

THE EFFECT OF DIET ON THE SUSCEPTIBILITY OF  
THE CANINE HEMATOPOIETIC FUNCTION TO  
DAMAGE BY AMIDOPYRINE

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PLATE 10

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The association of amidopyrine administration with the occurrence of acute agranulocytosis in human beings has been pointed out in a number of communications (1-4). However, attempts to confirm by experimentation the hypothesis of a toxic effect of amidopyrine on hematopoiesis have failed signally. Although in rare instances a decrease in the number of circulating granulocytes has been reported to follow the administration of amidopyrine to animals, such results have been so irregular as to throw serious doubt on the rôle of the drug as an inhibitor of blood formation.

Miller (5) and Climenko (6) have published evidence that in animals fed amidopyrine in solution, histological examination of the bone marrow discloses definite pathological changes even though no marked decrease in the numbers of granulocytes in the circulating blood is present. Miller and Rhoads (7), in a study of the hematological changes associated with acute black tongue in dogs, reported that the acute stomatitis was occasionally associated with a decreased number of granulocytes in the peripheral blood. Histological studies of bone marrow removed at the height of the granulopenia revealed a relative decrease of mature hematopoietic elements. The change observed presented certain similarities to the pathological alteration of the bone marrow in acute granulopenia of man.

In the case of acute stomatitis and granulopenia of the dog, the etiology is apparent; it is the feeding of a particular diet. In acute granulopenia of man the pathological changes are in certain respects similar to those of the dog, but the cause is obscure although amido-

pyrine administration appears to play some part. It seemed possible then, that a faulty dietary might well be one factor at least which increased the susceptibility to the toxic effect of amidopyrine. To substantiate this hypothesis experiment should prove that a dose of amidopyrine which is ineffective alone will cause a well defined disturbance of hematopoiesis when it is added to a diet which is not by itself causative of symptoms. Such experiments have been performed, but paradoxically, the resulting disturbance of the circulating blood cells manifested itself in the erythrocytes rather than in the leukocytes. The results are reported in this communication.

The experiments dealing with the effects of aromatic compounds on hematopoiesis have been reviewed recently in detail by Hamilton (8) and by Kracke (9) and need no discussion here. It suffices to state that the picture of progressive anemia, entirely similar to aplastic anemia in man, has not been encountered. Sudden and severe decreases in number of white cells have been the rule. This last fact led Kracke (1) to suggest that the rôle of amidopyrine in granulopenia was one similar to that of benzol in the blood dyscrasias which are due to that chemical.

The part played by diet in controlling the susceptibility of individuals to poisoning by aromatic compounds was suggested by studies of trinitrotoluol poisoning in Great Britain during the World War (10). Furthermore, Biberfeld (11) had observed that an inadequate dietary rendered rabbits susceptible to poisoning by amounts of methol which were non-toxic to animals fed a normal diet.

### *Methods*

The animals employed were mongrel dogs of about 7 kilos in average weight. They were kept under standard conditions in individual cages with bedding of wood shavings.

The so called normal diet was one which is fed as routine; and empirically is known to be capable of maintaining dogs in good health over a period of several years. It is a mixture of cooked beef, bread, and dog biscuit. The black tongue diet was that described by Goldberger (12). It is known to cause acute black tongue when fed, without supplement, for a period of from 5 to 10 weeks. In an extensive study the feeding of this diet has never been known to cause symptoms in previously normal dogs after a shorter interval. The diet was composed of the following ingredients.

Articles of diet	Quantity	Nutrients		
		Protein	Fat	Carbohydrate
	<i>gm.</i>	<i>gm.</i>	<i>gm.</i>	<i>gm.</i>
Corn meal.....	400	33.6	18.8	296
California black-eyed peas.....	50	10.7	0.7	30.4
Casein (purified).....	60	52.0		
Cane sugar.....	32			32.0
Cottonseed oil.....	15		15.0	
Cod liver oil.....	30		30.0	
Sodium chloride.....	3			
Calcium carbonate.....	10			
Total nutrients.....		96.3	64.5	358.4
Nutrients per 1000 calories.....		40.1	26.9	149.3

The corn meal, peas, and casein were mixed and cooked for 2 hours in a steam cooker. The remaining ingredients were then added and thoroughly mixed. The dogs were fed daily and were allowed to eat as much as they chose.

