HISTOLOGICAL STUDIES OF THE BONE MARROW IN FASTED AND POLYNEURITIC PIGEONS

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Plates 55 and 56

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In previous communications,[1] one of us has reported a series of studies on rice disease in pigeons and the effect of vitamin B, lactose, magnesium sulfate, and mineral oil on the anemia, weight loss, and bacteremia. In further study of this problem it was necessary to examine the histological changes in the bone marrow in rice disease and compare them with that in starvation and pure vitamin B deficiency. The present report concerns the results of this investigation.

Method

Adult healthy pigeons, which weighed about 350 gm., were used. Six were selected at random, fed on a normal diet of whole mixed grain and grit, and employed as controls. Eleven were deprived of all food except water until they had lost 40 to 50 per cent of the original body weight. Eight were allowed to eat polished rice until they had lost a similar weight. No attempt was made to force-feed these animals. Seven were fed a synthetic diet deficient only in vitamin B until definite polyneuritis developed. These animals were fed 12 to 18 gm. of the diet daily and watched to avoid regurgitation. This diet was prepared as follows:

Commercial casein was rendered vitamin B free by extraction three times with hot 80 per cent alcohol and mixed into a dough with the other constituents.

	per cent
Extracted casein	18
Starch	55
Crisco	20
Salt mixture	5
Cod liver oil	2

A 2 weeks supply was made at one time and kept in the ice box. When each animal developed a satisfactory experimental condition, it was sacrificed by di-

[1] Barlow, O. W., *Am. J. Physiol.*, 1930, **93**, 161.

vision of the neck and allowed to exsanguinate. The entire bone marrow from both radii was fixed in Zenker's solution, embedded in parafin, and cut in longitudinal section so that the full length could be examined. The marrow from other bones was not examined. Sections were also prepared of the liver, spleen, kidneys, heart, and lungs. All sections were stained with hematoxylin and eosin and some with the Prussian blue and ammonium sulfide reactions for iron. In a few instances smears of the marrow were stained with eosin and methylene blue.

RESULTS

The general state of the fasting and rice disease birds at the time of sacrifice is shown in Tables I and II. An average of the rice disease

TABLE I

Rice Series

No.	Per cent of normal weight	R.B.C.	Hemoglobin	Bacterial count total
			per cent	
B1	53.7	2,152,000	54	82,000
B2	65.3	3,008,000	68	67,000
B5	53	2,320,000	57	110,000
B12	62.5	2,832,000	62.5	108,000
C1	60	2,888,000	67	68,000
C3	63	2,304,000	60	72,622
C5	64.8	2,784,000	66	13,920
C10	63	2,788,000	65	58,790
C7	61.5	2,808,000	66	79,000
C13	66	3,048,000	70	40,000
Small	64	2,888,000	73	35,554
Median—11...	63	2,808,000	66	68,000

birds shows 63 per cent of normal body weight; 2,808,000 red cells per c.mm., 69 per cent of normal; 66 per cent hemoglobin; and a bacterial count of 68,000. In the fasted group the weight was 60.3 per cent of normal; the red cell count 2,656,000, 65 per cent of theoretical; the hemoglobin 68 per cent; and the bacterial count 10,360. The degree of anemia and weight loss in these two groups is about equal.

In the fasted series the changes are similar to those described by Doan, Cunningham, and Sabin.[2] There is a fat replacement of the

[2] Doan, C. A., Cunningham, R. S., and Sabin, F. R., *Carnegie Institution of Washington, Pub. No. 83, Contributions to Embryology*, 1925, **16**, 163.

marrow and marked decrease in hematopoietic elements. The myelocytic islands are for the most part absent except in the peripheral zones (Fig. 1, *b*). Erythrogenesis is almost in abeyance. In some animals (13, 20, 21, and 25) there is an increase in apparent vascularity and slight evidence of formation of red cells similar to the picture described by Doan, Cunningham, and Sabin as early regeneration (Fig. 2). With no refeeding which would produce this change, the conclusion is that it represents a failure of complete aplasia and not a regeneration after aplasia.

