PANMYELOPHTHISIS WITH HEMORRHAGIC MANIFESTATIONS IN RATS ON A NUTRITIONAL BASIS*

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PLATES 15 TO 20

INTRODUCTION

Vitamin B_6 is that part of the vitamin B_2 complex which is responsible for the cure of the specific dermatitis ("rat acrodynia"[1]) developed by young rats fed a vitamin B free diet supplemented with vitamin B_1 and lactoflavin (3).

A good and potent source of vitamin B_6, free from lactoflavin, is Peters' eluate (4), which is obtained essentially by elution of a charcoal adsorbate of an aqueous yeast extract with acid alcohol.

Lepkovsky, Jukes and Krause (5) have shown that rats need, in addition to vitamin B_6 and lactoflavin, a third, mainly growth-activating fraction of the vitamin B_2 complex. This is also called the "filtrate factor" (6), owing to the outstanding chemical properties revealed during the course of preliminary attempts at its purification.

In the synthetic diet first used (3) for the production of rat acrodynia, rice starch was employed as the source of food carbohydrate. When the experiments for purification of vitamin B_6, preliminary results of which have been reported (7), were resumed, rice starch was replaced in the diet first by corn starch, then by cane sugar. This substitution was made after a specimen of rice starch had proved toxic for rats and after it was found that corn starch, at least the brand

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[1] The term acrodynia which appears throughout this article refers to these skin manifestations in rats fed a diet deficient in vitamin B_6 (2).

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used, prevented or delayed the appearance of rat acrodynia, in confirmation of relevant observations by Richardson and Hogan (8).

Rats kept on a diet of this kind, deficient in vitamin B_6 and containing sugar, developed acrodynia in from 6 to 20 weeks. Treatment with purified, less crude B_6 preparations was in many cases followed not by complete recovery but by a very acute, fatal anemia and a great variety of symptoms of a concomitant hemorrhagic diathesis. Medication with more complex B_6 preparations, such as Peters' eluate, or with certain foodstuffs, such as milk, liver and yeast, assured final cure of the B_6 deficiency disease without the complication of abnormal hematopoiesis.

In several instances this peculiar hemorrhagic disease has been observed by us in rats when there was no change in the experimental conditions. On further study it became evident that this disease, which at first gave the impression of being purely accidental, was a distinct pathologic entity, with a well defined etiologic basis. We were able to show that one constituent of the vitamin B_2 complex plays, apparently, an important part in primary blood cell production.

EXPERIMENTAL DATA

Rats, mostly albino, 21 days of age, weighing not more than 35 gm., were placed on the following diet.

	per cent
Casein[2]	18
Cane sugar	68
Melted butter fat	9
Salt mixture (McCollum 185)	4
Cod liver oil	1

Each rat in an experiment received daily, in addition to the diet, 3 pigeon units of a highly purified vitamin B_1 preparation employed in previous studies (2) and 10γ of crystalline natural lactoflavin, which was replaced in later experiments by crystalline synthetic lactoflavin.[3]

Animals with symptoms typical of acrodynia were treated with vitamin B_6 preparations or with foodstuffs containing vitamin B_6.

In those animals in which anemia and hemorrhagic manifestations followed the successful treatment of vitamin B_6 deficiency, the first sign of the impending new disease consisted in renewed suspension of growth and in reduced food intake.

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[2] Purified casein chiefly was used, but the results were duplicated in some experiments with Merck's commercial casein.

[3] Vitamin B_1 and lactoflavin were furnished through the courtesy of Winthrop Chemical Company, Inc., New York.

In untreated animals, anemia and hemorrhagic symptoms were often preceded by spontaneous cure of a very mild acrodynia that usually started not more than 1 to 2 weeks earlier, and by further pronounced anorexia. Only exceptionally did we observe rats in which the hemorrhagic disease came about without such premonitory symptoms and in particular without preceding acrodynia.

Onset of the anemia was easily recognized in the albino rats by comparing the color of their eyes with that of normal rats. A drop of 10 to 20 per cent in the hemoglobin content of the blood could thus be estimated without difficulty. The hemorrhagic diathesis was indicated by variable signs. One of the most common symptoms was nosebleed. Usually this was not profuse and manifested itself only in slight bloody discharge which soon dried up. In albino animals the bleeding stained mostly the fur around the nose and, owing to repeated rubbing, also the forepaws. In other cases the tear fluid was hemorrhagic and stained the anterior corners of both eyes or even both eyelids, spectacle-like. Melena and profuse hematuria were rather rare occurrences. Mild hematuria is not necessarily connected with the hemorrhagic disease and can be observed as an independent complication, without any other hemorrhagic symptoms, in rats kept on a vitamin B_6 free basal diet.

The most impressive manifestations of the hemorrhagic diathesis in question were blood effusions into the skin, exhibiting all the characteristics of purpura (Fig. 1). In the gross there were patches of purpura of varying size, some confluent, resulting in larger areas of dark red or blue discoloration. These occurred mainly on the dorsum of the feet and, particularly, on the hind legs or on the face and neck. The first but rather hidden purpuric patches appeared usually on the back over the scapula or over the ribs close to the spine. We have observed pathologic disturbance of hematopoiesis in 72 rats thus far. Of these animals, 46 displayed visible purpura of a more or less severe degree. As a rule, purpura is a late manifestation of this specific disease. When it appears it gradually increases in severity until the fatal issue which normally follows in from 1 to 2 days.

Blood Morphology

Examination of the blood[4] of rats in the acute stage of hemorrhagic diathesis revealed (Table I), in the majority of the animals, low percentage of hemoglobin

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[4] Blood was obtained by snipping off the tip of the tail after it had been soaked for a minute in warm water. Hemoglobin percentage was determined by the Sahli hemoglobinometer. Red and white blood cells were counted according to standard methods, using the improved Neubauer counting chamber. Wright's stain was used for differential counts and brilliant cresyl blue for determination of reticulocyte percentage.

Platelets were counted according to the direct method involving the use of a hemacytometer. Blood was drawn up in the red cell-counting pipet to the mark 0.5 and diluted with Rees and Ecker's (9) diluting fluid to the mark 101. The platelets were then counted as in doing a red cell count and with the same calculations.

TABLE I

Blood Findings in Rats with Panmyelophthisis

Rat No.	Date	Hemo-globin	R.B.C. per c.mm.	W.B.C. per c.mm.	Platelets per c.mm.	Polymorphonu-clears	Lymphocytes	Monocytes	Eosinophiles	Nucleated red cells per 100 W.B.C.	Reticulocytes per 100 R.B.C.
		per cent	*millions*			*per cent*	*per cent*	*per cent*	*per cent*		
6627	July 2	46	4.83	—	—	—	—	—	—	—	—
6798	" 6	20	—	1,900*	—	—	—	—	—	—	—
6873	Sept. 4	46	—	5,200	—	—	—	—	—	—	—
	" 5	33	3.17	11,200	230,000	—	—	—	—	—	—
7098	Nov. 28	40	4.95	600	20,000	0	100	0	0	—	—
7129	" 21	55	—	700	—	0	100	0	0	—	—
7130	" 29	35	—	1,100	—	0	96	4	0	—	—
7132	" 27	—	—	600	—	12	88	0	0	—	—
7183	" 29	—	—	—	—	8	80	12	0	—	—
7296	" 28	—	—	550	—	4	75	20	1	—	—
7297	" 28	31	—	1,100	—	4	96	0	0	—	0.1
7298	Dec. 1	52	6.06	750	—	2	96	2	0	—	7.0
	" 4	—	—	<500	—	0	100	0	0	900	—
7300	Nov. 27	55	6.70	1,300	130,000	12	88	0	0	—	—
	" 28	26	—	800	—	8	92	0	0	1	6.0
7344	Dec. 5	69	7.83	600	50,000	0	100	0	0	—	—
7438	Mar. 3	73	8.20	1,100	130,000	4	92	2	2	—	—
	" 5	—	7.99	670	150,000	2	94	2	2	16	—
7553	Nov. 30	—	—	—	—	0	96	4	0	36	—
7570	Jan. 13	55	6.06	900*	40,000	8	88	4	0	5	4
7574	Feb. 4	60	9.30	2,450	200,000	0	94	6	0	—	—
	" 5	—	—	—	—	0	80	20	0	—	—
7577	Nov. 30	30	3.03	920	70,000	4	88	0	8	568	—
7675	Apr. 11	65	8.07	1,300	80,000	0	80	20	0	10	—
7716	" 10	60	6.35	750	40,000	4	76	20	0	24	—
7759	" 7	88	9.50	3,500*	90,000	6	89	4	1	—	—
	" 8	78	7.38	2,300*	80,000	2	96	2	0	—	—
7760	Mar. 24	49	5.80	1,150*	60,000	2	80	18	0	6	—
7765	" 22	—	4.62	1,800*	40,000	0	82	18	0	4	—
7766	Apr. 3	—	—	1,200	—	1	86	13	0	1	—
7770	Mar. 24	46	4.75	1,300*	20,000	0	100	0	0	—	—
7783	Apr. 13	52	5.40	2,600	60,000	7	88	5	0	7	—
7809	" 11	—	—	—	—	4	86	10	0	22	—

* Uncorrected.