Blood was taken from the jugular vein in a standard amount of potassium oxalate for routine examinations. Determinations of the number of erythrocytes and leukocytes were made in standard pipettes and counting chambers. The hemoglobin was estimated by the Sahli method, employing a glass standard. The Sahli tubes were carefully calibrated and checked at frequent intervals by the O<sub>2</sub>-combining capacity method of Van Slyke (13).

The amidopyrine used was lot 1,433, manufactured by the H. A. Metz Laboratories, New York. It was administered by stomach tube in a 5 per cent solution in water. Mild heating was required to effect a solution of that strength.

#### *General Results*

The effect of the administration of 0.5 gm. of amidopyrine daily was tested on two groups of dogs. One group of 12 was fed the Goldberger black tongue diet and the other group of 3 received the normal diet. In the first group (Table I), severe ulcerative stomatitis and profound anemia appeared between the 2nd and 9th weeks of treatment in 9. Three dogs, Nos. 2, 11, and 12, failed to develop severe anemia. Anorexia, loss of weight, and diarrhea were marked features. No striking granulopenia was observed. The bone marrows of the animals which died revealed a suppression of hematopoietic maturation. In the control group of animals (Table II) which were subjected to the

same medication but were fed the normal diet, no symptoms resulted. The experiment was repeated, employing larger doses of amidopyrine (Tables III and IV). Both stomatitis and anemia were severe in the animals fed the black tongue diet, but in those fed the normal diet only a mild anemia resulted. No distinct granulopenia occurred at any time in any experimental animal although histological study of the bone marrows of the animals which died showed a well defined interference with maturation of both red and white cells.

#### *Anemia*

The fall of erythrocyte levels was rapid in those animals in which anemia occurred. In the group fed amidopyrine plus the diet producing black tongue, the anemia was profound, the erythrocyte count in some instances reaching 50 per cent or less of the original level. In general, the fall of hemoglobin levels paralleled the fall in erythrocytes, giving a color index which is approximately normal for the dog. Marked variation in size and shape of erythrocytes was a feature at the height of the anemia. Reticulocyte counts were not done. There was no increase in the bilirubin content of the serum as indicated by the icterus index.

#### *Stomatitis*

The stomatitis appearing in those dogs fed the black tongue diet supplemented by amidopyrine was somewhat similar to that seen when the diet alone was fed for a longer period of time. The first manifestation was a marked dusky red coloration of the entire oral and pharyngeal mucous membrane. After a short time, small, discrete ulcerations appeared, most frequently just anterior to the pharyngeal pillars. These showed a base covered by yellow necrotic material and a raised, injected margin. At this stage, salivation was severe. In some instances the ulceration remained localized, and in others it progressed until multiple, coalescent lesions were present and were covered by a membrane of necrotic material. The loss of tissue was more severe, though more localized, than that seen in most instances of black tongue due to feeding alone.

*Pathological Changes of the Bone Marrow*

In Figs. 1 and 2 are shown low and high power photomicrographs of the bone marrow of dog 3, which developed severe anemia and died following the combination of feeding the black tongue diet and the daily administration of 0.5 gm. of amidopyrine. Similar changes were seen in other animals which died.

The normal arrangement of femoral bone marrow in the dog is almost completely obliterated. Instead of well defined islands of orderly hematopoiesis, the cells are scattered irregularly throughout the marrow tissue (compare with Fig. 3 which shows normal canine, femoral marrow). There is a striking suppression of maturation, particularly as concerns the cells of the erythropoietic series. Normoblasts and late erythroblasts are almost completely absent. A very definite increase in young cells has taken place as shown by the presence of many undifferentiated forms which vary somewhat in size. The most common one is a small, round cell with a small amount of basophilic cytoplasm and a deeply staining nucleus containing a very large amount of chromatin. This is held to be the primitive erythropoietic cell of Sabin (14). Various modifications of the primitive cell are present; some have more cytoplasm and a looser arrangement of nuclear chromatin. All of the stages in development up to that of the erythroblast are represented but only a few examples of more mature red cells are at hand.