In the rice disease birds the histological changes in the bone marrow are strictly comparable to those of the fasted animals (Fig. 1, *c*). Again,

TABLE II

Fasting Series

No.	Per cent of normal weight	R.B.C.	Hemoglobin	Bacterial count total
			per cent	
A	54	1,984,000	62	12,864
B	64.6	3,136,000	72	9,408
C	61.6	2,512,000	65	12,560
D	52	1,760,000	62	10,560
E	68	3,088,000	76	10,190
F	53.2	2,032,000	59	10,160
G	55	1,920,000	68	10,360
H	59	3,256,000		
C8	63.4	2,800,000	74	14,000
C11	69	3,208,000	78	9,728
Median — 10...	60.3	2,656,000	68	10,360

in Animals 9 and 12 there is a similar type of slight erythrogenesis. Thus on the basis of histological examination the conclusion must be, that the anemia of rice disease, in which the animals are not force-fed, is essentially a starvation anemia and not at all, or only indirectly, related to the absence of vitamin B in polished rice.

In the group of pigeons fed a synthetic diet deficient only in vitamin B, the type of change is entirely different. Hematopoiesis of both the granular and red cell series is abundant and shows all stages, but many of the adult and immature cells show degenerative changes (Fig. 1, *d*). The nuclei of myelocytes and megaloblasts show karyorrhexis, pyk-

nosis, and karyolysis and the cytoplasm of these cells is granular and vacuolated. (Fig. 3.) The cytoplasm of some erythrocytes stains a light bluish green and the cell walls are indistinct. This change is disposed throughout the marrow in irregular islands not over 500 microns in diameter. There is also an edema of the walls of the arterioles and venules with swelling and early proliferation of the endothelium of these vessels. It is not possible at this time to explain these changes on a definite etiological basis, but it is possible that the degenerative changes are secondary to the vascular disease. In the seven animals which we have examined, there is no alteration in erythrogenesis and no change similar to that observed in the rice disease birds.

Both the polished rice and synthetic diets are deficient in vitamin B, but the rice is also deficient in many other factors. On the basis of our histological observations, the changes in the bone marrow are unrelated and hence we believe that the anemia of rice disease, cannot be accepted as the result of vitamin B deficiency alone.

Sections of the liver, spleen, lungs, and heart show no pathological change other than a variable deposit of an iron-containing pigment in the endothelial cells of the hepatic and splenic sinusoids. We have been unable to distinguish the fasted and rice disease birds on the basis of the quantity or type of this pigmentation.

CONCLUSIONS

1. The histological changes of the bone marrow in fasted and rice disease pigeons are essentially the same.

2. The histological changes of the bone marrow in pure vitamin B deficiency consist of degeneration and edema and slight endothelial proliferation of the small vascular channels, but with active hematopoiesis.

3. The anemia of rice disease in pigeons is in large part a starvation anemia and not directly related to vitamin B deficiency.

We wish to thank Doctor C. A. Doan for aid in this investigation.

EXPLANATION OF PLATES

PLATE 55

FIG. 1. (*a*) normal bone marrow, (*b*) bone marrow from fasted pigeon, (*c*) bone marrow from rice disease pigeon, (*d*) bone marrow from polyneuritis pigeon. × 90.

PLATE 56

FIG. 2. From fasted pigeon. Note the apparent increase of vascular channels with many immature red cells within the lumina. × 325.

FIG. 3. Island of hematopoietic tissue from pigeon fed a diet deficient in vitamin B. Note the swelling, granularity, and nuclear degeneration in the immature blood cells with edema of the artiolar wall. × 560.