TABLE I—*Concluded*

Rat No.	Date		Hemo-globin	R.B.C. per c.mm.	W.B.C. per c.mm.	Platelets per c.mm.	Differential count				Nucleated red cells per 100 W.B.C.	Reticulocytes per 100 R.B.C.
							Polymorphonuclears	Lymphocytes	Monocytes	Eosinophiles		
			per cent	*millions*			*per cent*	*per cent*	*per cent*	*per cent*		
7853	Apr.	29	10	1.47	370	30,000	0	100	0	0	250	—
7867	"	26	83	9.10	330	100,000	0	89	11	0	4	—
7870	"	9	—	8.02	1,550	30,000	4	80	16	0	—	—
7884	"	16	48	—	1,000	140,000	0	92	8	0	32	—
7914	Mar.	30	31	—	1,800*	—	4	84	12	0	—	—
7948	Apr.	5	39	4.16	600	70,000	0	100	0	0	50	—
8033	May	21	83	8.10	1,900	20,000	0	86	14	0	5	—
8039	"	12	39	4.42	1,250	10,000	0	89	11	0	7	—
8040	"	23	54	5.45	850	70,000	0	76	24	0	12	—
8044	"	14	42	4.15	900	30,000	2	93	5	0	6	—
8105	"	15	—	4.95	1,150	90,000	0	84	16	0	4	—
8140	"	21	58	7.00	1,400	120,000	3	82	15	0	7	—
8152	"	25	54	4.90	1,250	50,000	0	92	8	0	30	—

and low platelet, white blood cell and red blood cell counts, with a very pronounced granulocytopenia. The leucopenia and the disappearance of the granulocytes may even be regarded as regular features of the disease and were missing in only 2 rats (6873 in Table I and 7237 in Table III) in the whole series. The polymorphonuclear cells when present were always segmented, frequently showing vacuolation and other signs of progressive disintegration.

In the control animals, which were kept on the same vitamin B$_6$ free diet as were the anemic rats, we found constantly high hemoglobin, red blood cell and white blood cell values, with a very distinct *granulocytosis* (Table II). These animals had no signs of a pathologically impaired hematopoiesis, whether they did or did not display symptoms of acrodynia. In view of the generally very high platelet count in rats that had no hemorrhagic manifestations (Tables II and III), a count of 100,000 or less can be considered pathologically diminished. This assumption is borne out by the fact that the bleeding time after clipping the tail to obtain blood for examination, in rats with a platelet count of 100,000 or less, was very markedly prolonged. The clotting time was as a rule within normal limits.

From the foregoing summary of the hemorrhagic manifestations observed and the morphologic blood findings, it becomes evident that we are dealing here with a profound disturbance of the primary blood-

producing tissue, the reticulo-endothelium, in its transformation into the three distinct types of blood cells, *viz.*, into red blood cells, white blood cells and megakaryocytes (platelets). Generally the disease is ushered in by granulocytopenia but is followed soon by thrombocytopenia, affecting the red blood cells only at a later stage in the form of a progressive anemia. This combination of symptoms and their consecutive appearance are characteristic of the clinical entity called

TABLE II

Blood Findings in Control Rats

Rat No.	Date	Hemo-globin	R.B.C. per c.mm.	W.B.C. per c.mm.	Platelets per c.mm.	Differential count				Nucleated red cells per 100 W.B.C.	Reticulocytes per 100 R.B.C.
						Polymorphonu-clears	Lymphocytes	Monocytes	Eosinophiles		
		per cent	*millions*			*per cent*	*per cent*	*per cent*	*per cent*		
6331	Jan. 14	—	9.28	21,000	1,000,000	68	32	0	0	—	—
6547	Dec. 21	100	11.98	13,800	140,000	86	14	0	0	—	—
6611	Apr. 22	104	11.90	22,700	580,000	76	18	6	0	—	—
6770	Dec. 2	—	—	10,550	—	72	25.5	2.5	0	—	—
6909	" 7	98	9.95	12,700	540,000	53	43	4	0	—	—
6943	" 11	90	9.52	6,700	710,000	50	49	1	0	—	—
6957	Aug. 13	95	12.31	16,400	—	80	19	1	0	—	—
6959	Dec. 18	72	9.67	15,500	170,000	57	35	8	0	—	—
7126	" 4	90	9.10	12,900	440,000	37	60	2	1	1	—
7203	" 2	—	—	—	—	47	52	1	0	—	—
7348	Jan. 12	105	14.25	11,900	1,020,000	80	18	2	0	—	<0.1
7696	Mar. 5	82	9.93	10,400	830,000	54	45	1	0	2	—

by Frank (10) aleukia hemorrhagica, which again is more or less synonymous with the term aplastic anemia.

The hematological data given in Tables I and III suggest in 2 rats already mentioned (6873 in Table I and especially 7237 in Table III) the presence of pure red cell anemia as a sign of a lesion involving only the red cell-producing system. In exceptional cases, for instance in rats 7438, 7574 and 7867 (Table I), granulocytopenia seems to be the leading symptom, and platelet production and erythropoiesis are normally active or are interfered with only to a mild degree. This manifestation could be classified as uncomplicated agranulocytosis.

Anatomical and Histological Examination

Mucous Membranes.—Necrotic and ulcerative changes around the mouth (Fig. 2) and on the mucous membranes, almost specific signs of human agranulocytosis, were observed rather infrequently in the rats which displayed signs of granulocytopenia. Furthermore, changes of this kind have been encountered in rats that were kept on the vitamin B_6 free basal diet but did not show signs of disturbed hematopoiesis.

Bone Marrow.—Fresh biopsy specimens of bone marrow (femur, humerus, vertebrae) from a healthy animal showed that the marrow was very cellular and included cells of all three blood systems: white blood cells, red blood cells and megakaryocytes. In contrast to this normal finding, bone marrow smears from femur, humerus and vertebrae of a rat which succumbed to the experimental aleukia hemorrhagica (rat 7570) showed distinctly reduced cell content. In the gross the bone marrow cavities seemed to be filled with fluid blood. The bone marrow of the vertebrae and femur could be considered very hypoplastic, and that of the humerus even more so. Myelocytes and myeloblasts were only rarely seen in the smears. Megakaryocytes were missing in the humerus and vertebrae; only one was found in the femoral marrow. Normoblasts and early and late erythroblasts were present and were more common than cells of the granulocytic series. There were also phagocytic cells and, in the vertebral marrow, cells that were apparently lymphoid.

Similar findings were recorded in fresh bone marrow smears obtained from a second animal that was in a rather less advanced stage of the disease (rat 7870).

The results of the examination of fresh bone marrow have been substantiated by histological studies carried out on 25 rats in diverse stages of the disease. Appearance of the marrow varied in different bones and in the bones of different animals. In some, the only abnormalities were intense hyperemia, almost entire absence of megakaryocytes and a reduction in the number of cells, chiefly the granulocytes, in the marrow (Fig. 3). In others, in addition to the severe hyperemia, there was a varying amount of hemorrhage and edema, accompanied by more pronounced reduction in the number of cells in the marrow, and the granulocytes and megakaryocytes were altogether missing. Many of the patches of edema contained deposits of fibrin (Fig. 4). Small deposits of brown pigment, which did not give the reaction for iron but in many instances showed crystalline formation in the characteristic burrs of hematoidin, were repeatedly found. In the marrow of some bones of most of the animals and in that of all the long bones of some of the animals megakaryocytes and granulocytes were entirely absent and the hematopoietic cells in the tissue of the marrow had almost completely disappeared (Fig. 5).

Suprarenal Bodies.—A frequent postmortem finding in the internal organs, and the most striking, was hyperemia and hemorrhage in the suprarenal bodies (Fig. 6). The hemorrhage was easily recognizable in 24 out of 72 animals and

varied in extent from small patches, in the cortex or medulla or both, to diffuse hemorrhage with obliteration of the natural architecture of the entire organ.

Microscopically, in the regions of hyperemia alone, the vessels were distended with blood and the cells between them showed a varying degree and amount of degenerative change. In those parts where the hyperemia was intense and also in the regions of extravasation of blood, the parenchyma was almost or entirely destroyed. The destruction of parenchyma was evidently secondary to the hyperemia or hemorrhage because in the parts that were not affected by these processes the parenchyma was quite well preserved (Fig. 7).

In the hyperemic and hemorrhagic suprarenal bodies of 2 of the animals there were masses of bacteria in the cortex but no surrounding inflammation. Most of the masses of bacteria were within the blood vessels, and the parenchyma immediately surrounding them showed coagulation necrosis.