An interference with the maturation of the granulocytes is less marked but is definite, in spite of the absence of granulopenia in the circulating blood. Granular leukocytes and their precursors are present in reduced numbers in the marrow. Furthermore, they show well defined evidence of damage as indicated by pyknosis of nuclei, abnormal size and shape, and irregular staining. No interference with the megakaryocytes can be made out.

*Experiment 1. Table I. Text-Fig. 1*

In this experiment, 12 dogs fed the black tongue diet received 0.5 gm. of amidopyrine daily. The results can best be seen by examination of the protocol of an animal which is typical of the series. It should be noted that in this instance the oral lesions appeared in less

than 10 days after the experimental diet feeding was begun, whereas such changes have never been observed in this laboratory from diet feeding alone before the 5th week.

*Dog 4.*—

5-31-34. Black tongue diet feeding begun. Amidopyrine administration 0.5 gm. daily begun.

6-12-34. Multiple small shallow punched out ulcers have appeared on the buccal mucosa. These range from 0.2-0.5 cm. in diameter and show a yellow

TABLE I  
*High and Low Blood Levels in Dogs Receiving Goldberger Diet and Amidopyrine, 0.5 Gm. Daily*

Dog No.	Before amidopyrine		Time on amidopyrine days	Time on diet days	After amidopyrine		Remarks
	R.B.C. per c. mm.	Hb			R.B.C. per c. mm.	Hb	
	millions	per cent			millions	per cent	
1	560	62	8	8	249	39	Amidopyrine discontinued. Recovered
2	576	81	12	12	390	70	Died
3	672	111	29	29	175	30	"
4	669	102	33	33	356	62	"
5	558	86	35	55	293	44	Amidopyrine discontinued. Recovered
6	747	107	31	51	271	57	" "
7	704	93	30	50	288	55	" "
8	660	95	28	48	168	29	" "
9	589	87	19	33	291	41	" "
10	539	80	41	41	164	29	" "
11	459	90	57	57	399	73	—
12	783	103	30	40	527	92	—

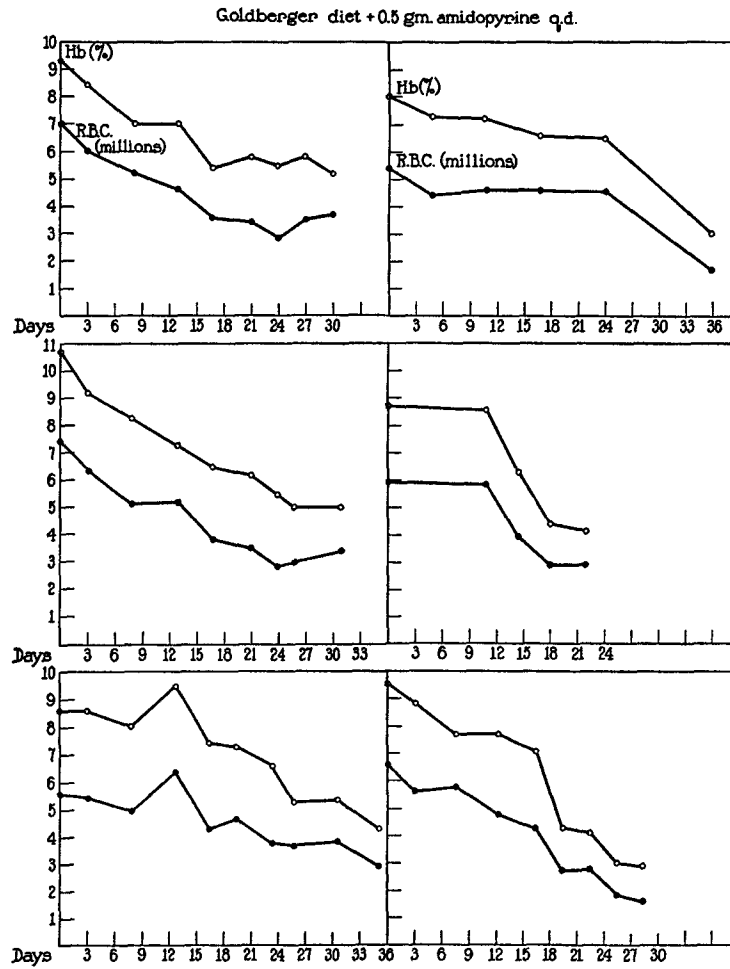
base with a reddened margin. There is active salivation. The whole oral and pharyngeal mucous membrane is a deep and dusky red. There is fairly active diarrhea. The animal refuses food.

