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Research Article

ANEMIA, HYPOPROTEINEMIA, AND CATARACTS IN SWINE FED CASEIN HYDROLYSATE OR ZEIN. COMPARISON WITH PYRIDOXINE-DEFICIENCY ANEMIA [1]

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Certain investigators (1) have recently confirmed the earlier limited data (2 to 4) which indicated that rats maintained on a diet in which the protein was supplied in the form of an acid hydrolysate of casein became anemic and that this anemia was relieved by the administration of tryptophane. The anemia observed by these workers was very slight in degree. The hemoglobin was reduced below 12.5 grams in 10 out of 16 animals but only in 1 of these was it as low as 10.0 grams. In addition, these investigators noted that a reduction in plasma protein preceded the fall in hemoglobin. These findings differ from those of another worker (5) who placed 9 rats on a tryptophane-low diet consisting of equal parts of acid hydrolyzed casein and zein and observed anemia in only 2 of the animals. The former workers criticise the latter's work on the ground that the diet was not entirely free from tryptophane and the experiments were not of sufficiently long duration.

Observations in other species are even more limited. Evidence from experiments in dogs indicates that tryptophane plays a rôle in the formation of plasma proteins (6) and hemoglobin (3, 4, 7). In tryptophane deficiency produced experimentally for periods of 2 to 5 weeks in human subjects, one of us (M. M. W.) working with the first mentioned workers (1) was unable to demonstrate significant alterations in the red cells. The following observations in swine, although also limited in number and in scope, are reported because of the pronounced anemia and hypoproteinemia which developed when casein in the diet was replaced by acid hydrolysed casein.

We have also been interested in studying the relationship of the anemia developing in pigs fed diets poor in tryptophane with that seen in pyridoxine deficiency since, in the latter, abnormal metabolism of tryptophane occurs (8, 9, 10). Pyridoxine-deficient swine excrete xanthurenic acid, "kynurenine," and another indole derivative in the urine in abnormal quantities. It therefore seemed desirable to ascertain the effect of low tryptophane intake on the course of pyridoxine deficiency as well as to compare the anemias developing in each type of deficiency.

MATERIAL AND METHODS

Full details of the experimental method have been published elsewhere (11). Pigs were obtained from the Bureau of Animal Industry, the United States Department of Agriculture, Beltsville, Maryland, except animals 8-01 to 8-05, inclusive, which were obtained from a private source. These animals were of Chester White breed and suckled until approximately 3 weeks of age, when the experiment was commenced.

The hydrolysate of casein [3] was an acid hydrolysate and by analysis was free of tryptophane. When supplemented with tryptophane, it permitted growth of at least 10 grams per week in rats.

The standard diet consisted of casein,[4] 26.1 per cent; sucrose, 57.7 per cent; lard, 11.0 per cent; swine salt mixture no. 3 (11), 5.2 per cent; 36.4 grams of this mixture constitutes one "kilo unit" and furnished 152 calories. When the casein was replaced by casein hydrolysate or zein,[5] it is so stated in the tables and protocols. When less than 26 per cent casein or casein hydrolysate was used, the caloric deficit was made up

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[3] Products 89-9 and 89-10, prepared by Mead Johnson and Company from HIP quality casein of the Casein Corporation of America.

[4] Sheffield "New Process," Sheffield By-Products Company.

[5] Corn Products Company.

with sucrose. In addition, the animals received cod liver oil[6] (1800 units A, 175 units D per gram), 0.5 gram per kgm. body weight daily. Vitamins[7] were supplied in crystalline form by placing them in capsules and administering them orally. The quantities of crystalline vitamins given were as follows (mgm. per kgm. body weight daily): thiamin hydrochloride, 0.25; riboflavin, 0.12; nicotinic acid, 1.20; pyridoxine hydrochloride, 0.20; calcium pantothenate, 0.50; p-aminobenzoic acid, 0.50; inositol, 1.20; choline chloride 10.0. The animals were fed the above standard diet supplemented with all 8 vitamin supplements for 1 week. After this time, the appropriate protein substitutions were made and the pyridoxine hydrochloride was omitted from the vitamin supplements given the groups which were to become pyridoxine-deficient. All animals receiving acid hydrolyzed casein took the diet poorly when it was first offered and, for this reason, for the first 2 weeks of the experiment, animals 7–98 and 7–99 were given half casein and half casein hydrolysate while pigs 8–01 and 8–02 were given half zein and half casein hydrolysate.

Serum iron determinations were made by the method of McKibbin *et al.* (12) by precipitating 5 milliliters of serum with trichloroacetic acid at 90° C. The filtrate was then adjusted to pH 6 with ammonium hydroxide and the iron determined with alpha-alpha dipyridyl after reducing with thioglycollic acid. A correction factor was used for the amount of iron which was carried down by the protein precipitate. Serum iron determinations by this method are accurate within $\pm$ 10 per cent. Great care was taken in cleaning glassware and in preparing reagents free of iron.