Kidneys.—Only 2 of the animals showed abnormalities recognizable in the gross. These consisted of massive necrosis of the upper pole of both kidneys in one animal and patches of necrosis in the upper pole of one kidney in the other animal. They were the same animals in which the suprarenal bodies also showed foci of necrosis with masses of bacteria in them. The kidneys of some of the animals showed a varying amount of hyperemia and some were slightly swollen, but many appeared quite normal.

Microscopically, the only change of a general nature was a varying degree of degeneration of the tubular epithelium, which in no case was very severe. In the portion of the kidneys of the 2 animals necrotic in the gross, the tissue showed the typical picture of coagulation necrosis. In this necrotic tissue, however, and mostly within the dilated blood vessels there were masses of bacteria. Surrounding these bacterial masses there was no inflammatory reaction. With the exception of the bacterial emboli no other cause of the necrosis was observed in the sections. The large blood vessels were patent.

Spleen.—At macroscopical examination the spleen showed only hyperemia. There were no striking changes of size and no other characteristics recognizable in the gross.

Microscopically the sinusoids were distended with blood. In the pulp, especially around the lymphoid follicles (Fig. 8), there were zones of hemorrhage. Most of the follicles had no germinal centers. The most striking abnormality was the absence of megakaryocytes. In the pulp there was moderate cellularity with a varying amount of histiocytic hyperplasia and fibrosis (Fig. 9). In the zones of hemorrhage the pulp cells showed varying degrees of degenerative change but in other portions they were well preserved. In the pulp of some of the spleens there were small deposits of brown pigment similar to those in the bone marrow.

Intestine.—In several animals that had clinical melena the large intestine showed, microscopically, some extravasation of blood in the mucosa without accompanying inflammation.

Testes.—In 2 of the rats the testes, in the gross, were intensely hyperemic. Microscopically, these testes showed great dilatation of the blood vessels and

some extravasation of blood in the interstitial tissue. The parenchyma was well preserved, but there were no spermatozoa in the lumen of the tubules.

Skin.—Microscopically, in the regions of purpura, the epidermis varied in thickness. Especially was this true of the stratum granulosum and the stratum corneum. The stratum spinosum showed varying degrees of parakeratosis and acanthosis, presumably as the last remnant of the acrodynia-like skin manifestation which usually preceded the purpura. Extravasation of blood was present within the epidermis and in the corium immediately beneath the epidermis. There was no deposition of blood pigments, and little or no inflammatory reaction was apparent around the extravasated blood. The deeper portions of the corium and the subcutaneous tissue showed only occasional small foci of hemorrhage. The upper part of the corium was hyperemic and edematous (Fig. 10).

The anatomical and histological findings are in complete accordance with the conclusions drawn from observations made on the sick animals and from the appearance of the blood smears. In brief, it becomes evident that this experimental disease in the rat, having for its anatomical basis a more or less advanced panmyelophthisis, resembles most closely the characteristic syndrome of aleukia hemorrhagica in man. As to its cause, three possibilities have to be taken into consideration: (*a*) nutritional etiology, (*b*) bacteriological etiology or (*c*) eventually a combination of both,—bacterial infection with an underlying nutritional factor.

Bacteriological Studies

Bacteriological studies[5] have been carried out to clarify the question of a possible infection. 5 rats were examined, all of which were in a rather advanced stage of the disease. Blood, obtained by heart puncture, and spleen tissue served as material; brain broth, blood agar plates and Endo plates were used as culture media. The results were not conclusive and were even rather negative. In 2 rats slightly pathogenic *Staphylococcus aureus* was found; in one rat the bacteriological culture yielded apathogenic staphylococcus, in one rat *Bacillus coli* and *Bacillus proteus*, and in the 5th rat the bacteriological cultures were completely negative. Anemia and hemorrhagic manifestations were not observed when intraperitoneal injection was made into 2 rats of 0.3 cc. of blood, taken again by heart puncture from a moribund anemic animal (rat 7159), or when injection was made into eight rats of 0.5 cc. of a 48 hour brain broth culture of mannite-fermenting *Staphylococcus aureus* obtained from a rat that showed typical panmyelophthisis (rat 7298).

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[5] We are indebted to Dr. E. E. Ecker of the Institute of Pathology, Western Reserve University, for his valuable aid in these studies.

The 2 rats that received the blood of rat 7159 and 6 of the 8 rats injected with the brain broth culture of the staphylococcus isolated from rat 7298 were on the same vitamin B_6 free diet that was fed the experimental animals. Several displayed specific symptoms of acrodynia. The basal diet of the 2 remaining injected rats was deficient in lactoflavin; that is, it was supplemented with vitamin B_1 and Peters' eluate but not with lactoflavin. One rat on the vitamin B_6 free diet died in the first 12 hours after intraperitoneal injection of the brain broth culture without exhibiting any sign of anemia or hemorrhagic diathesis. The remaining 7 animals on the B_6 deficient diet and the 2 on the lactoflavin deficient diet were observed for several weeks. No untoward effect was seen except for a slight local abscess formation at the place of injection of the brain broth culture in some of the rats, accompanied by temporary loss in weight. Blood examination 24 hours after injection revealed no alteration in the direction of panmyelophthisis.

	Rat 6331 (B₆ deficient diet)	Rat 7038 (Lactoflavin deficient diet)	Rat 7076 (Lactoflavin deficient diet)
Hemoglobin, *per cent*.........................	94	100	88
Red blood cells, per c.mm., *millions*.........	10.6	8.8	8.0
White blood cells, per c. mm................	23,900	12,400	8,800
Differential count:			
Polymorphonuclears, *per cent*............	83	63	35
Lymphocytes, *per cent*..................	14	36	65
Monocytes, *per cent*.....................	3	1	—
Platelets, per c. mm.......................	310,000	290,000	150,000

In view of these facts we are inclined to believe that the bacterial invasion found in the majority of the panmyelophthisic rats examined for bacteria was a secondary process due to generally lowered tissue resistance. The same conclusion may be drawn with regard to the coagulation necrosis in the suprarenal glands and in the kidneys in which bacteriological emboli were found during histological examination, as mentioned above. The absence of more pronounced inflammatory changes is only a natural consequence of the breakdown in cellular defense and tallies well with the histological picture in human agranulocytosis given by Piette (11).

Great care was taken to exclude the possibility of *Bartonella* infection, which has been proved to be an important and frequently even a misleading complication in rats (12) and in dogs (13). The anemia produced by *Bartonella* infection in rats and dogs is always accompanied by leucocytosis, in particular by granulocytosis. Hemorrhagic manifestations were never recorded. In our anemic rats that manifested hemorrhagic diathesis and granulocytopenia, *Bartonella* bodies were missing, as was to be expected.

The negative or at least inconclusive results of the bacteriological search for the possible etiology of the panmyelophthisis encountered

in our rats bring again to the foreground the possibility that a nutritional deficiency is the main causative factor.

Summary of Experimental Data

In previous studies on the vitamin B_2 complex reported by one of us (3) in which a synthetic diet was used consisting of rice starch, casein (Glaxo), melted butter fat, cod liver oil, salt mixture and, as supplements, purified vitamin B_1 and lactoflavin preparations, the syndrome of anemia and hemorrhagic diathesis was never observed. In the studies now reported, rice starch was replaced in the diet by cane sugar and the purified lactoflavin preparation by crystalline natural, later synthetic, lactoflavin, while the vitamin B_1 concentrate was of the same source and of the same degree of purification as that used in our previous experiments. Under these conditions panmyelophthisis was noticed in 33 animals. Of the rats which manifested acrodynia when they were kept on the vitamin B_6 free basal diet and which were subsequently treated with purified vitamin B_6 concentrate prepared[6] from wheat germ, cane molasses and rice polishing, 39 developed anemia and hemorrhages. In contrast to these facts, signs of aregenerative anemia were completely missing in vitamin B_6 deficient rats that died from acrodynia or in those that were treated with cruder B_6 concentrates such as Peters' eluate, yeast, cow's milk, human milk (both milks in daily doses $\geq$ 3.0 cc.), liver or wheat germ autolysate. Anemia and hemorrhagic manifestations were also absent in rats that received, in addition to the vitamin B_6 free diet plus vitamin B_1 concentrate, Peters' eluate instead of lactoflavin. The lactoflavin deficient rats remained under observation for several months and never showed any signs of pathologic hematopoiesis or hemorrhages. This group included 85 animals. Of these, 49 have been successfully treated with lactoflavin or with human milk and cow's milk, 16

[6] These B_6 preparations represented different degrees of concentration. In the case of wheat germ the purification was extended generally over autolysis, precipitation with lead acetate, adsorption on fuller's earth, elution with barium hydroxide to precipitation with phosphotungstic acid and regeneration of this precipitate with barium hydroxide. Details are given in the paper by Birch and György (7). Similar preparations have been made from cane molasses and rice polishing.

succumbed to the lactoflavin deficiency, 6 to diarrhea, 2 to pneumonia and 12 to other infections.

