THE EXPERIMENTAL PRODUCTION IN DOGS OF ACUTE STOMATITIS, ASSOCIATED WITH LEUCOPENIA AND A MATURATION DEFECT OF THE MYELOID ELEMENTS OF THE BONE MARROW

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PLATES 5 AND 6

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The characteristic features of the rapidly progressive and frequently fatal disease of human beings known as acute agranulocytosis are leucopenia, granulopenia, stomatitis, and a suppression of maturation of the myeloid elements of the bone marrow. Many attempts have been made to reproduce the syndrome in animals, but although the granulopenia has been successfully simulated, an experimental condition presenting all of the features of the human disease has not been obtained.

In the course of a study of chronic black tongue in dogs (1) it was observed that in the animals dying of an acute form of the disease, pronounced leucopenia was frequently associated with the acute stomatitis. Moreover in certain instances the appearance of the oral lesions was strikingly similar to that seen in acute agranulocytosis of human beings. This observation prompted a more detailed study of the pathological changes of acute black tongue in order to ascertain whether more fundamental similarities existed.

Previous attempts to produce in animals a complex similar to acute agranulocytosis of man may be divided into two main groups: those employing bacteria, or their soluble products, and those depending upon the use of certain toxic chemical substances. Among the authors reporting experiments of the first type are Lovett (2), Piersol and Steinfield (3), Fried and Dameshek (4), and Dennis (5) whose work failed of confirmation by Meyer and Thewlis (6). In spite of the very considerable number of experiments reported, the results have been somewhat inconstant and leucopenia associated with stomatitis has not been observed.

The second group, in which toxic agents were used to cause agranulocytosis,

includes the work of Selling (7), Kline and Winternitz (8), Weiskotten (9), Turley (10), and Kracke (11). All depended upon the administration of some aromatic compound, in most instances benzol, but occasionally such substances as phenobarbital or amidopyrine. In these experiments again, the combination of stomatitis and leucopenia was not obtained, although a well defined suppression of maturation of the myeloid elements of the bone marrow was established.

The pathological changes in the bone marrow of human beings dying of acute agranulocytosis have been described by Schultz (12), Uffenorde (13), Dameshek (14), and Fitz-Hugh and his coworkers (15, 16). Widely varying degrees of cellularity have been reported but there is essential agreement that irrespective of the presence of hyperplasia or aplasia, the characteristic feature is a suppression of maturation of myeloid cells at some stage of their development.

Methods

Mongrel dogs weighing from 7 to 15 kilos were used. The diet fed was the modification of the Goldberger black tongue-producing diet described by Rhoads and Miller (1).

Articles of diet	Quantity	Nutrients		
		Protein	Fat	Carbohydrate
	gm.	*gm.*	*gm.*	*gm.*
Corn-meal	400	33.6	18.8	296
California black-eyed peas	50	10.7	0.7	30.4
Casein (purified)	60	52		
Cane sugar	32			32
Cottonseed oil	15		30.0	
Cod liver oil	30		15.0	
Rice polishings	40			
Sodium chloride	3			
Calcium carbonate	10			
Total nutrients		96.3	64.5	358.4
Nutrients per 1,000 calories		40.1	26.9	149.3

Samples of blood were taken from the jugular vein at regular intervals, and the blood was collected in a standard amount of potassium oxalate to prevent coagulation. For counting the formed elements of the blood carefully calibrated pipettes and counting chambers were used. Smears for differential counts were stained by Wright's method.

Necropsies were done on all the animals. Specimens of bone marrow from the femur, tibia, rib, and sternum were fixed in Zenker's fluid with 5 per cent acetic acid as well as in Zenker's fluid containing 10 per cent formalin. The Zenker-

acetic acid-fixed tissue was stained with eosin-methylene blue while that fixed in Zenker-formol was stained by Giemsa's method. Similar procedures were employed in the study of other organs.