Xanthurenic acid was measured presumptively and qualitatively by neutralizing the urine to litmus, adding a few drops of ferric ammonium sulfate and filtering (8). The depth of the green color was then noted.

Serum non-protein-nitrogen was determined by the method of Dr. M. V. Buell which makes use of a persulfate digestion followed by nesslerization in the presence of potassium gluconate. This is read in the photoelectric colorimeter at wave length 500. Total serum protein was determined by a macro Kjeldahl analysis as adapted by Dr. Buell in which selenium is used as a digestant and the ammonia collected in boric acid. Albumin and globulin separations were performed by the method of Kingsley (13) and then measurements were made by the macro Kjeldahl method.

RESULTS

Groups I and II—"Acid Hydrolyzed Casein" and "Control"

Group I consisted of 3 pigs (7–98, 7–99, and 7–05), while Group II consisted of only 1 animal (7–03). This animal was fed the casein used in making the acid hydrolysate for 7–05 and was given that amount of diet which 7–05 consumed the day preceding. In this manner, it served as an inanition control. Both animals were given a diet containing 26 per cent casein and received in addition a supplement of 0.07 gram cystine per kgm. body weight per day. The 2 remaining animals of Group I were fed a diet containing only 15 per cent acid hydrolyzed casein. The results are summarized in Table I.

The animals receiving acid hydrolyzed casein ate poorly, failed to show any appreciable growth, and died on the 74th, 115th, and 117th days of the deficiency. Definite anemia developed which was normocytic, or slightly microcytic, and normochromic. The anemia appeared early and was slowly progressive. There was no evidence of increased hemolysis as determined by icteric index and qualitative urobilinogen determinations. The serum iron levels remained at normal limits even at the height of the anemia and there was no reticulocytosis. Terminally, that is a week or less prior to death, leukopenia appeared and persisted in all 3 animals. Differential leukocyte counts, however, revealed no consistent or significantly greater reduction in the number of cells of one series of leukocytes as compared with another. Blood platelets were not reduced in number.

These animals also developed pronounced hypoproteinemia with reduction of both albumin and globulin. The hypoproteinemia and anemia appeared to develop simultaneously. The results of Tiselius electrophoretic studies are given in Table II.[8] For comparison with these determinations, the blood serum of a pig (7–94) fed the standard diet in reduced amounts ("inanition control") was also examined. The globulin fractions are designated as proteins Nos. 2, 3, and 4 since these appeared in slightly different arrangement as compared with those of human serum. The most pronounced change was in the albumin fraction which showed a marked reduction and some change from normal composition as indicated by a broad asymmetrical curve.

When the blood changes described above had become quite pronounced, pig 7–98 was given 0.5 gram *d,l*-tryptophane daily (Figure 1). The animal died 18 days later. This small amount of

[6] Mead Johnson and Company.

[7] Merck and Company.

[8] We are indebted to Dr. John Luetscher, Jr., of the Chemical Division for these determinations.

TABLE I

Summary of the data on blood studies

Group:	I			II	III		IV			V			VI
Protein, *type*	Acid hydrolyzed casein			Casein	Acid hydrolyzed casein		Zein			Casein			Casein
Protein, *per cent*	15	15	26	26	18		18			26			26
Vitamin supplement	Complete			Complete	Pyridoxine omitted		Pyridoxine omitted			Pyridoxine omitted			Complete
Animal number	7–98	7–99	7–05	7–03	8–01	8–02	8–03	8–04	8–05	7–80	7–82	7–84	
Duration experiment, *days*	117	115	74	74	69	46	59	64	90	66	78	73	66
Termination[1]	D	D	D	K	D	D	D	D	D	K	K	K	K
RBC, *millions per c. mm.*	3.87	4.36	5.53	7.80	4.80	6.20	5.60	3.12	3.86	5.25	6.40	8.30	7.93
Hemoglobin, *grams per cent*	6.8	8.6	9.3	16.7	7.7	9.3	9.0	5.4	5.9	6.1	8.4	10.1	14.0
Volume packed red cells, *ml. per 100 ml.*	20.4	24.0	27.6	49.0	25.7	29.5	29.2	17.6	20.2	21.6	27.8	34.5	46.0
MCV[2], *cubic microns*	53	55	50[3]	63[3]	53	48	52	56	53	41	43	42	58
MCH[2], *micromicrograms*	18	20	17	21	16	15	16	17	15	12	13	12	18
MCHC[2], *per cent*	33	36	34	34	30	32	31	31	29	28	30	29	33
WBC, *thousands per c. mm.*	5.8	6.9	4.0	13.8	6.4	6.3	14.0	12.4	10.3	12.2	16.6	18.5	18.0
Serum iron, *μg. per cent*	143	120			145	144	116	72	75	422	465	370	142
Serum NPN, *mgm. per cent*	23	31	50	42	32	31	23	18	32	32	23	29	19
Total serum protein, *grams per cent*	2.81	4.44	4.41	6.97	4.38	4.06	3.19	2.63	3.00	6.44	6.63	6.31	6.33
Albumin, *grams per cent*	1.38	2.44	2.05	3.19	2.88					4.06	4.00	4.12	3.69
Globulin, *grams per cent*	1.43	2.00	2.36	3.78	1.50					2.38	2.63	2.18	2.64
Albumin-Globulin ratio	0.92	1.22	0.87	0.85	1.92					1.71	1.52	1.89	1.39

