content of circulating leukocytes indeed reflects the Vit. B_6 stores of the mammalian body. Therefore pyridoxal phosphate levels were determined in various tissues as well as in leukocytes, plasma and total blood in young rats on a synthetic Vit. B_6 deficient diet and simultaneously in control rats that had been kept on the same synthetic diet with added Vit. B_6.

Material and method. Young male rats of Wistar strain, weighing about 120 g were placed on a synthetic diet obtained from Nutritional Biochemicals Corp., Cleveland, O., which contained 18% casein supplemented with all essential vitamins with the exception of Vit. B_6. Control animals received the same formula to which had been added 2.2 mg pyridoxin hydrochloride/100 g of diet. Groups of rats on the deficient and supplemented diet were sacrificed after 1, 2, 3, 4, 5 and 6 weeks. A separate group of deficient and control animals were pair-fed and killed after 3 weeks. At sacrifice, weight of the animals was recorded. Heart, kidneys, liver and gluteal muscles were excised, blotted on filter paper to remove excess blood, and the organs weighed and frozen, if not used immediately. Pyridoxal phosphate was determined in total blood, plasma and leukocytes after blood had been collected in siliconed test tubes containing a small amount of disodium versenate. Leukocytes were isolated by a variation of the method of Lie and Osgood(3) using freeze dried hemagglutinin prepared from navy beans. Tissues were homogenized in a bacterial grinder, and subjected to alkaline hydrolysis and analysed at once. Estimation of pyridoxal phosphate was performed according to the method of Boxer *et al.*(1), based on the coenzyme function of B_6-al-PO_4 in a tyrosine decarboxylase system from *Streptococcus faecalis*(4). All analyses were made in duplicate.

Results. In animals on a complete diet the concentration of pyridoxal phosphate is of comparable magnitude in heart, liver, kidney and muscle (Table I). It averages 0.82 (± 0.09) γ/g for heart, 0.86 (± 0.10) γ/g for liver, 0.93 (± 0.06) γ/g for kidney and 1.15 (± 0.21) γ/g for muscle. Pyridoxal phosphate in leukocytes averages 1.34 (± 0.13) mγ million cells, in plasma 116.4 (± 24.1) mγ/cc and in whole blood 122.6 (± 19.2) mγ/cc.

The effect of a nutritional B_6 deficiency on body weight and the relative weight of heart, kidney and muscle is clearly evident (Tables II and III). There was a lack of gain in body weight in the deficient animals, accom-

TABLE II. Total Weight Gained and Organ Weight/100 g Body Weight in B_6 Deficient and Control Rats Fed Synthetic Complete Diet *Ad Lib.*

Group	Wk on diet	No. of animals	Wt (g) gained	% body wt		
				Heart	Liver	Kidney
Control	5–6	4	98	.28 ± .03	3.54 ± .21	.63 ± .09
Deficient	"	7	35	.34 ± .02	4.19 ± .34	.80 ± .08
Significance of diff.				<.01	<.01	<.01

TABLE III. Pyridoxal Phosphate in Organs and Blood of Vit. B_6 Deficient and Control Rats Fed Synthetic Complete Diet *Ad Lib.*

Group	Wk on diet	No. of animals	Wt (g) gain	Organ wt (g)			Concentration (γ/g)								Total content (γ)		
							Heart		Liver		Kidney		Muscle				
				Heart	Liver	Kidney	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Heart	Liver	Kidney
C*	1–2	6	33	.43	5.26	.88	.98	.90–1.10	1.27	1.00–1.80	1.20	.96–1.70	1.59	.88–2.83	.43	6.71	1.06
D	"	10	7	.38	3.87	.84	.61	.56– .80	.57	.26– .82	.44	.33– .55	.63	.32– .95	.23	2.21	.37
C	3–4	5	69	.68	7.31	1.44	1.00	.61–1.35	1.02	.75–1.73	1.00	.66–1.60	1.18	.86–1.56	.68	7.45	1.44
D	"	8	20	.53	6.75	1.24	.23	.21– .51	.26	.12– .38	.26	.15– .38	.27	.14– .37	.12	1.76	.30
C	5–6	4	98	.73	9.28	1.56	.62	.56– .70	.67	.65– .68	.63	.56– .68	.71	.67– .76	.46	6.22	.98
D	"	7	35	.68	8.20	1.56	.15	.07– .19	.14	.06– .18	.11	.01– .19	.14	.06– .18	.11	1.15	.17

Group	Wk on diet	No. of animals	Wt (g) gain	Leucocytes, mγ/mill.		Plasma, mγ/cc		Whole blood, mγ/cc	
				Mean	Range	Mean	Range	Mean	Range
C	1–2	6	33	1.39	.76–2.78	126.2	96.7–245.4	121.1	86.4–232.5
D	"	10	7	.60	.28– .85	31.8	7.5– 72.9	35.2	6.8– 72.0
C	3–4	5	69	1.42	1.17–1.56	99.3	84.0–108.9	127.9	73.0–175.2
D	"	8	20	.39	.15– .55	29.6	8.3– 42.0	22.8	7.2– 35.8
C	5–6	4	98	1.18	.87–1.42	122.4	103.5–137.5	118.2	90.0–157.5
D	"	7	35	.25	.11– .48	20.7	15.0– 24.1	16.5	~3.7– 28.8

* C = Control; D = Deficient.