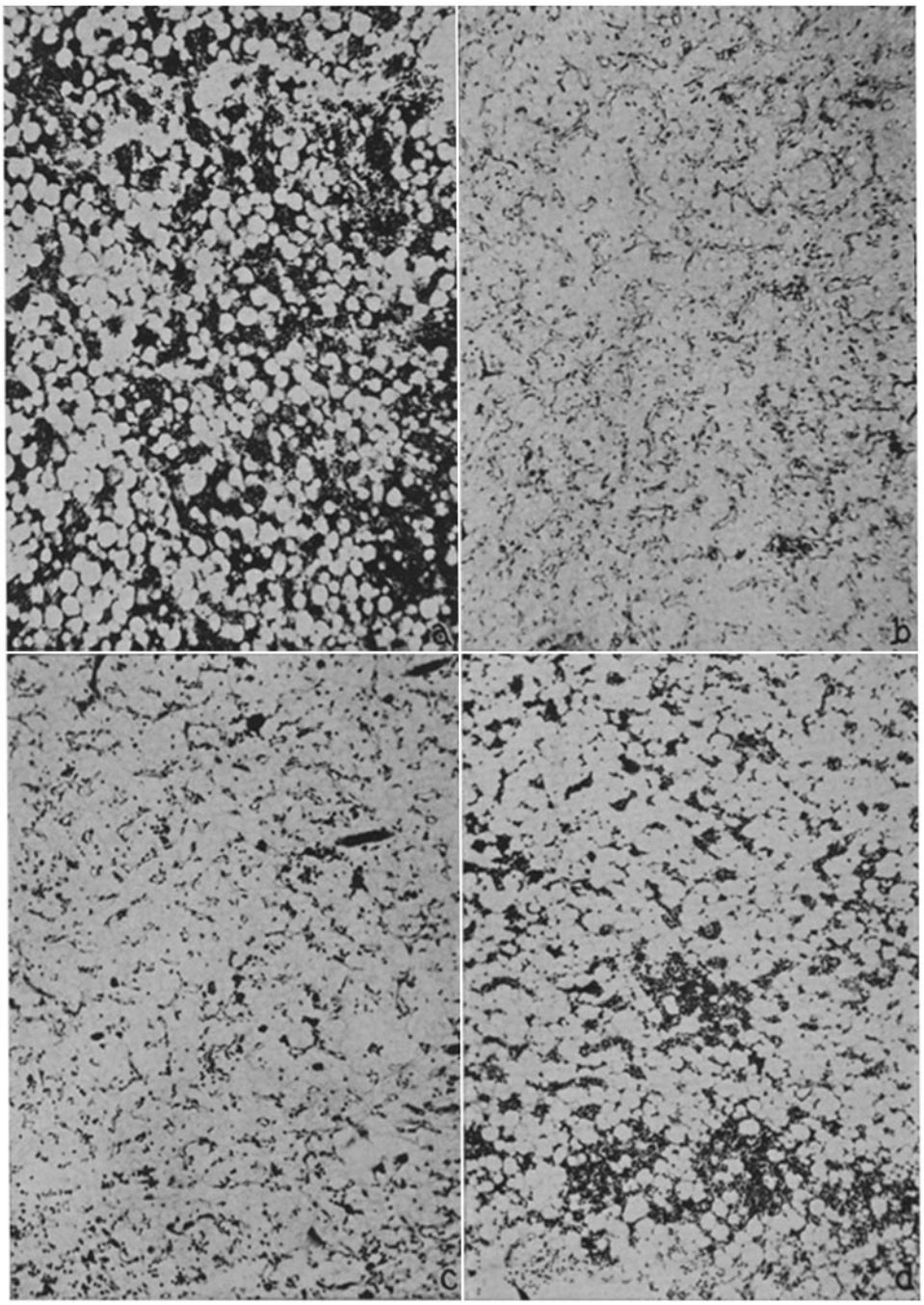

FIG. 1

(Moore and Barlow: Bone marrow in polyneuritic pigeons)