As to the incidence of panmyelophthisis, it has to be borne in mind that the current experiments served primarily for purification of vitamin B_6. The group of vitamin B_6 deficient rats included 319 animals. Of these 52 were cured of acrodynia, 70 died from the same disease, 41 succumbed to pneumonia, 30 to septic infections comprising kidney and lung abscesses, 54 to diarrhea and other infections the cause of which could not be determined and, finally, 72 developed panmyelophthisis. This number appears to be a high percentage considering the fact that in the majority of animals the B_6 deficiency was not eliminated and acted as a complicating factor.

Panmyelophthisis developed in several of our animals that had been previously treated with vitamin B_6 preparations of varying purity and cured of the B_6 deficiency disease. They were kept continuously on the basal diet after treatment had been discontinued. In view of these complications it is difficult to evaluate the significance of the length of the preparatory period needed for the development of the hemorrhagic disease in question. We have observed the onset of the disease after the following periods of time had elapsed.

Number of weeks elapsed.............	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	26	28
Number of rats in which disease onset was observed.........	1	1	3	3	5	6	6	7	8	9	3	1	4	2	3	2	4	2	1	1

Thus, on an average, 14 weeks elapsed before the first symptoms of panmyelophthisis could be noticed, although they might occur after 5 to 6 weeks of a preparatory period.

As to the influence of sex, panmyelophthisis was observed in almost even distribution: 38 female and 34 male animals.

Seasonal variations were a more pronounced factor. There were two distinct peaks, one in spring and one in late fall, with a definite minimum in August, September and October. The period of observations covered 12 months, from June, 1936, through May, 1937.

June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May
2	5	0	2	0	15	6	1	4	5	12	20

The influence of seasonal variations cannot be explained by temperature differences, for the temperature of the animal room was kept at a fairly constant level, except perhaps on days of very warm outside temperature when regulation was not possible. In view of the depressing influence of cold environmental temperature on the bone marrow, shown by Huggins and Blocksom (14), one would have suspected that the same physical factor should at least accelerate the production of panmyelophthisis. Contrary to these expectations, out of 34 rats which for varying periods up to 26 weeks we exposed daily between 5 p.m. and 9 a.m. to cold temperature (about 40°F.) only one animal developed anemia (rat 7237 in Table III). Surprisingly, the same rat was one of the 2 animals that manifested "pure red cell anemia" without diminution of the white cell and platelet counts.[7]

The fact that panmyelophthisis has never occurred in rats that have received Peters' eluate was considered by us a possible clue to the nutritional etiology of panmyelophthisis. In order to prove this assumption we tried administering Peters' eluate to several rats ill with panmyelophthisis. Unfortunately many animals were already in a moribund state when the very acute, usually almost fulminant breakdown in hematopoiesis was discovered. They died despite treatment, from 1 to 2 days after it had been initiated, frequently refusing any medication or food. In animals in which aleukia hemorrhagica was diagnosed at an early stage, administration of Peters' eluate in doses corresponding to 10 to 100 gm. of fresh yeast daily prevented the fatal issue and restored normal conditions. Out of 12 rats which survived the first 2 days of treatment, 4 died with very severe manifestations of progressing aleukia hemorrhagica (3 on the 3rd and 1 on the 4th day of the therapeutic assay). 8, however, were saved. They showed a gradual improvement in the morphologic blood composition (see Table III) and in the permeability of the vessels (purpura). It is of interest to note that in rat 7882 the increase in the white cell count and in the granulocyte count was the first sign that a positive effect was exercised by Peters' eluate, the hemoglobin value, red cell count and platelet count at the same time having still shown a decline. In rats 7811 and 7984 subcutaneous medication proved equally beneficial.

[7] In contrast to the negative results with regard to the possibility of accelerated production of panmyelophthisis, symptoms of acrodynia appeared distinctly earlier in the cold-treated rats than in the control animals (15).

In comparison to the pathologic findings in the animals ill with panmyelophthisis, it is of essential importance to note that in rat 6946, which had made a complete recovery after treatment, the microscopic appearance of the bone marrow and of

TABLE III

Blood Findings before and after Specific Treatment of Panmyelophthisis in Rats

Values recorded after treatment are in italics

Rat No.	Date	Hemo-globin	R.B.C. per c.mm.	W.B.C. per c.mm.	Platelets per c.mm.	Polymorphonu-clears	Lymphocytes	Monocytes	Eosinophiles	Nucleated red cells per 100 W.B.C.	Treatment (Peters' eluate)
		per cent	*mil-lions*			*per cent*	*per cent*	*per cent*	*per cent*		
6946	Dec. 4	72	7.53	2,600	16,000	6	93	1	—	—	
	" 15	68	6.17	8,400	1,010,000	48	51	1	—	—	By mouth
7237	Feb. 3	41	3.80	11,400	440,000	61	39	—	—	8	
	" 6	30	2.97	4,650*	310,000	28	53	18	1	Numer-ous	
	" 9	43	3.60	5,100	280,000	31	64	4	—	—	By mouth
	" 13	76	5.43	4,400	440,000	40	55	4	1	4	
7703	" 6	45	4.12	1,950	60,000	4	76	20	—	—	
	" 9	38	—	2,100	—	32	48	20	—	—	By mouth
	" 13	60	5.28	5,350*	340,000	71	25	4	—	12	
7579	Mar. 25	42	4.05	1,800	60,000	8	80	11	1	80	
	Apr. 1	72	5.10	11,000	210,000	59	33	8	—	3	By mouth
7811	May 2	—	—	1,100	—	3	81	16	—	12	
	" 10	—	—	11,400	—	49	43	7	1	—	By injection
	" 18	81	6.13	12,200	610,000	50	44	5	1	—	
7882	" 1	73	10.40	1,975	110,000	2	87	11	—	12	
	" 6	57	5.58	5,965	20,000	11	81	4	4	21	By mouth
	" 18	84	6.18	8,600	1,000,000	21	73	5	1	2	
7984	" 15	57	5.85	600	70,000	2	76	22	—	11	
	" 24	65	4.68	3,500	180,000	55	34	7	4	1	By injection

*Uncorrected.

the spleen was entirely normal (Figs. 11 and 12), with numerous megakaryocytes and other blood cell-producing elements.[8]

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[8] An incidental finding in the autopsy specimens of bone marrow and spleen from one patient who received Peters' eluate was a great increase in the number of megakaryocytes and megakaryoblasts. We do not attempt to 'evaluate this finding, but it is of interest because of similar findings in rats treated with the same preparation.

DISCUSSION

From the foregoing résumé of the experimental findings recorded for 72 rats it becomes evident that a new disease of the rat has been found. The hematological data, controlled by study of fresh bone marrow smears and by histological examination of the bone marrow and the spleen, together with a great variety of hemorrhagic manifestations, correspond to the classical syndrome of aplastic (aregenerative) anemia (aleukia hemorrhagica). It is now generally accepted that the human counterpart of this disease, aplastic anemia, or panmyelophthisis as its histological equivalent, is the final combined result of three more or less separate, pathologic reactions involving transformation of reticulo-endothelial cells into (a) red blood cells, (b) white blood cells and (c) megakaryocytes. This conception is borne out by the fact that morbid conditions that affect only one specific system of blood cell production are widely known in human pathology; we have (a) agranulocytosis or granulocytopenia from deficient production of white cells, especially of granulocytes, (b) thrombocytopenia from lack of platelets and (c) pure red cell anemia from diminished production of erythrocytes caused by arrest in maturation of red blood cells in the first stage of their formation.

These considerations apply equally well to our animal experiments, especially with regard to the gradual transition of a partial aplasia, as, for instance, agranulocytosis into fully developed panmyelophthisis.

In rats, partial or total arrest in maturation of the reticulo-endothelial cells has been produced by nutritional deficiency, and in particular by lack of one constituent of the vitamin B_2 complex, a component which in this connection may be regarded as a specific maturation factor. In some animals panmyelophthisis developed when they were kept on the diet used by us, usually after spontaneous improvement of a mild acrodynia which had just started. In the greater part of our observations, however, the specific blood dyscrasia followed the addition of a more or less purified vitamin B_6 concentrate, which was administered in order to cure the acrodynia in progress. In the majority of these cases the vitamin B_6 deficiency disease (acrodynia) was in distinct remission, or even completely cured, before symptoms of aleukia hemorrhagica became manifest. Therefore we have to infer that the maturation factor must be different from vitamin B_6.

Furthermore, in uncomplicated deficiency of the filtrate factor, symptoms of anemia or of a hemorrhagic diathesis were not noticed and not reported (5). We could neither prevent nor cure panmyelophthisis merely by addition of the fuller's earth filtrate of a rice bran extract which was supposed to be rich in the filtrate factor (5). We believe, therefore, that this factor cannot be the determining cause of the specific disturbance in hematopoiesis. In our opinion it appears more likely that the maturation arrest in the hematopoietic tissues that occurred as a preliminary reaction to panmyelophthisis in our rats is related to lack of a hitherto unknown factor (or factors) of the vitamin B_2 complex.