GENERAL RESULTS

Ten animals which developed an acute febrile disease characterized by stomatitis, leucopenia, granulopenia, and characteristic lesions of the bone marrow, are included in this report. In Table I is presented a summary of the essential findings. Four of the animals developed symptoms after more than 140 days of the experimental diet feeding, whereas less than 100 days sufficed in the case of the others. In two instances leucopenia accompanied the first attack of stomatitis, while in the others it did not occur until subsequent attacks. In all instances the leucopenia was observed together with, or soon after, the onset of the mouth lesions. No evidence is at hand to show that leucopenia preceded the stomatitis. Four of the dogs recovered from the first attack of leucopenia, and of these two died in a second attack, whereas one subsequently developed stomatitis which terminated in death without leucopenia. The fourth animal died of intercurrent disease without either stomatitis or blood dyscrasia.

Symptomatology.—The appearance of the stomatitis of acute black tongue has been described by Goldberger (17) and his coworkers and the pathological changes by Lillie (18).

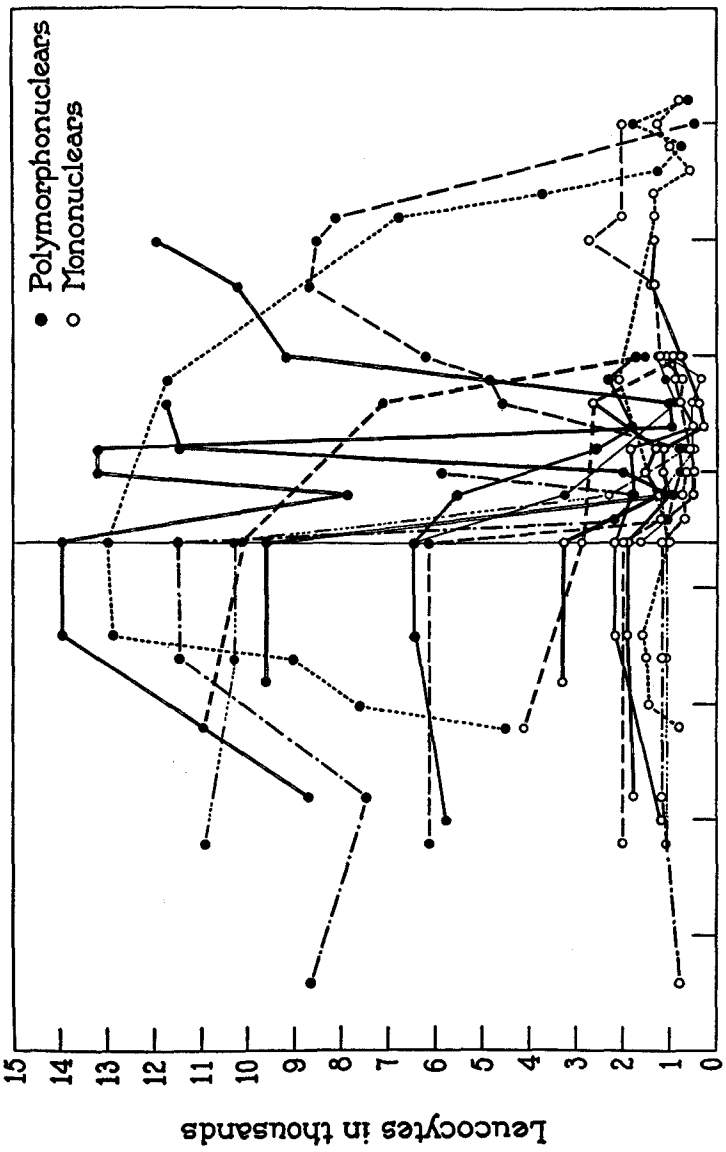
The earliest manifestation is a more or less extensive reddening of the mucosa of the lips, the floor of the mouth, or the cheeks. In the course of 12 to 24 hours the injection becomes a deep reddish purple color and soon after areas of superficial ulceration develop. Necrotic areas then form, composed of a grayish yellow center surrounded by a red margin. These areas extend rapidly and coalesce to involve the deeper layers of tissue with a change of the bright red color to a dirty yellow, with underlying deep purple, injected surfaces. A membrane of necrotic tissue, which can be scraped away easily, forms as a terminal feature. The odor is very fetid and salivation is marked.