[1] D indicates that the animal died, K that it was sacrificed.

[2] MCV refers to mean corpuscular volume; MCH, mean corpuscular hemoglobin; and MCHC, mean corpuscular hemoglobin concentration.

[3] The mean red cell diameter at this time was 5.09 microns in pig 7–05 and 5.48 μ in pig 7–03.

tryptophane had no effect on the anemia and there was no reticulocytosis. However, the total serum protein rose from 2.81 grams per cent to 4.19 grams per cent.

One of these animals developed ocular lens opacities. When the experiment had been in progress 80 days, a fine, somewhat wavy line of opacity was noted near the equator of the lenses of pig 7–98. A week later opacities were noted along the anterior suture lines and also at the posterior suture lines. In another week, the anterior suture line opacities were quite pronounced, and posterior capsular or subcapsular opacities as well as peripheral equatorial opacities were clearly visible, especially in the right eye. By this time, the hypoproteinemia and anemia were quite severe. In pig 7–99, only several small vacuoles located nasally below and near the equator of the lens were seen. The hypoproteinemia in this animal was never as pronounced as in 7–98. Pig 7–05 was not found to have cataracts.

TABLE II

Tiselius electrophoretic analysis of serum proteins of pigs fed casein hydrolysate

Condition	Animal number	Total serum protein	Albumin fraction	Globulin fraction			
				Total	No. 2	No. 3	No. 4
		grams per cent	*grams per cent*	*grams per cent*			
Inanition control casein, 15 per cent	7–94	5.00	2.42	2.58	1.11	0.64	0.83
Casein hydrolysate, 15 per cent	7–98	2.81	0.83	1.98	1.08	0.33	0.57
Casein hydrolysate, 15 per cent	7–99	4.69	1.87	2.82	1.36	0.67	0.79

Neither epileptiform convulsions nor ataxia, such as occur in pyridoxine-deficient swine (14), were observed in these animals but they became very weak. The gait of 7–05 was described as "staggering" in character and in all the animals the muscular development was very poor. They were very thin and the hair coats were untidy. The hair came out very readily. Edema was present, especially in pig 7–98, where it appeared as a swelling in the under portion of the neck.

At autopsy the tissues were edematous. Histologically the muscle of 7–98 showed large areas where muscle fibers were atrophied or had completely disappeared (Figure 2). Such areas were very cellular. The nuclei resembled those of