Such a theory of specific nutritional disturbance is borne out by the fact that panmyelophthisis was regularly prevented and, in cases where it was recognized in an early stage, also cured by a watery yeast extract concentrate, represented by Peters' eluate. It was not prevented or cured, however, by lactoflavin or by purified vitamin B_6 concentrates or by a supposedly active filtrate factor preparation.

It is a common occurrence in vitamin studies that only one disease becomes apparent in cases of a combined deficiency while the other deficiencies remain more or less suppressed. For instance, rats kept on a diet deficient in the whole vitamin B complex do not show symptoms of acrodynia but show merely symptoms of polyneuritis or even only of dystrophy. Production of acrodynia is accelerated by the addition of a sufficiently large amount of vitamin B_1 to the diet (Kellogg and Eddy (16), György (3)). By assuming the presence of a combined deficiency in the basal diet used in our present studies, we may surmise that, under the conditions chosen, vitamin B_6 deficiency was the dominant disease, while panmyelophthisis was relegated to a more latent position. This prevalence is not absolute, however, as we were able to observe in several animals in which the concurrence of the two diseases ended in favor of panmyelophthisis. The dominance of the vitamin B_6 deficiency also satisfactorily explains the fact that aplastic anemia was more frequently encountered in rats in which treatment of acrodynia had been instituted with purified B_6 concentrates (presumably deficient in the maturation factor or factors) than in the untreated rats.

Our investigations failed to find support for the assumption that, in the production of panmyelophthisis, specific infection in addition to basic nutritional disturbance plays an important etiologic rôle. Although this negative result does not exclude with final certainty the interaction of an undetermined bacteriological cause, which is then

subject to nutritional influence, we have to regard an interaction of this kind as a very remote possibility.

Whereas partial symptoms of panmyelophthisis, produced in animals by nutritional means, have been described prior to our own observations, they were either not recognized as such or at least were not recognized in their relation to the common denominator of maturation arrest in the primary blood-producing tissue, the reticulo-endothelium.

In chronological order the first contribution we have to mention is a paper by Shipley, McCollum and Simmonds (17) in which it is stated that in rats kept on a vitamin B complex free diet there might develop lesions in the bones "which are essentially identical with those seen in guinea pigs suffering from acute and uncomplicated scurvy," hemorrhages in the medullary cavity and, finally, complete destruction of the cellular marrow elements. Disappearance of the bone marrow generally precedes the hemorrhages. Hemorrhages in other organs and, in particular, purpura-like blood effusions were not described, however, and Happ (18), who carried out blood examinations on 2 rats of this group, failed to demonstrate even a slight degree of anemia but found some leucopenia, significantly without agranulocytosis. Shipley, McCollum and Simmonds attributed the pathologic findings in bone and bone marrow to beriberi and compared them with scorbutic manifestations in guinea pigs deficient in vitamin C. At the time of publication of their findings, the composite nature of vitamin B was not realized. In view of our recent observations we may consider that the bone marrow changes, including the medullary hemorrhages, were a *forme fruste* of panmyelophthisis and therefore were manifestations of deficiency of a specific maturation factor.

In nursing young of mother rats which during pregnancy and lactation were kept on a diet with an apparently suboptimal content of the vitamin B complex, present as wheat germ or yeast, Sure and Schilling (19) and Moore, Brodie and Hope (20) have reported, in addition to paralytic conditions, hemorrhages in the osteogenetic tissues and also, with far less regularity, in the subcutis (petechiae) and in the internal organs. Blood findings and histological bone marrow examinations were not reported.

More closely resembling our own observations, but still far from the fully developed picture of panmyelophthisis, are those recently described by Miller and Rhoads (21) in dogs and by Day, Langston and Shukers (22) in monkeys. The former authors emphasized the analogy of the clinical syndrome of agranulocytosis with the condition encountered in dogs fed a modification of the black tongue producing diet of Goldberger. Hematological data are given for 10 dogs. In only one dog, however, did the granulocytes drop to a level as low as 15 per cent. They were on the average >50 per cent, with a very definite total leucopenia. The histological picture of the bone marrow given by Miller and Rhoads showed also far from complete destruction of the granulocytes or other blood cell-producing elements.

In 6 young macaques, Day, Langston and Shukers obtained evidence of anemia and leucopenia resulting, in their opinion, from vitamin deficiency. Addition of yeast to the diet prevented the disturbance in morphologic blood composition. In general, as leucopenia developed in monkeys kept on the basal diet, "the decrease in white cells appeared to be more at the expense of the neutrophils than of the lymphocytes." But inasmuch as the differential counts were very variable, the authors admitted that "it is difficult to make any broad statement that holds true for all the animals." This statement excludes also the diagnosis of a true agranulocytosis, in spite of the unquestionable tendency to it.

Hemorrhages and low platelet counts, indispensable attributes of panmyelophthisis, were reported neither by Miller and Rhoads nor by Day, Langston and Shukers. In a personal communication Day points out that the hitherto unpublished platelet counts of his animals were usually within or only slightly below the physiological range during the disease but that they always dropped distinctly before death. Hemorrhages were absent even in this stage.

In spite of the incomplete symptomatology, the disturbance in hematopoiesis described by Day, Langston and Shukers in monkeys and by Miller and Rhoads in dogs may be considered to be probably related to the classical picture of aleukia hemorrhagica encountered by us in rats. This analogy is substantiated by the seemingly identical nutritional etiology.

Several authors (Witts (23), Beck (24), Fitz-Hugh (25)) have assumed that a hypothetical maturation factor influences the regulation of the primary blood production also in man. As to the origin of this factor, endogenous (hormonal) or exogenous (dietary) sources have been taken into consideration. For purely theoretical reasons Witts regarded the second possibility as more consistent with clinical facts, thus putting the pathogenesis of panmyelophthisis on a qualitative basis similar to that of pernicious anemia, although he was at the same time unable to identify the "maturation factor" for which he searched.[9]

However, in man this supposedly nutritional etiology represents, certainly to a greater degree than can be inferred from our animal experiments, at least in the majority of cases, merely the background or basis for the bone marrow depression and its clinical manifestations. Although it is now generally accepted that bacterial influence is of only secondary importance and that infections follow rather than precede aplastic anemia (Frank (10)) or agranulocytosis (Roberts and Kracke (26), Baldridge and Needles (27), Thums (28)), toxic factors

[9] See Witts's scheme of anhematopoietic anemias showing point of action of the substances essential for blood formation (23, page 549).

in many cases seem to activate or to release the chain of reactions leading finally to the clinical syndrome of aplastic anemia and its subgroups. Lately the presence of such a causal toxic influence, as well as that of the well known roentgenologic effect, has been proved for organic arsenic compounds (29), for benzene (30), dinitrophenol (31), gold preparations (32), quinine (33) and for amidopyrine (34), the latter particularly with regard to agranulocytosis.

The question whether these substances exert their toxic influence directly or through a secondary reaction, based on specific sensitivity, is not yet finally settled. The observations of Squier and Madison (35) as well as those of Disselmeyer and Zorn (36) seem to substantiate the presence of amidopyrine sensitivity in cases of agranulocytosis during or after medication with this drug. It is particularly interesting that administration of even one small dose of amidopyrine to these patients after they had recovered had a definite depressive action on the bone and on the bone marrow (35, 36, 37), an effect that could be closely followed in bone marrow smears of biopsy material (Plum (38)). The same deduction applies with regard to thrombocytopenic purpura that occurs as result of quinine sensitivity (33 b). On the other hand several authors, in the first place Kracke and his coworkers (34), maintain that most of the chemical compounds prominent in the etiology of panmyelophthisis and its subgroups act chiefly through their benzene ring which, after it is oxidized in the body, acquires direct toxic properties.

On the whole, the experimental production of agranulocytosis in animals was not successful in spite of several attempts. Infections (39), bacterial toxins (40) and also, with rare exceptions (41), the drugs (42) that played a definite rôle in suppression of the bone marrow function in man failed to produce conclusively relevant disturbances in animals.

We tried to produce panmyelophthisis by administering amidopyrine to rats kept under experimental conditions that were the same as those found necessary for spontaneous manifestation of the disease. Unfortunately we encountered difficulties in administration of amidopyrine to rats by mouth. It was impossible to pursue medication with 2 cc. of a 1 per cent solution of amidopyrine in 4 rats for longer than 1 week. To 2 other animals 0.5 cc. of the same solution was fed for 4 weeks. In all these 6 rats the results were completely negative. The morphologic blood picture presented normal data, and no symptoms of hemorrhagic diathesis could be detected. These negative results may be explained either by refractory behavior of our rats with regard to a directly toxic action of amidopyrine or by the fact that it is impossible to sensitize rats against this drug. The latter explanation

appears especially likely to us in view of the general unsuitableness
of the rat for allergic experiments. But be that as it may, so far we
have not been able to demonstrate in rats ailing with panmyelophthisis
the presence of etiologically important toxic factors. Such non-toxic
forms of the disease occur also not infrequently in man. The con-
clusion drawn as to the intimate relation between inhibited formation
of bone marrow cells in man and that experimentally produced in
rats is therefore valid for the time being for these non-toxic, so called
idiopathic forms.