In the studies here reported the tendency to the formation of deeply ulcerated, localized lesions was striking (Fig. 1). The necrosis was superficial at first but increased rapidly in extent to involve the deeper tissues. At the death of the animal gangrene had frequently occurred of a large part of the tonsillar fossa or floor of the mouth. Spiral and fusiform organisms morphologically similar to those found in ulcerative stomatitis of human beings were often present (Fig. 2). Masses of these organisms were injected into and under the gingival and buccal membranes of normal dogs without causing any perceptible reaction.

TABLE I

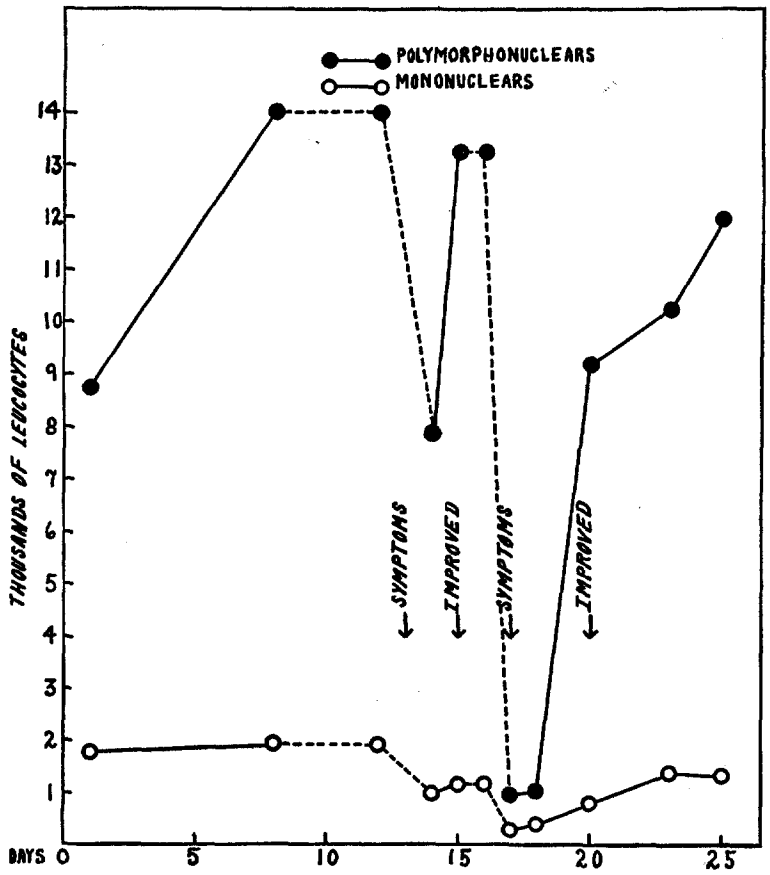
Summary of the Blood Findings before, during, and after Attacks of Induced Stomatitis with Leucopenia