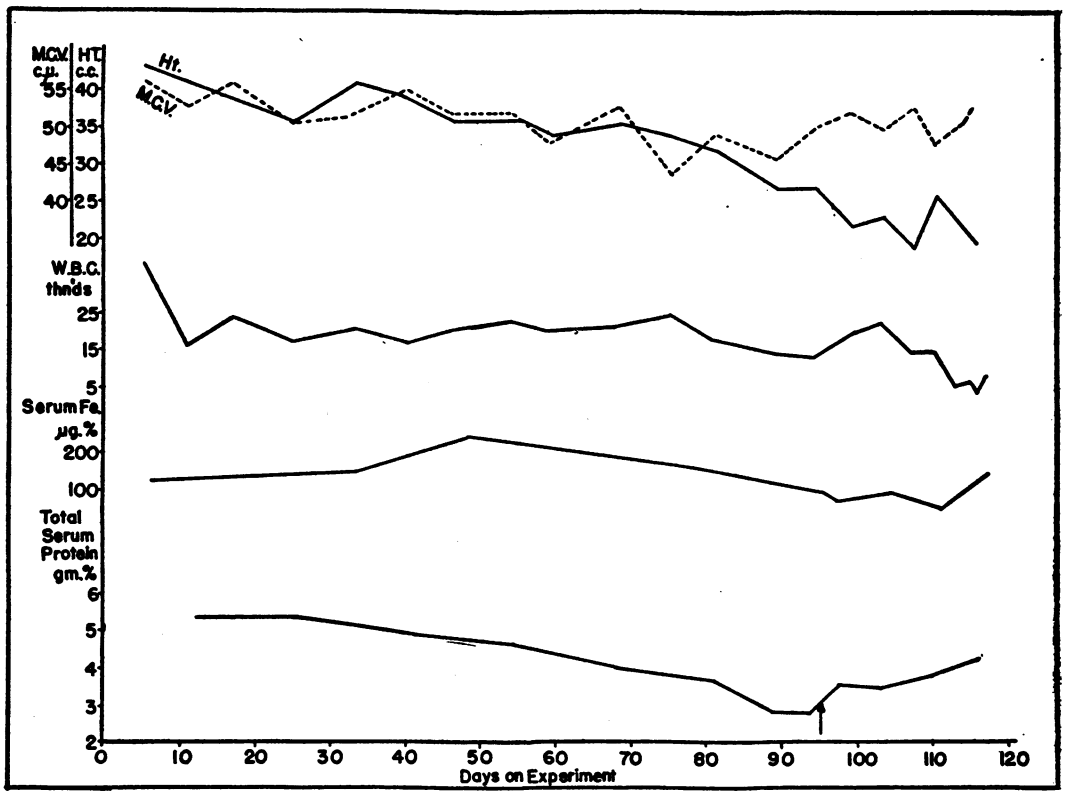


FIG. 1. DEVELOPMENT OF NORMOCYTIC ANEMIA AND HYPOPROTEINEMIA, AS WELL AS TERMINAL LEUKOPENIA, IN A PIG (7–98) FED ACID CASEIN HYDROLYSATE IN THE PLACE OF NATURAL CASEIN

Note the increase in total serum protein following administration of a small quantity (0.5 gram) of *d,l*-tryptophane (arrow). At no time was there a significant rise in serum iron.

MCV refers to mean corpuscular volume; Ht to volume of packed red cells, ml. p. 100 ml. blood. The leukocyte count (WBC) is expressed in thousands per c. mm.

sarcolemma and connective tissue cells. There were a few hyaline muscle fibers but on the whole the changes were not fresh. Muscle from 7–99 showed extreme atrophy and a few fresh focal necroses. In the muscle of pig 7–05, one lesion consisting of a focus of several hyaline muscle fibers was found. Muscle tissue from 7–03 showed no abnormalities.

The bone marrow was pale and, in 7–98, was hypoplastic on microscopic examination (Figure 4). There was no hemosiderosis in the spleen, liver, or bone marrow and no fatty changes were found in the liver. These data are summarized in Table III.

Groups III and IV—"Acid Hydrolyzed Casein, Pyridoxine-Deficient," and "Zein, Pyridoxine-Deficient"

The purpose of these 2 groups was to ascertain the effect of low tryptophane intake on the course of the pyridoxine deficiency. Pyridoxine was omitted from the vitamin supplement of both groups. Group III consisted of 2 animals receiving a diet containing 18 per cent acid hydrolyzed casein and group IV consisted of 3 animals given a similar proportion of zein in the place of casein. All animals failed to grow and died between the 46th and 90th day of the experiment. A moderately severe anemia developed which was normocytic, or slightly microcytic, and normochromic. The 2 animals of group III developed a terminal leukopenia but the animals maintained on zein failed to show this. As in group I, there were variations in the differential leukocyte counts but no significant alterations could be correlated with the changes in the total count. The serum iron remained within normal limits. There was a pronounced reduction in total serum proteins.

Epileptiform convulsions or ataxia were not observed. Furthermore, no xanthurenic acid

could be demonstrated in the urine of these animals. As in the case of the pigs of group I, weakness and edema characterized these animals but in the pigs fed zein in the place of casein (group IV), edema was much more pronounced. This was especially noticeable about the eyelids, the under part of the neck and the genitalia (Figure 3). The swelling about one eye of pig 8–04 was so great that the eyeball was hidden. Postmortem examination revealed extreme edema of all the subcutaneous tissue, and in pig 8–05, there was serous fluid in the abdominal cavity.

The bone marrow of pigs 8–01 and 8–02 was slightly to moderately hyperplastic (Table III). A slight to moderate degree of hemosiderosis was present. These changes seemed less pronounced than we would have expected in animals not given pyridoxine for the same length of time, as judged by past experience (14). The findings in pigs 8–03, 8–04, and 8–05 (Figure 6) were similar. These last 3 animals had received pyridoxine, 200 micrograms intravenously daily, respectively, for the 7, 3, and 8 days preceding the autopsies.

The muscle fibers in pigs 8–01 and 8–02 were very atrophic. No necroses were found. In pigs 8–03 and 8–05, there were a few small foci consisting of extremely atrophic hyaline muscle fibers surrounded by mononuclear cells. Pig 8–04 was found to have extreme atrophy with hyalinization of the muscle fibers so that virtually no transverse striations could be found. Whether due to the decrease in muscle tissue or to a true proliferation of sarcolemma nuclei, the tissue was very cellular.