The experiments here reported cast new light on the so called Water-
house-Friderichsen (W.-F.) syndrome that is characterized clinically
by sudden onset, prostration, high fever, hyperpnea, rapidly fatal
course and, as a special feature, by purpura in addition to monolateral
or more frequently bilateral suprarenal hemorrhage found at post-
mortem examination. The first case was mentioned rather casually
by Marchand (43) in 1880; later, Little (44), Dudgeon (45), Langmead
(46) and others have given similar reports, but only since the analysis
offered by Waterhouse (47) and later by Friderichsen (48) has its
character as a specific and independent entity been recognized. The
syndrome is usually encountered in young infants, from 6 months to
3 years of age, but it has also been found in older children and in
adults (49). It is certainly different in its genesis from the suprarenal
hemorrhage of the newly born, as the latter condition is determined
essentially by purely mechanical causes (50).

Purpura, suprarenal hemorrhage, prostration and a rapidly fatal
course frequently occurred in our rats as peculiarly grouped mani-
festations of panmyelophthisis. Furthermore, in a case of the W.-F.
syndrome Glanzmann (51) found progressive thrombocytopenia and
distinct diminution of the granulocytes with degenerative changes in
the remaining polymorphonuclear cells. Although these blood find-
ings seem to be rather exceptional, and leucocytosis prevailed in
similar cases (50 b), the close analogy between the W.-F. syndrome
and the appearance of some of our rats that were ill with panmyelo-
phthisis can be regarded nevertheless as exceedingly striking.

Etiology of the W.-F. syndrome is now generally attributed to a
septic infection and in particular, certainly in the majority of cases,
to a fulminant meningococcus sepsis which ends fatally before the

local alterations of meningitis may develop. However, in rats ill with the corresponding syndrome we were unable to prove the existence of a primary infection as a decisive etiologic factor. Whereas a negative observation such as this does not exclude with certainty the presence of an unrecognized infection, the nutritional control of the production of the syndrome in rats permits us to make at least another important suggestion with regard to its human equivalent. From the rat experiments we may hypothesize that the bacterial, septic etiology of the W.-F. syndrome in man is built up on the basis of a nutritional deficiency similar to that which we have found necessary for the production of the analogous disturbance in rats. With this assumption it becomes conceivable why meningococci provoke the specific W.-F. syndrome only in certain persons.

The possible identity of the maturation factor active in our rat experiments with vitamin K (52) is ruled out by the morphologic blood composition and the normal clotting time in rats ill with panmyelophthisis as well as by the difference in solubility, vitamin K being fat-soluble, the maturation factor apparently water-soluble. The same conclusion applies with regard to the identity of the maturation factor with so called vitamin P (53), the biological function and clinical use of which certainly seem to be different from those of our maturation factor.

SUMMARY

During the 12 months ending May, 1937, 72 rats were observed that manifested typical symptoms of panmyelophthisis. The disease may start as agranulocytosis, thrombocytopenia or pure red cell anemia, leading progressively, often rapidly, to aleukia hemorrhagica with its typical manifestations (epistaxis, melena, hematuria, purpura).

Blood examinations revealed correspondingly low white cell, red cell and platelet counts with very pronounced granulocytopenia (0 to 4 per cent). Bone marrow smears and histological findings were consistent with the diagnosis of panmyelophthisis.

Suprarenal hemorrhage was a frequent postmortem finding.

The pathogenesis of this experimental panmyelophthisis and this hemorrhagic diathesis is confined to special nutritional conditions. These diseases have been observed by us in rats kept on a diet deficient

in vitamin B_6, containing cane sugar and supplemented with vitamin B_1 and crystalline natural or synthetic lactoflavin. Lack of vitamin B_6, however, is not a necessary condition, since the disease was encountered in the majority of the animals after the specific deficiency disease which became apparent in rats kept on the B_6 free diet was successfully treated with purified B_6 preparations. Even in the untreated animals kept on the B_6 deficient diet acrodynia was, as a rule, in distinct remission before symptoms of panmyelophthisis and hemorrhagic diathesis became manifest.

By means of the addition of Peters' eluate to the basal diet, panmyelophthisis could be prevented and, in animals where it was recognized in an early stage, cured. In view of these facts it is suggested that Peters' eluate contains a specific maturation factor for the primary blood-producing tissue, the reticulo-endothelium, a factor which, being different from lactoflavin, vitamin B_6 and probably also from the so called filtrate factor, constitutes another distinct component of the vitamin B_2 group.

Bacteriological studies brought forward no conclusive positive evidence for the infectious etiology of the experimental panmyelophthisis in our rats.

The possible relation of this new disease in rats to aleukia hemorrhagica and its partial manifestations in man, as well as to the so called Waterhouse-Friderichsen syndrome, is discussed.

Administration of amidopyrine, at least under the conditions chosen, failed to provoke panmyelophthisis in rats kept on the same diet as that given to rats in which the disease spontaneously developed.

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EXPLANATION OF PLATES

Plate 15

Fig. 1. Hemorrhagic diathesis in rat 6722, showing effusion of blood into the skin (typical purpura).

Fig. 2. Noma-like gangrene in and around the mouth of rat 7703, seen in advanced stage of panmyelophthisis.
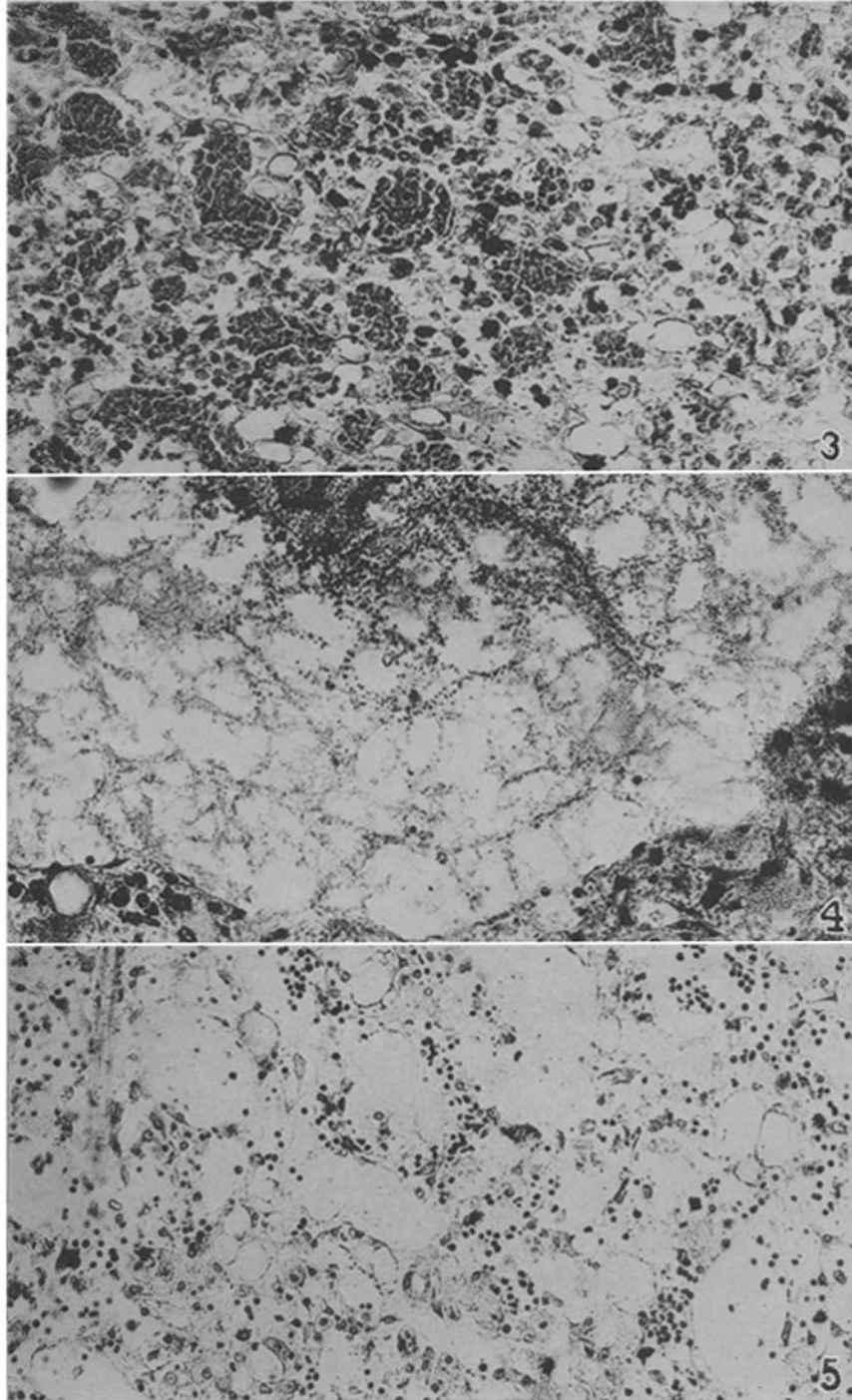
(György *et al.*: Panmyelophthisis)

FIG. 3. Section of bone marrow from humerus of rat 7152; Giemsa stain used. There is intense hyperemia, with moderate edema. Marrow cells are greatly decreased in number and megakaryocytes or granulocytes are almost entirely absent. × 297.