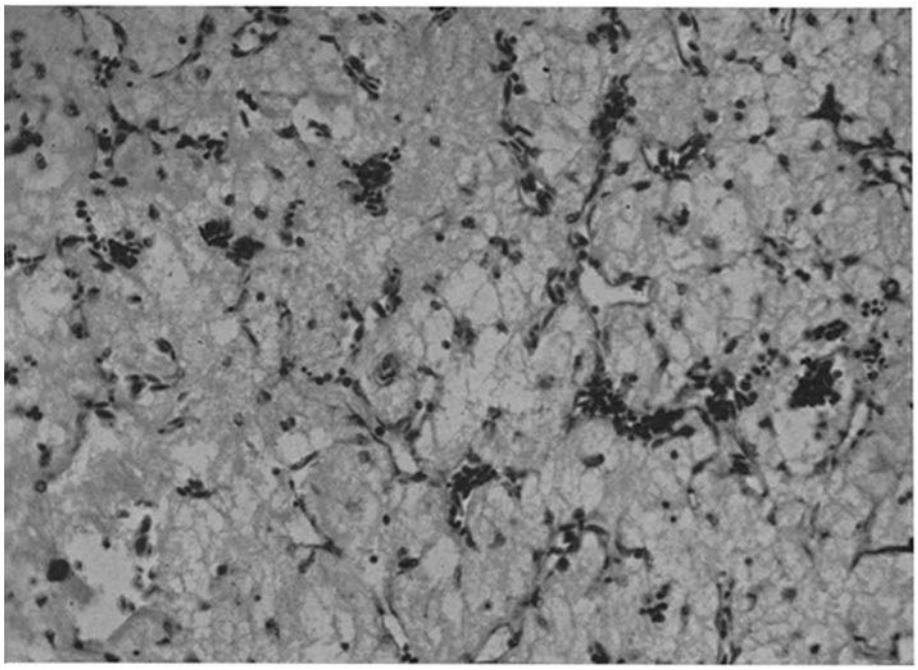

FIG. 2

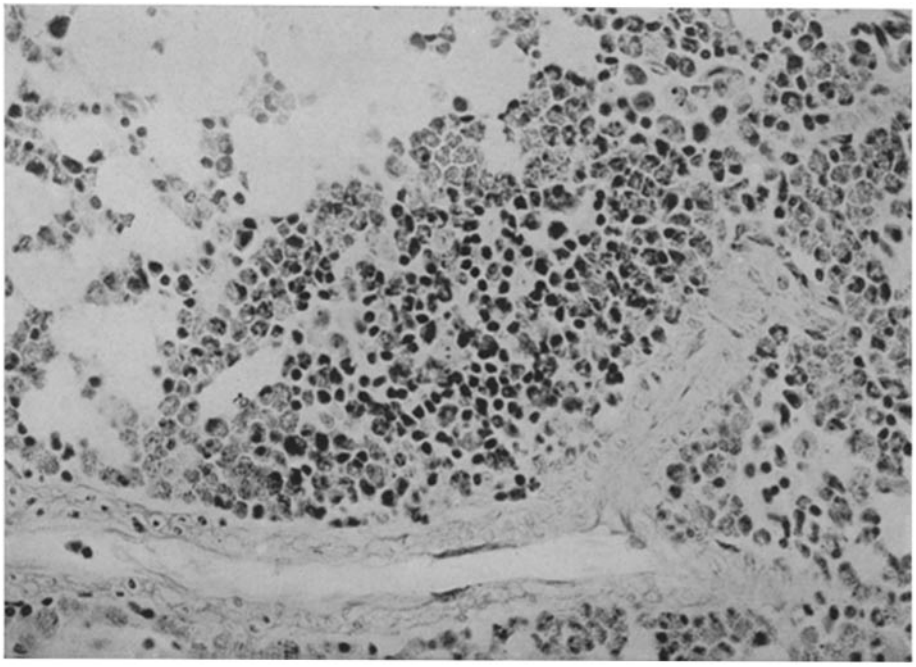

FIG. 3

(Moore and Barlow: Bone marrow in polyneuritic pigeons)