Dog No.	Length of time on diet	No. of attacks of stomatitis	Attack of stomatitis associated with leucopenia	Total W.B.C. before attack, per c.mm.	Absolute number of polymorphonuclear cells before attack, per c.mm.	Absolute number of mononuclear cells before attack, per c.mm.	Total W.B.C. during attack, per c.mm.	Absolute number of polymorphonuclear cells during attack, per c.mm.	Absolute number of mononuclear cells during attack, per c.mm.	Total W.B.C. after attack, per c.mm.	Remarks
	days										
1	85	2	2nd	8,100	6,460	1,640	1,350	830	520		Died
2	142	4	4th	9,800	7,150	2,650	1,800	1,120	680		Died
3	196	5	4th	8,150	6,050	2,100	1,250	750	500	9,300	Recovered
			5th	11,200	8,500	2,700	2,400	360	2,040		Died
4	101	2	1st	10,500	7,550	2,950	2,450	1,100	1,350	44,000	Recovered
											Died in subsequent attack without leucopenia
5	94	3	3rd	11,350	10,200	1,150	1,300	700	600		Died
6	79	2	1st	15,000	13,200	1,800	3,700	2,800	700	17,000	Recovered
			2nd	13,900	11,500	2,400	1,350	590	760		Died
7	97	3	3rd	12,600	11,500	1,100	2,500	1,900	600		Died
8	93	4	4th	7,300	5,500	1,750	2,350	1,750	550		Died
9	186	4	3rd	14,400	13,100	1,300	1,250	960	290	13,300	Recovered
											Died in subsequent attack without leucopenia
10	147	2	2nd	11,450	8,450	3,000	1,450	590	860		Died
Average...				11,146	9,100	2,045	1,927	1,135	791		



TEXT-FIG. 1. Composite graph of the changes in polymorphonuclear and mononuclear leucocyte levels of ten dogs which developed acute black tongue. The perpendicular line indicates the time of onset of symptoms.

All of the animals were definitely ill from the time of onset of the stomatitis and refused food and fluid. The temperature was uniformly elevated and frequently reached 104–105°. Death usually occurred in from 2 to 3 days after the appearance of well defined disease manifestations.



TEXT-FIG. 2. Graph showing the change of leucocyte levels in a dog which developed acute black tongue with leucopenia and recovered.

Leucopenia.—A pronounced leucopenia with a marked decrease in the absolute number of polymorphonuclear cells was observed in all instances although in none was complete agranulocytosis present. In Table I are presented for all the animals the total and absolute numbers of polymorphonuclear and mononuclear cells present both before and during each attack of stomatitis and leucopenia. The lowest total leucocyte count observed during an attack was 1,250 per c. mm.,

whereas the lowest absolute polymorphonuclear count observed was 360 per c. mm. The average total leucocyte count of all the animals before symptoms had occurred was 11,146 per c. mm. At the same time the average absolute polymorphonuclear count was 9,100 per c. mm. and the average absolute mononuclear count was 2,045 per c. mm. During the attacks of stomatitis and leucopenia the average total leucocyte count fell to 1,927 per c. mm., a decrease of 82 per cent. The average absolute polymorphonuclear count fell to 1,135 or a decrease of 90 per cent, while the average absolute mononuclear count fell to 791 per c. mm., or a decrease of 61 per cent. Of the four animals which recovered from attacks of leucopenia, one showed a total leucocyte count of 44,000 after recovery, a second of 17,000, and those of the remaining two dogs were not above normal.

Bone Marrow Alterations.—The femoral marrow of the normal dog is reddish yellow in color. Microscopically, islands of hematopoiesis (Fig. 3) are fairly widely separated by groups of fat cells. In the areas of hematopoiesis, erythrocytes, normoblasts, myelocytes, and polymorphonuclear cells with their various transition forms, bear a fairly constant numerical relation to each other. The normoblasts and the polymorphonuclear cells are the predominating cell types, but occasionally a small undifferentiated cell is seen. This cell has a small amount of cytoplasm which usually takes a mildly basophilic stain. The nucleus is round or slightly oval, contains a heavy chromatin network, and often one or more nucleoli. Sabin (19) considers this cell to be primitive in type and perhaps a precursor of both red and white blood cells.