FIG. 4. Section of bone marrow from humerus of rat 7133; Giemsa stain used. Varying amount of hemorrhage and edema is shown, with deposits of fibrin in many of the patches of edema. × 297.

FIG. 5. Section of bone marrow from humerus of rat 7130, stained with hematoxylin and eosin, showing exhaustion of marrow and severe edema. There is some deposition of fibrin. Marrow cells have almost completely disappeared, and no megakaryocytes are seen. There is some extravasation of red blood corpuscles. × 297.

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PLATE 17

FIG. 6. Entire section of suprarenal body from rat 6722, stained with hematoxylin and eosin, showing patchy hemorrage and diffuse hyperemia. ×60.

FIG. 7. Section of cortex of suprarenal body shown in Fig. 6. Degeneration of cortical cells is seen in the region of hemorrhage and intense hyperemia. × 199.
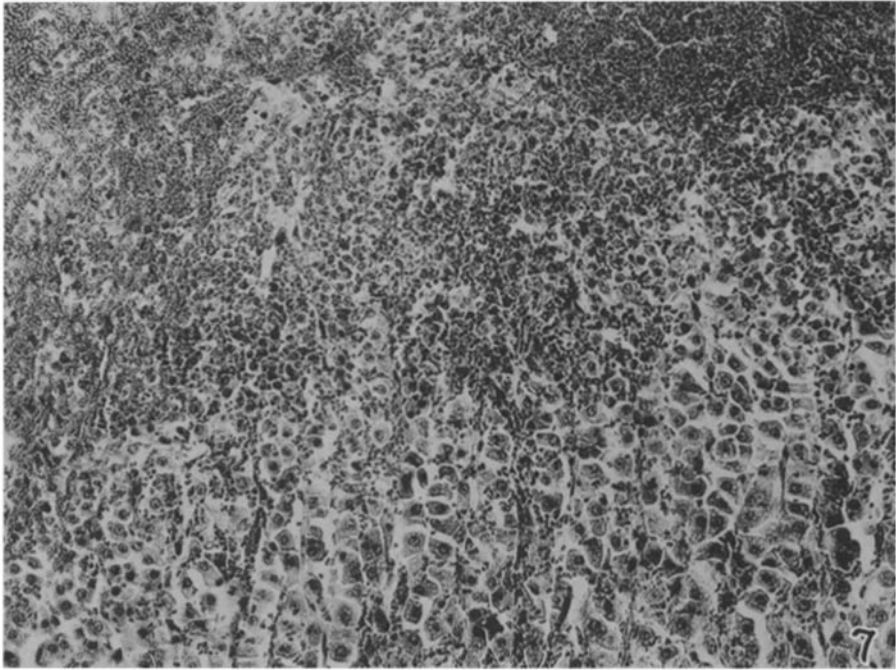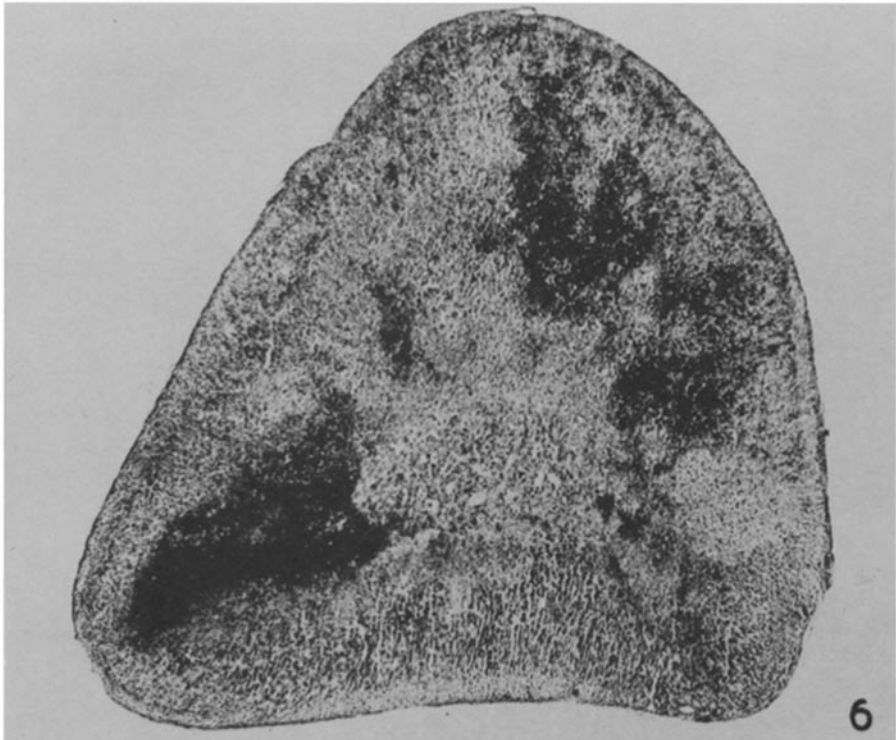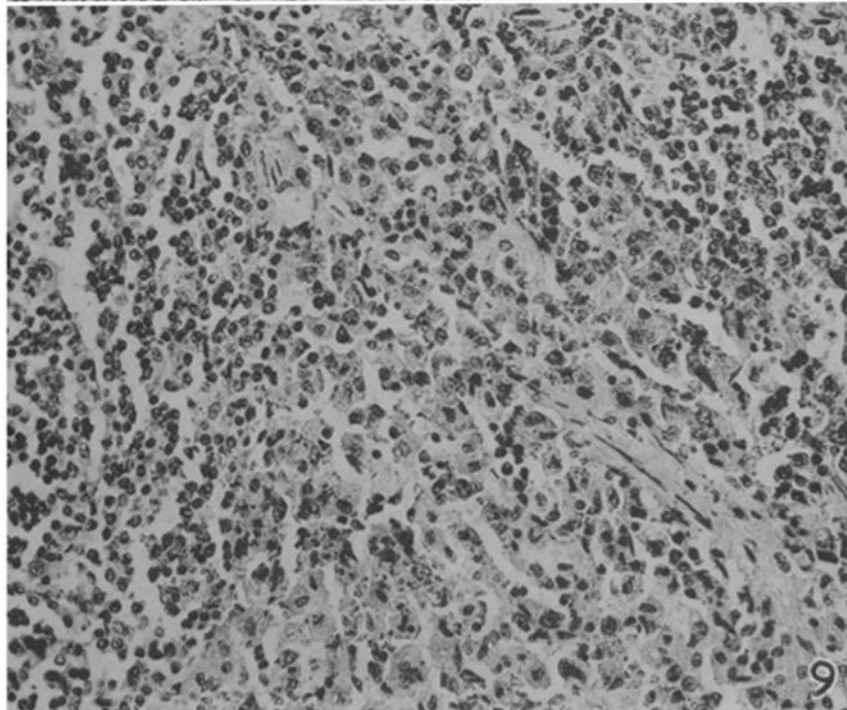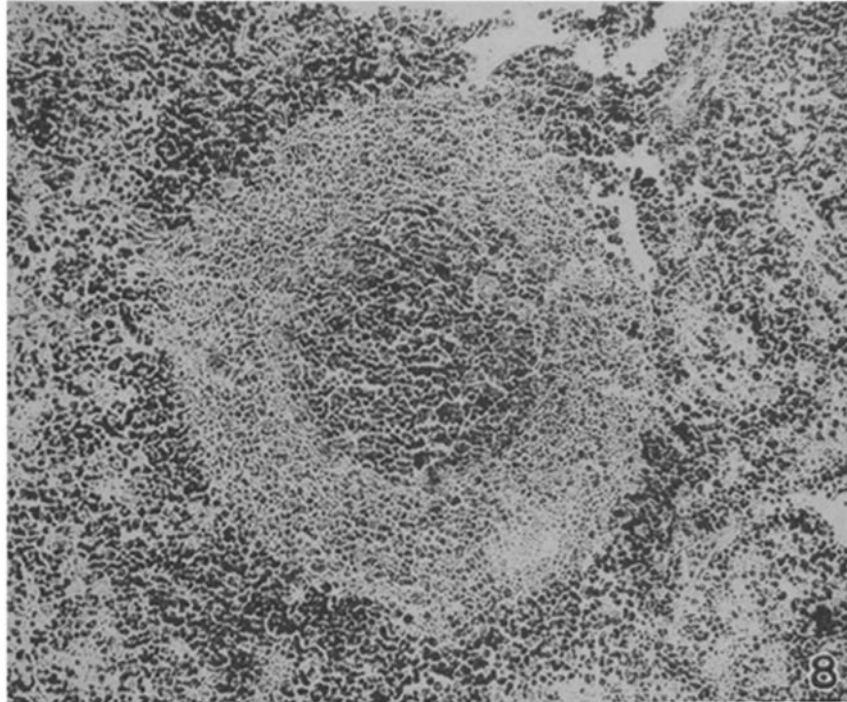
(György *et al.*: Panmyelophthisis)

PLATE 18

FIG. 8. Section of spleen from rat 6227; Giemsa stain used. Sinusoids are distended with blood and zones of hemorrhage are seen around the lymphoid follicles. Megakaryocytes are absent. × 158.