There were no lens changes in pig 8–03. Animal 8–04 developed in the lens of the right eye peripheral cortical opacities above and temporally, as well as lens fiber dissociation and one group of vacuoles nasally. In the lens of the left eye, there was 1 group of vacuoles in the peripheral cortex nasally and there were 2 patches of opacities temporally and above. These were possibly also in part vacuolized. In pig 8–05, when the experiment had been in progress 37 days and the serum proteins were 4.19 grams per cent, very marked cortical lens fiber dissociation was noted in both eyes and a small number of groups of vacuoles were visible near the equator. As the deficiency progressed, the lens fiber dissociation became more pronounced and after another 23 days both lenses showed advanced posterior capsular cataracts, particularly around and at the posterior pole. Sixteen days later there was a very outspoken posterior rosette-type opacity in each eye and a streaky appearance throughout the lenses. The changes in the right eye were more pronounced than in the left eye.

Groups V and VI—"Pyridoxine-Deficient" and "Normal Controls"

Group V consisted of 3 animals fed the standard diet containing 26 per cent casein but with pyridoxine omitted from the vitamin supplements. These animals have been considered elsewhere (9) and the data are presented here only for comparison. By the 66th day of the deficiency, these animals had developed microcytic anemia and manifested epileptiform convulsions from time to time. The urinary test for xanthurenic acid was positive. Serum iron levels were greatly elevated but there was no reduction in total serum protein and no evidence of edema.

PLATE I

FIG. 2. MUSCLE FROM TONGUE OF PIG (7–98) FED ACID HYDROLYSED CASEIN.

Note normal muscle fiber and hyaline necrotic ones. The latter are infiltrated by leukocytes, mostly mononuclears. There are also occasional giant cells. Areas such as these were focal and diffuse.

FIG. 3. MARKED EDEMA IN A PIG (8–04) FED ZEIN IN THE PLACE OF CASEIN AS A SOURCE OF PROTEIN.

Note especially the edema in the neck and about the penis. The total serum proteins at this time were 2.63 grams per cent (normal over 6.00 grams).

Figures 4, 5, and 6. Bone Marrow. Custer Stain, × 100

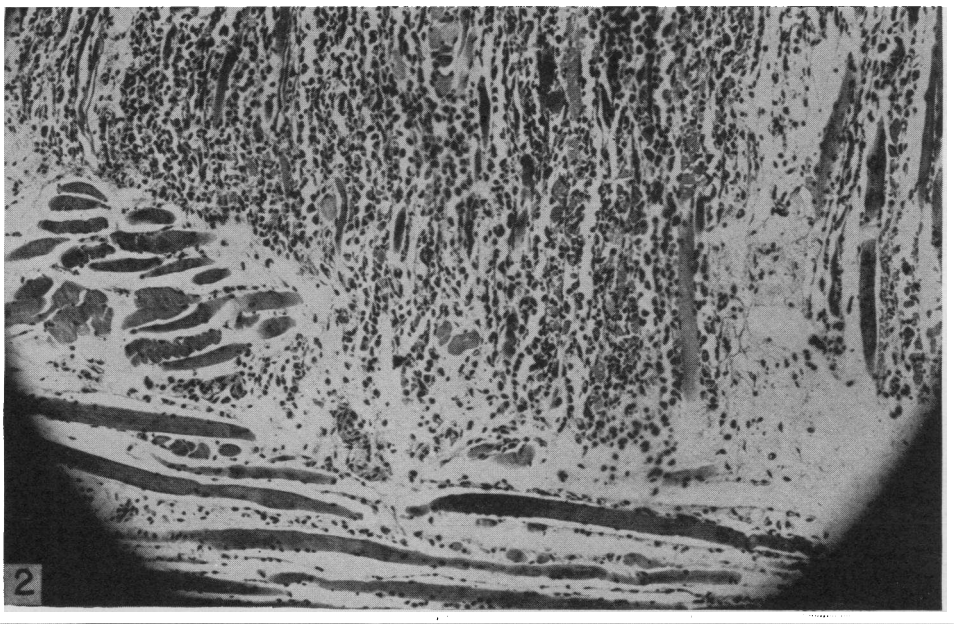
FIG. 4. PIG (7–98) FED ACID HYDROLYSED CASEIN.

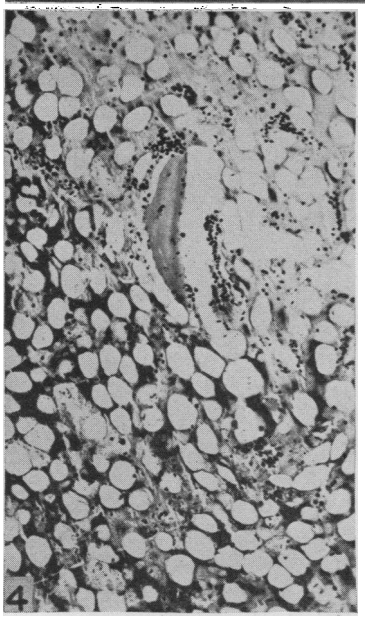
There is no hyperplasia. (The dark areas between the fat cells contain no marrow cells.)