A photomicrograph of a section of the bone marrow of a patient who died of acute agranulocytosis is presented (Fig. 4) which may be compared with the histological picture present in the marrows of the dogs which died with leucopenia and stomatitis (Figs. 5 and 6). In those animals the bone marrow shows a striking deviation from the normal. There are practically no adult polymorphonuclear cells, and few adult myelocytes. Most of the cells present are normoblasts and the so called primitive cells discussed above. The latter are present in far greater numbers than in the normal dog (Fig. 3) or human marrow (Fig. 4).

The degree of cellularity was not the same in all of the animal marrows; in some it was strikingly increased and in others it was even less than normal. However, the cell types were the same regardless of the cellular activity. No polymorphonuclear cells were seen; myelocytes and myeloblasts were not common; normoblasts and primitive cells predominated. In Fig. 6 primitive cells are present and certain forms suggest the transition of the primitive cell to the myeloblast. The maturation of erythropoietic cells also appears to have ceased, but the duration of the change was too short to be reflected in the peripheral blood. A striking feature was an intense vascular congestion, a change which is not uncommonly seen in the marrows of patients with acute agranulocytosis.

DISCUSSION

Our report deals with a group of experimental animals which died of acute black tongue following the feeding of a modification of the Goldberger black tongue-producing diet. The terminal features were an ulcerative, gangrenous stomatitis, in which spiral and fusiform organisms were found, leucopenia, granulopenia, and a suppression of maturation of the hematopoietic elements of the bone marrow.

The lesions of the mouth in acute black tongue have been described (18) and need not be discussed from a pathological viewpoint. The constant and persistent presence of spiral and fusiform organisms has suggested that they might be causative. Since the inoculation of masses of these organisms into and under the labial mucous membrane of normal dogs uniformly failed to produce lesions, it was concluded that they were secondary invaders. Lillie (18) has demonstrated lesions of the myelin of the nerves leading to the affected areas in canine black tongue, and hence it is assumed that the mucous membrane change is trophic in nature.

Two points concerning the leucopenia deserve special emphasis. Though pronounced in our animals, total or even almost complete absence of granulocytes was never observed. Secondly, in no instance did the sharp decrease in the number of circulating leucocytes precede the appearance of symptoms. It invariably followed or was coincidental with the earliest lesions of the mucous membranes. The leucopenia and oral lesions may be concomitant manifestations of a general disorder.

The histological changes of the bone marrow were characteristic. A similar dilatation of capillaries and cessation of maturation of hematopoietic cells have been observed in experimentally produced granulopenia following the administration of a number of toxic substances of the group of aromatic compounds. The marrow changes observed were clearly causal of the decrease of circulating leucocytes in the peripheral blood.

No explanation is at hand concerning the mode of action of the Goldberger black tongue-producing diet in causing suppression of hematopoiesis. The content of the diet in various dietary constituents has been discussed at length elsewhere (20) and need not be

detailed here. It suffices to state that the effects of feeding the diet may be prevented by the administration of a number of substances, among them meat and yeast. Conclusive evidence that the diet is lacking in some requisite dietary constituent capable of identification is not available. Pending further experiments, any speculation concerning the mode of action of the diet will be inconclusive at best.

SUMMARY

An ulcerative stomatitis associated with leucopenia and granulopenia can be induced in dogs by means of a diet causing black tongue. The decrease of circulating leucocytes is due to a suppression of maturation of the erythropoietic elements of the bone marrow. The changes as a whole have a resemblance to those occurring in human beings with acute agranulocytosis.

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EXPLANATION OF PLATES

PLATE 5

FIG. 1. Photomicrograph of a cross-section of an ulcerated lesion of the pharyngeal mucous membrane of a dog with acute stomatitis. Necrosis of the mucous membrane extending to the muscular layer is shown. Eosin-methylene blue. ×4.

FIG. 2. Photomicrograph of the spiral and fusiform organisms present in the mucous membrane lesion shown in Fig. 1. ×1000.