FIG. 9. Section of spleen from rat 6383, stained with hematoxylin and eosin, showing moderate cellularity in the pulp, with varying amount of hyperplasia of histiocytes. × 280.
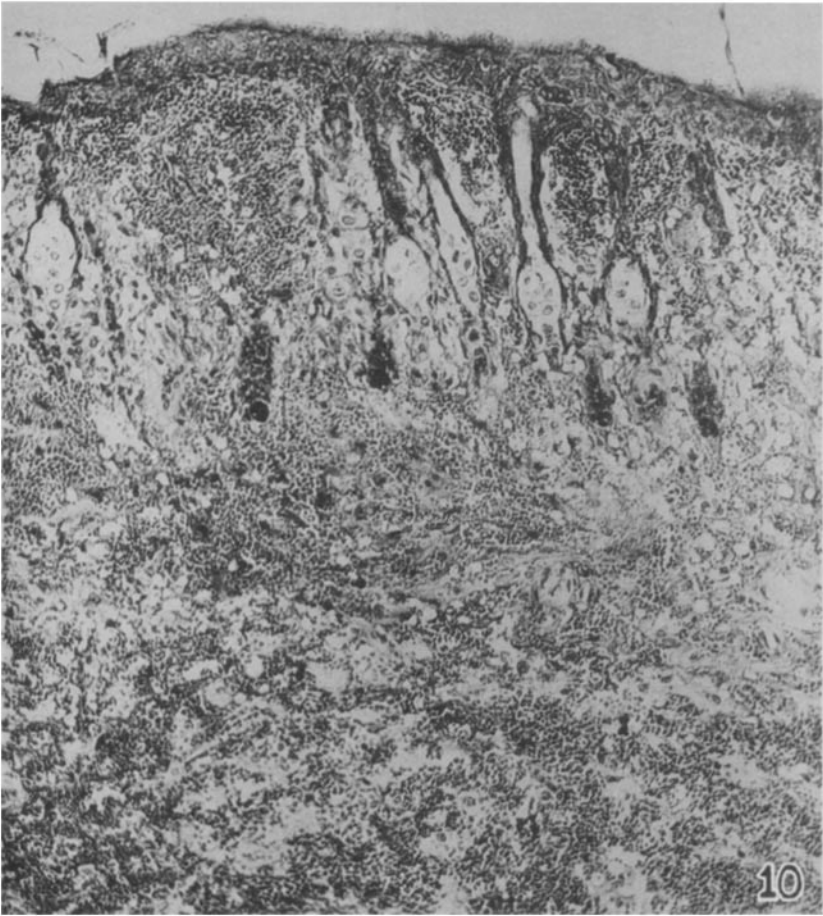
(György *et al.*: Panmyelophthisis)

PLATE 19

FIG. 10. Section of skin of rat 7159, stained with hematoxylin and eosin, showing hyperemia, hemorrhage and edema in upper portion of corium.   × 100.

PLATE 20
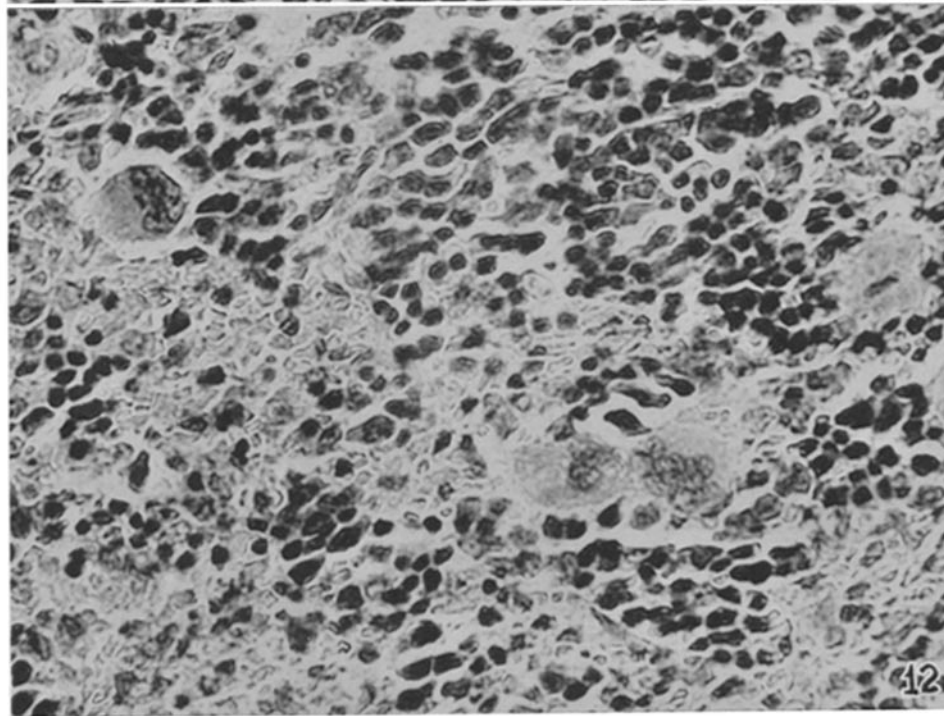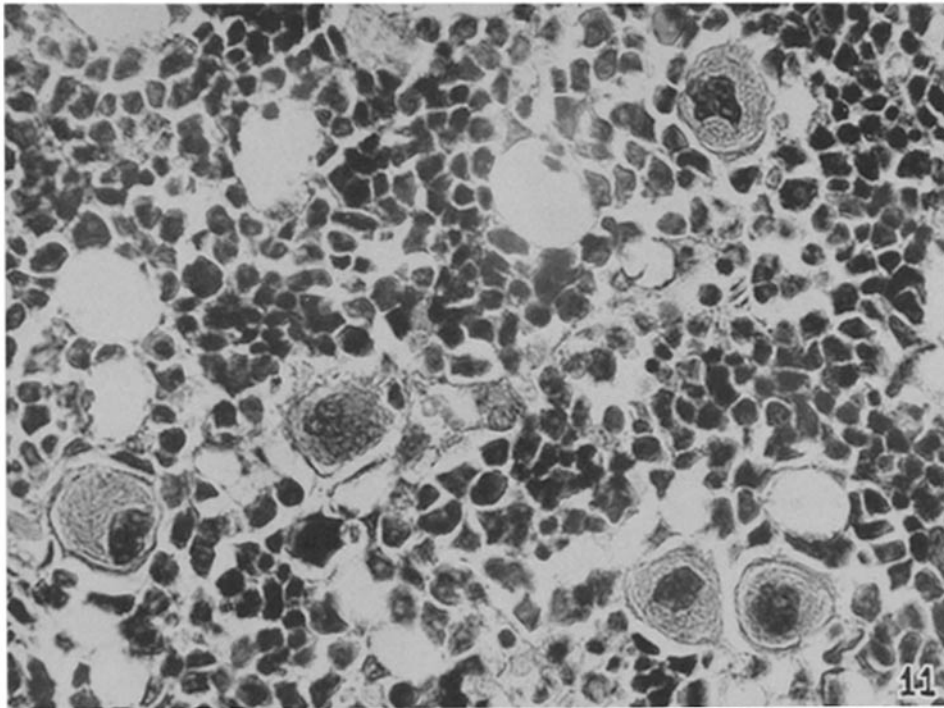
FIG. 11. Section of bone marrow from humerus of rat 6946 which had made a complete recovery after treatment. Hematoxylin and eosin stain used. There is a normal number of megakaryocytes and the cellular marrow shows many granulocytes. × 570.

FIG. 12. Section of spleen from rat 6946 which had completely recovered after treatment. There is a normal number of megakaryocytes. × 495.

(György *et al.*: Panmyelophthisis)