TABLE IV. Pyridoxal Phosphate in Organs and Blood of Vit. B_6 Deficient and Pair Fed Control Rats Given Synthetic Complete Diet.

Group	Wk on diet	No. of animals	Wt (g) gain	Organ wt (g)			Concentration (γ/g)								Total content (γ)		
							Heart		Liver		Kidney		Muscle				
				Heart	Liver	Kidney	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Heart	Liver	Kidney
C*	3	4	76	.60	8.42	1.38	.81	.78–.88	.76	.64–.90	.76	.68–.90	1.12	1.04–1.20	.49	6.40	1.05
D	3	4	31	.63	10.06	1.62	.19	.14–.25	.10	.08–.12	.10	.08–.12	.29	.22– .48	.12	1.01	.16

Group	Wk on diet	No. of animals	Wt (g) gain	Leucocytes, mγ/mill.		Plasma, mγ/cc		Whole blood, mγ/cc	
				Mean	Range	Mean	Range	Mean	Range
C	3	4	76	1.25	.92–1.54	81.2	72.0–97.5	75.3	61.0–95.0
D	3	4	31	.21	.11– .35	31.9	24.0–40.5	16.4	11.4–21.3

* C = Control; D = Deficient.

panied by a paradoxical increase in wet weight of heart, liver and kidneys(5,6). A prompt decrease in concentration of B_6-al-PO_4, occurred in all tissues and in leukocytes and blood within 1 to 2 weeks of the developing B_6 deficiency (Table III). In this interval concentration of pyridoxal phosphate decreased to about 70% in heart, to about 45% in liver and muscle and to 30% in kidney. Total vitamin content of heart decreased to about 50%, of liver and kidney to about 35%. Concentration of B_6-al-PO_4 in leukocytes dropped to 40% and in plasma and whole blood to about 30% of control values. A marked further decrease of coenzymatically active form of Vit. B_6 occurred in the following depletion period. After 5 to 6 weeks, concentration of pyridoxal phosphate in heart averaged 15%, in liver and muscle 12% and in kidney 10% of normal. Total amounts dropped to about 20% in heart, 18% in liver and 10% in kidney. B_6-al-PO_4 levels in blood, plasma and leukocytes diminished to 15% of control values. In the pair-fed group sacrificed after 3 weeks similar results were obtained (Table IV).

Discussion. Data in the literature indicate much higher levels of Vit. B_6 in various tissues of normal rats(6,7) than those found by us for pyridoxal phosphate. Liver for instance, was reported to contain an average of 7.7 γ/g, kidney 6.6 γ/g and heart 4.6 γ/g(7). These higher values are not unexpected, since the biological assay methods in these investigations measure total Vit. B_6 content, both metabolically active and inactive components.

Boxer *et al.*(1) determined the pyridoxal phosphate content of blood of various mammalian species and found it to be highest in the rat, varying from 135 to 240 mγ/cc, (average 187 mγ/cc). This is in good agreement with our findings of 73 to 232 mγ/cc, (average 122.6 mγ/cc), when one considers the fact that our rats were on a synthetic diet, while animals used by Boxer *et al.* had consumed an optimal nonsynthetic food mixture (personal communication).

A relationship between pyridoxin supplied in the diet and codecarboxylase (pyridoxal phosphate) content of rat tissue has been reported(8). Our experiments revealed a very ready response of B_6-al-PO_4 levels in organs, leukocytes and blood to restriction of dietary Vit. B_6. This response is much more marked with the metabolically active form than with total amount of Vit. B_6. Beaton and McHenry(7), for instance, found in liver a decrease of total Vit. B_6 to about 50% within 6 weeks of deficiency. In contrast, the metabolically active pyridoxal phosphate declined to about 12% during an equally long period of nutritional B_6 depletion. The relatively greatest decrease of total Vit. B_6 content(7) and of B_6-al-PO_4 occurred in the kidney. The lowest concentration and lowest total content of pyridoxal phosphate was found in this organ, averaging only about 10% of control values after 6 weeks of B_6 deficiency. After this period the concentration of B_6-al-PO_4 in all other tissues as well as of leukocytes, blood and plasma, was not greater than 15%.