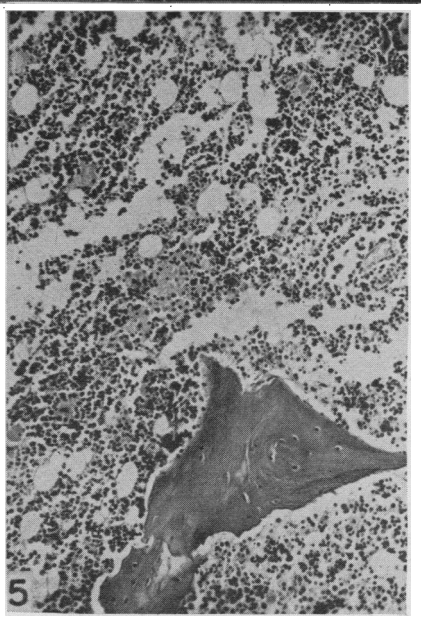
FIG. 5. PRONOUNCED HYPERPLASIA IN THE MARROW OF A PIG (7–82) FED A DIET DEFICIENT IN PYRIDOXINE.

FIG. 6. PIG 8–05 FED ZEIN AND ALSO NO PYRIDOXINE UNTIL 8 DAYS PRECEDING AUTOPSY WHEN 200 MICROGRAMS WERE GIVEN DAILY BY VEIN.

There is very minimal hyperplasia, as would be expected following treatment with pyridoxine.







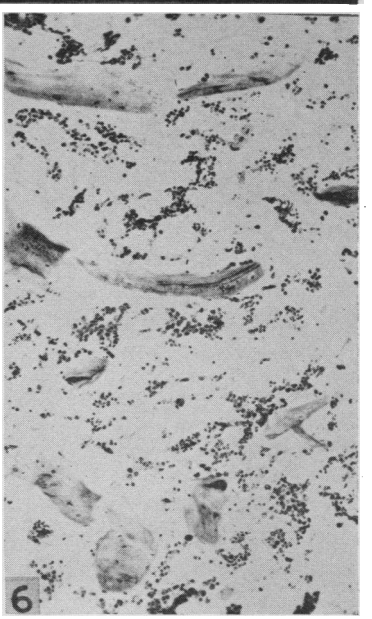


PLATE I

TABLE III

Summary of clinical and pathological studies

Group	I			II	III		IV			V			VI
Protein, *type*	Acid hydrolyzed casein			Casein	Acid hydrolyzed casein		Zein			Casein			Casein
Protein, *per cent*	15	15	26[1]	26[1]	15		18			26			26
Vitamin supplement	Complete			Complete	Pyridoxine omitted		Pyridoxine omitted			Pyridoxine omitted			Complete
Animal number	7–98	7–99	7–05	7–03	8–01	8–02	8–03	8–04	8–05	7–80	7–82	7–84	
Average daily food intake, *units*[2]	0.7	0.6	0.6	0.6	0.6	0.6	0.9	0.8	0.9	0.9	0.9	1.0	1.0
Total weight gain or loss, *kgm.*	+1.3	+0.5	−2.4	+6.7	−0.1	−0.6	+1.0	+1.2	+0.9	+10.6	+13.1	+4.0	+12.0
Ataxia	0	0	0	0	0	0	0	0	0	+	+	+	0
Convulsions	0	0	0	0	0	0	0	0	0	+	++	++	0
Edema	++	+	+	0	++	++	++	++++	++++	0	0	0	0
Bone marrow, hyperplasia[3]	0	0	0	+	±	+		±[4]	0[4]	+	+	+	0
Hemosiderosis liver	0	0	0	0	0	+	0	+	0	0	0	0	0
Spleen, pulp	0	0	0	0	+	++	0	++	++	+++	+++	±	0
trabeculae	0	0	0	0	0	0	0	0	0	++	++	0	0
Bone marrow	0	0	0		0	0			0	0	0	0	0
Fatty liver	0	0		0	0	0	+[7]	++[7]	0	+	++	0	0
CNS degeneration	0	0			0	0	0	0	0	+++[5]	+++[5]	+[5]	0
Muscle degeneration	++++	++	+	0	++	++	+	+++	+	0[6]	0[6]	0[6]	0
Lens opacities	+	+	0	0	0	0	0	+	+	0	0	0	0

[1] Plus cystine 0.07 grams per kgm. per day.
[2] One "unit" is an amount of the dietary mixture furnishing 152 calories per kgm. body weight daily.
[3] Under Bone Marrow Hyperplasia, + indicates "moderate," ± "slight," 0 "none," and 0 "hypoplastic."
[4] Pigs 8–03, 8–04, and 8–05 were given intravenously 200 micrograms pyridoxine per kgm. body weight daily during their 7, 3, and 8 last days, respectively.
[5] The degenerative changes in the nervous systems of these animals were found only in the peripheral nerves.
[6] Muscle tissue was not examined in these animals but in numerous other pyridoxine-deficient pigs which have been examined no degeneration has been encountered.
[7] This was periportal rather than central, as occurs in pigs deficient only in pyridoxine.