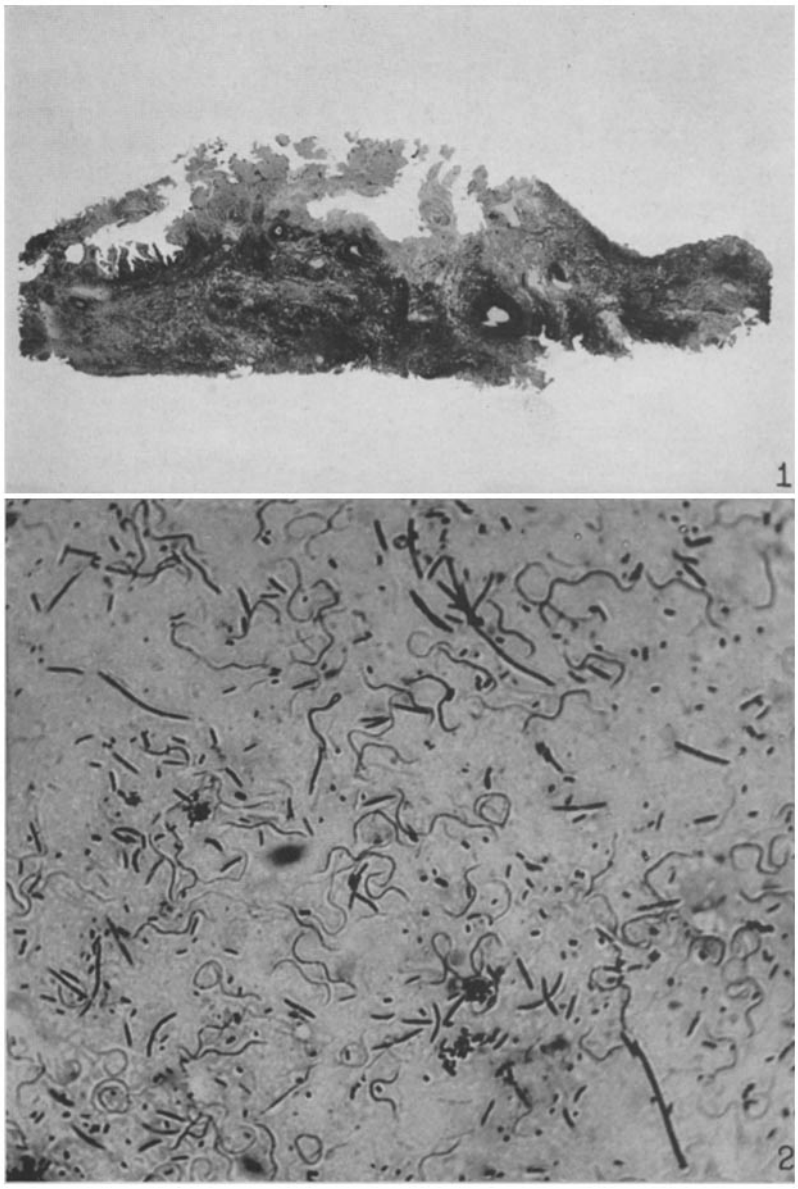
PLATE 6

FIG. 3. Photomicrograph of the femoral bone marrow of a normal dog. Both granulopoiesis and erythropoiesis are active. ×450.

FIG. 4. Photomicrograph of the sternal bone marrow of a patient with acute stomatitis and granulopenia. Granulopoiesis is almost completely absent and erythropoiesis markedly reduced. ×450.