6-16-34. The ulcerated areas which have shown little change since 6-12 have now extended and show a tendency to coalesce. They are much deeper and show more necrotic tissue at the border.

6-20-34. The buccal and pharyngeal mucous membrane is almost replaced by an extensive coalescent, deep ulceration with gangrene of the submucous tissues. Salivation is very active and the animal appears ill. Diarrhea has been present every day. There is marked loss of weight, complete anorexia, and progressive

weakness. Amidopyrine is discontinued. From this time on there is a distinct tendency of the ulcerated areas to heal. Weakness and anorexia persist, however.

7-6-34. After a progressive increase in weakness the animal is found dead.

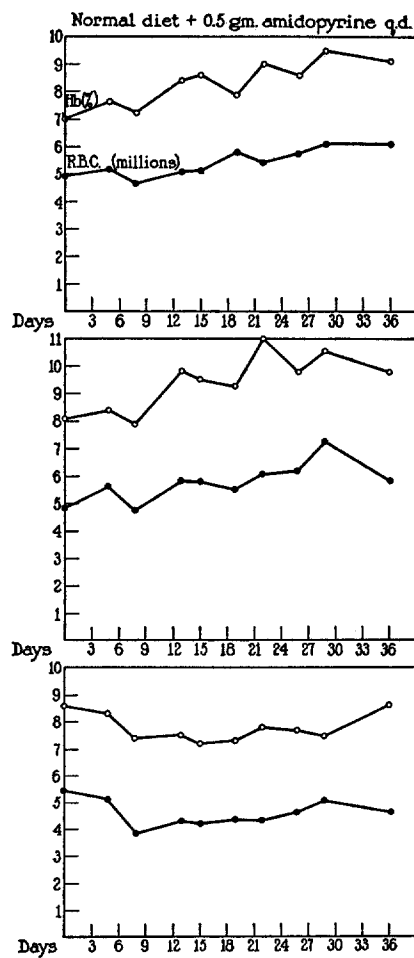


TEXT-FIG. 1

*Autopsy.*—An extensive gangrenous stomatitis is present. The marrow of the femur is a reddish yellow in color. No other gross lesions of the organs are seen. The microscopical changes are described under pathological alterations.

TABLE II  
*High and Low Blood Levels in Dogs Receiving Normal Diet and Amidopyrine,  
 0.5 Gm. Daily*

Dog No.	Before amidopyrine		Time on amidopyrine	After amidopyrine		Remarks
	R.B.C. per c. mm.	Hb		R.B.C. per c. mm.	Hb	
	<i>millions</i>	<i>per cent</i>		<i>millions</i>	<i>per cent</i>	
1	644	86	36	468	86	Well
2	492	70	36	608	91	"
3	487	81	36	586	98	"



TEXT-FIG. 2



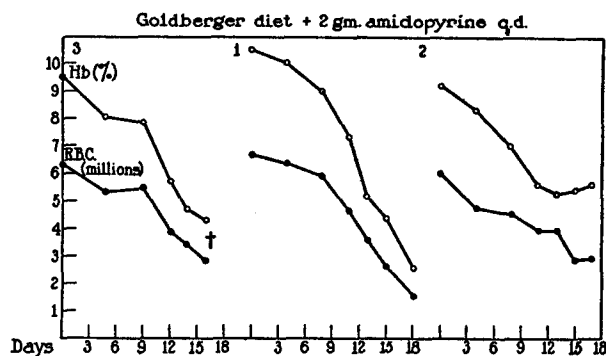
*Experiment 2. Table II. Text-Fig. 2*

This experiment served as a control on Experiment 1. 3 dogs fed the normal diet were given 0.5 gm. of amidopyrine daily. The two experiments were performed in parallel and the conditions differed in no detail other than the diet.

In no instance did either mouth lesions or blood dyscrasia occur in this group of animals. They remained in excellent health throughout the period of observation.