There was no terminal leukopenia. When these animals were sacrificed, no edema could be demonstrated at autopsy. There was hyperplasia of the bone marrow (Figure 5), hemosiderosis of the spleen, the livers showed central fatty infiltration, and there were demonstrable changes in the central nervous system. No cataracts were observed in these pigs.

Group VI consisted of a large number of control animals receiving the standard diet containing 26 per cent casein, and fed a complete supplement of the 8 "B" vitamins listed under Material and Methods. Average findings are shown in Table I. In such animals, we have found no reduction in hemoglobin or serum proteins. At autopsy, the edema and muscle necrosis, the malnutrition, the bone marrow hyperplasia, and the hemosiderosis described in groups I, III, IV and V, were not encountered.

DISCUSSION

Circumstances have made it impossible to extend these studies to a larger series of animals and to include the various experimental groups which would be desirable in a complete experiment. Nevertheless, it seems clear from these data that in swine, the feeding of a diet composed of acid hydrolyzed casein instead of the natural product, but presumably adequate in other respects, is associated with the development of marked hypoproteinemia and anemia. This occurred even when the hydrolyzed casein was fed at a 26 per cent level rather than 15 per cent. The anemia was normocytic or slightly microcytic but the concentration of hemoglobin in the red corpuscles remained unchanged. The hypoproteinemia, which was due more to a reduction of albumin than of globulin, was associated with the development of edema. At the same time, there were failure to gain weight, focal atrophy and necrosis of muscles, and cataracts in the lenses of the eyes.

The pigs fed 18 per cent zein in the place of acid hydrolyzed casein were also given no pyridoxine, but comparison with pyridoxine-deficient animals fed natural casein suggests that the changes associated with the feeding of a zein diet are similar to those associated with the feeding of acid hydrolyzed casein. In fact the hypoproteinemia and edema were even more pronounced.[9]

[9] This greater degree of hypoproteinemia may have been due to the effect of lysine deficiency in addition to tryptophane deficiency. Lysine deficiency has been shown recently to be associated with the development of hypoproteinemia (Harris, H. A., Neuberger, A., and Sanger, F., Biochem. J., 1944, **37**, 508).

The most striking difference between the findings in the pigs fed acid hydrolyzed casein and in those fed zein was the development of leukopenia terminally in the former.

The presence of lens changes in pigs receiving a diet furnishing acid hydrolyzed casein or zein instead of natural casein, confirms the findings of others (15 to 18). Certain investigators (17, 18) reported that rats maintained on a diet deficient in tryptophane (acid hydrolyzed casein) developed cataractous changes. These changes could be prevented by supplementing the diet with tryptophane. Also described (17) were cataractous changes in rats maintained on a diet of zein. These changes were not influenced by the concurrent lysine deficiency but could be prevented by feeding tryptophane.

The fact that similar changes took place in our animals when they were maintained on acid hydrolyzed casein or on zein, suggests that these effects were due to a lack of tryptophane rather than to lysine which zein lacks in addition to being deficient in tryptophane. We can offer no direct proof of this as sufficient tryptophane was not available to determine whether such a supplement to the diet would completely restore the animals to normal. That the changes were not due to inanition is indicated by the fact that animal 7–03 was fed the same quantity of a complete diet as 7–05 consumed of the deficient one. Pig 7–03 nevertheless failed to develop either anemia or hypoproteinemia.

That a disturbance in tryptophane metabolism occurs in pyridoxine deficiency is indicated by the fact that pyridoxine-deficient swine excrete xanthurenic acid, "kynurenine," and at least one other indole derivative in abnormal quantities (9, 10). Since either a lack of tryptophane or of pyridoxine leads to the development of anemia, the question arises whether pyridoxine anemia might indirectly be due to a lack of properly metabolized tryptophane. If such were true, the 2 anemias should be similar.