Determination of pyridoxal phosphate in either plasma, whole blood or leukocytes, can serve as a sensitive guide for assessing the nutritional status of Vit. B_6. Estimation of B_6-al-PO_4 can be done in 1 cc of blood and thus repeated examinations can be performed without necessity of sacrificing the animal. For clinical purposes, it would obviously be advantageous to use blood or plasma instead of leukocytes to avoid the time-consuming separation and counting procedures. Boxer *et al.* have, however, pointed out that the level of pyridoxal phosphate in total blood of many normal human beings is less than 10 mγ/cc, the detection limit of the method for determination of B_6-al-PO_4. Attempts are under way to improve the technic so that it can be used for analysis of such small quantities of the vitamin.

Summary. Pyridoxal phosphate levels were determined in the heart, liver, kidney, muscle, circulating leukocytes, plasma and whole blood of rats on a synthetic diet with and without added Vit. B_6. Concentration of B_6-al-PO_4 decreased in all tissues and in leukocytes, blood and plasma significantly within 1 to 2 weeks in animals on the deficient diet. The levels of pyridoxal phosphate diminished within 5 to 6 weeks to 10 to 15% of control values in all organs, circulating leukocytes, plasma and blood. It is concluded that measurement

of pyridoxal phosphate in organs as well as in leukocytes, plasma and whole blood can serve as a sensitive indicator for assessment of the nutritional state of the animals in regard to Vit. B_6.

1. Boxer, G. E., Pruss, M. P., Goodhart, R. S., *J. Nutrition,* 1957, v63, 623.

2. Wachstein, M., Moore, C., Graffeo, L. W., PROC. SOC. EXP. BIOL. AND MED., 1957, v96, 326.

3. Li, J. G., Osgood, E. E., *Blood,* 1949, v4, 670.

4. Umbreit, W. W., *The Vitamins, Chemistry, Physiology, Pathology,* edited by Sebrell, Jr., W. H., and Harris, R. S., Academic Press, N. Y., 1954, v3, 239.

5. Agnew, L. R. C., *J. Path. and Bact.,* 1951, v63, 699.

6. Olsen, N. S., Martindale, W. E., *J. Nutrition,* 1954, v53, 329.

7. Beaton, G. H., McHenry, E. W., *Brit. J. Nutrition,* 1953, v7, 357.

8. Umbreit, W. W., Gunsalus, I. C., *J. Bact.,* 1945, v50, 124.

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Hematologic Manifestations of Lysine Deficiency in Swine.* (23919)

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Lysine deficiency has been produced experimentally in rats and swine by feeding diets deficient in lysine(1), by feeding diets containing "deaminized casein" as a source of protein(2,3), and by feeding diets low in lysine to which hexahomoserine (6-hydroxy-DL-norleucine), a lysine antagonist, was added (4-7). Under all of these conditions anemia was observed. In rats Smith and Stohlman (3) found a macrocytic megaloblastic type of anemia, whereas Gillespie, Neuberger and Webster(1) reported that the anemia was hypochromic. The morphologic characteristics of the anemia in swine have not been recorded(6).

The purpose of this report is to describe the hematologic manifestations of lysine deficiency in swine.

Methods. Twenty-three swine of the Chester-White breed were housed in individual cages and were started on the experimental diet at 3 weeks of age. The diet consisted of yellow corn, 71.1%; cottonseed meal, 25%; salt mixture 3.8%; terramycin feed supplement (Bi-Con TM 10), 0.1%; and a B vitamin mixture, 0.02%. The diet was fed in an amount of 35 g/kg of body weight/day. In addition, all animals received 10 mg of choline chloride/kg of body weight daily, and 3000 units of Vit. A, 600 units of Vit. D, 1 mg of Vit. E, and 1 mg of Vit. K/kg of body weight by mouth each week. The salt mixture consisted of calcium carbonate, 1.5 g; calcium phosphate, 1.4 g; sodium chloride, 0.5 g; iron citrate, 0.3 g; manganese sulfate, 0.04 g; zinc chloride, 0.03 g; copper carbonate, 0.005 g; and cobalt carbonate, 0.0006 g. The B vitamin mixture consisted of the following (mg): thiamine hydrochloride, 0.25; riboflavin, 0.12; nicotinic acid, 1.20; pyridoxine hydrochloride, 0.20; calcium pantothenate, 0.50; inositol, 0.20; para-aminobenzoic acid, 0.10; biotin, 0.10; pteroylglutamic acid, 0.10; and cobalamine, 0.01. Five pigs were given hexahomoserine (6-hydroxy-DL-norleucine) after they had been taking the experimental diet for 112 days. The hexahomoserine was added to the diet in an amount of 0.5% of the diet. The control animals were fed a casein, sucrose, and lard diet which has been described previously(8). Plasma iron was determined by the method of Hamilton, *et al.*(9). Plasma copper was determined by the method of Gubler, *et al.*(10). Serum protein estimations were performed by a modification(11) of the method described by Weichselbaum(12).

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