TABLE III  
*High and Low Blood Levels in Dogs Receiving 2 Gm. Amidopyrine Daily and Goldberger Diet*

Dog No.	Before amidopyrine		Time on amidopyrine days	After amidopyrine		Remarks
	R.B.C. per c. mm.	Hb		R.B.C. per c. mm.	Hb	
	millions	per cent		millions	per cent	
1	6.63	105	19	1.55	26	Amidopyrine discontinued. Recovered
2	6.00	92	19	2.88	54	" "
3	6.29	95	16	2.89	43	Died after convulsion



TEXT-FIG. 3

*Experiment 3. Table III. Text-Fig. 3*

Three dogs were fed the black tongue diet and received 2 gm. daily of amidopyrine. The results of the treatment are presented in Table III and in Text-fig. 3. All of the animals developed a severe grade of

anemia and one died. In the remaining 2, treatment was discontinued and recovery ensued. One striking discrepancy between this experiment and Experiment 1, in which only 0.5 gm. of amidopyrine was administered daily, was observed. The animals receiving a smaller amount of the drug all showed ulceration of the oral mucosa, whereas those given a larger amount did not manifest that change. No explanation is at hand for this difference.

*Dog 3.—*

4-23-34. Black tongue diet administration begun.

5-9-34. Amidopyrine, 2 gm. daily begun.

5-16-34. A tonic and clonic convulsion occurs lasting several minutes following the amidopyrine administration. This is followed by a period of marked weakness of the extremities.

5-18-34. Severe salivation follows the amidopyrine administration. No more convulsions have been observed.

5-26-34. The animal has a severe anemia and is weak and pale. Amidopyrine administration is again followed by a convulsion. Later the dog is found dead.

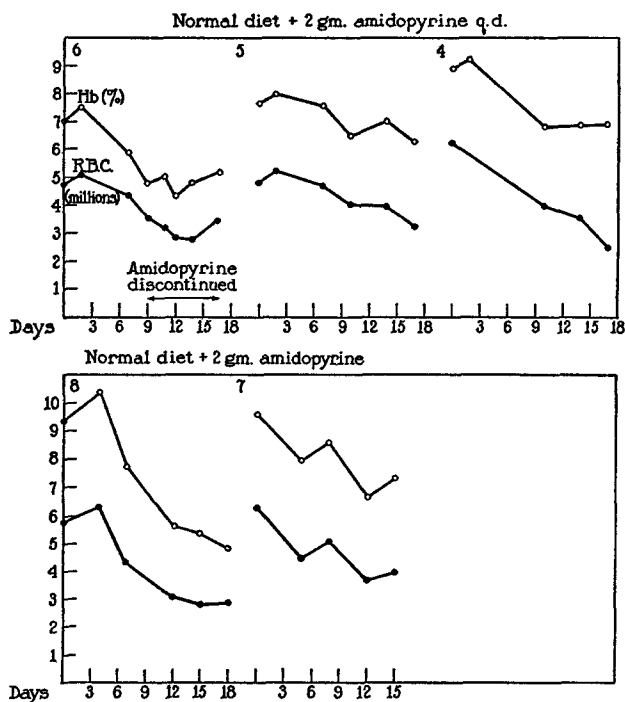
*Autopsy.*—No abnormality of the organs except the bone marrow is found other than a very marked degree of pallor. The bone marrow appears redder and more cellular than normal to gross examination.