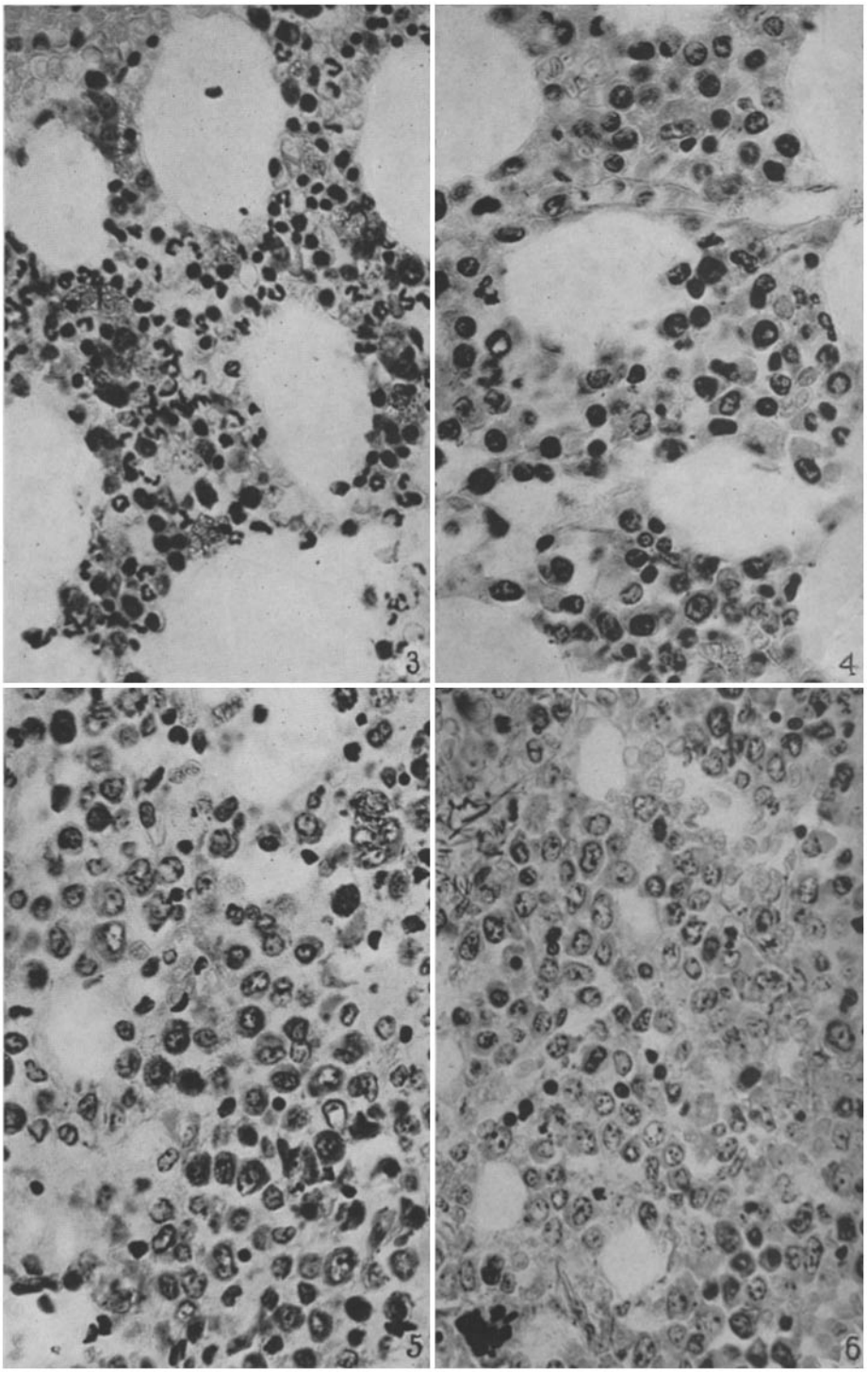
FIG. 5. Photomicrograph of the femoral bone marrow of a dog with acute stomatitis and granulopenia. Both granulopoiesis and erythropoiesis are decreased. ×450.

FIG. 6. Photomicrograph of the femoral bone marrow of a second dog with acute stomatitis and granulopenia. Changes similar to those in Fig. 4 are present. ×450.



Photographed by Louis Schmidt

(Miller and Rhoads: Production of acute stomatitis)



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