The 2 types of anemia, however, are very different from one another. The anemia of pyridoxine deficiency is microcytic and slightly hypochromic and is accompanied by an elevated serum iron, hyperplastic bone marrow, and hemosiderosis of the spleen, liver, and bone marrow. The anemia associated with the feeding of acid hydrolysed casein and presumably due to tryptophane deficiency is essentially normocytic and normochromic, the bone marrow appears to be hypo- or normo-plastic, the serum iron level is normal and there is no hemosiderosis of the tissues. Furthermore, the 2 deficiencies are quite different clinically. "Tryptophane" deficiency causes cessation of growth and is accompanied by marked hypoproteinemia and edema. Pyridoxine deficiency causes only limitation of growth and the quality and quantity of the serum proteins are unaffected.

It is of interest to speculate in what manner tryptophane, pyridoxine, and iron are utilized in blood formation. A simple explanation of the rôle of these substances is that union of tryptophane, iron, and other factors (X) takes place ($T + Fe + X \rightarrow RBC$), with pyridoxine acting as an essential component of an enzyme system promoting this reaction. When T is lacking, Fe is still bound to X, the enzyme being present, and no ferremia occurs. Anemia develops, however, because T is lacking. When Fe is lacking, T is still bound to X, since the enzyme is present. No ferremia occurs and products of tryptophane metabolism are not excreted although anemia occurs owing to iron deficiency. We have found (9) these to be the actual facts in pigs fed iron-poor diets. On the other hand, when the theoretical enzyme system involving pyridoxine is lacking, no union of the building stones takes place. As a result, ferremia occurs and substances derived from tryptophane are lost in the urine. If this hypothesis is correct, it should be expected that feeding tryptophane to pyridoxine-deficient animals will result in increased excretion of xanthurenic acid. This has actually been found to be the case (8, 9). It may be that other substances, derived from X, are also excreted. The theory presupposes that total body iron is normal in pyridoxine deficiency, which the obvious hemosiderosis would appear to deny. In current experiments, attempts are being made to study this question.

It is interesting that, although the degree of anemia is much the same, the bone marrow in pyridoxine deficiency is hyperplastic whereas the marrow of the pigs maintained on acid hydrolysate of casein was normo- or hypo-plastic. The factors governing the cellularity of the bone mar-

row are unknown. Certainly anemia *per se* is not the only governing factor. It is recognized that anemia occurs in the presence of hyperplastic, normoplastic, or hypoplastic marrow.

Our results, although very limited, suggest that the development of pyridoxine deficiency may be retarded when the tryptophane intake is decreased. The pyridoxine-deficient animals fed casein acid hydrolysate and those given zein failed to develop certain signs of pyridoxine deficiency in 46 to 90 days, namely: pronounced microcytosis, elevated serum iron, epileptiform convulsions, and ataxia. Histologic evidence of changes in the nervous system was also lacking. Hemosiderosis was not as marked as might have been expected in pyridoxine deficiency of this duration. Pyridoxine-deficient pigs, started on experiment at a similar age and fed 26 per cent casein, have shown these changes in 4 to 10 weeks (14). These results are in harmony with those of others (19) who studied pyridoxine deficiency in rats fed diets containing 15, 30, and 45 per cent casein. They found that at the low level of protein intake, little dermatitis developed in 70 days, while, at the intermediate level, rats developed dermatitis after 30 days. At a protein level of 45 per cent casein, severe dermatitis developed in 26 days and was followed shortly by death. Apparently, high protein intake in rats seemed to increase the severity of the nutritional disorder due to inadequate intake of pyridoxine. Further studies by these investigators (20) revealed that tryptophane delayed the onset of symptoms and prolonged the life of the animals, whereas cystine aggravated them.

Whether tryptophane is ever the limiting factor in the formation of hemoglobin in humans is not known.

The changes observed in the muscles of the animals fed acid casein hydrolysate or zein in the place of casein, will require much further study. In one pig, there were extensive lesions in which there were atrophy and absence of fibers. Such areas contained very cellular tissue which probably arose from sarcolemma cells. In all the animals, the muscle fibers were extremely atrophic. The fibers in some were hyaline.

SUMMARY

1. Swine maintained on a synthetic diet, in which the protein was supplied in the form of an acid hydrolysate of casein or by feeding zein, failed to grow and developed normocytic, or slightly microcytic, normochromic anemia which was accompanied by a normal serum iron level, hypo- or normo-plastic bone marrow, and marked hypoproteinemia and edema.

2. Lenticular opacities developed in 2 out of 3 animals maintained on acid hydrolysed casein and in 2 of 3 pigs maintained on zein.

3. It is thought most likely that these changes were caused by a deficiency of tryptophane.

4. Although faulty tryptophane metabolism occurs in pyridoxine deficiency, comparison between "tryptophane" and pyridoxine anemia reveals marked differences. A hypothesis is offered to explain the rôle of tryptophane and pyridoxine in hematopoiesis.

5. The data presented suggest that a low intake of tryptophane retards the course and diminishes the severity of the nutritional disorder due to pyridoxine deficiency in swine.

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