*Experiment 4. Table IV. Text-Fig. 4*

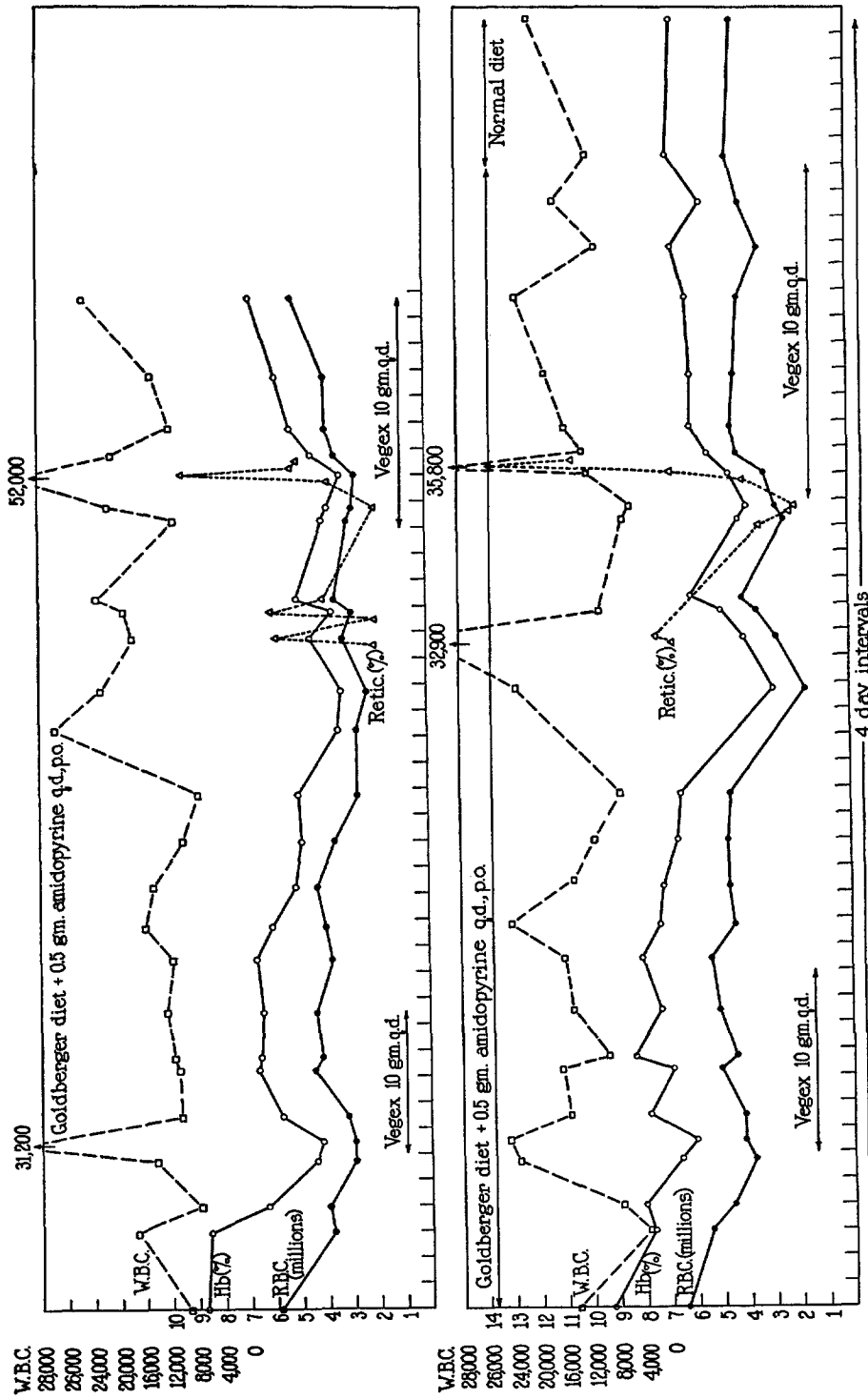
This experiment served as a control on Experiment 3. 5 animals were fed the normal diet and at the same time given 2 gm. of amidopyrine daily. The results are shown in Table IV. In contrast to the animals of Experiment 2, which received the same diet but only 0.5 gm. of amidopyrine daily, those given the larger amount of the drug did evince a definite fall in erythrocyte values. At no time, however, were mouth lesions or evidence of gastro-intestinal tract involvement, such as diarrhea, present. The animals continued to eat well and remained in relatively good health. The grade of anemia encountered in this group was not as severe as that seen in the animals fed the black tongue diet. In further experiments of this type similar results have been encountered. Whereas some depression of erythrocyte levels can be caused by the prolonged administration of large amounts of amidopyrine, it is irregular and of mild degree.

TABLE IV  
*High and Low Blood Levels in Dogs Receiving Normal Diet and Amidopyrine,  
 2 Gm. Daily*

Dog No.	Before amidopyrine		Time on amidopyrine	After amidopyrine		Remarks
	R.B.C. per c. mm.	Hb		R.B.C. per c. mm.	Hb	
	millions	per cent	days	millions	per cent	
4	6.29	89	15	3.59	69	Amidopyrine discontinued. Recovered
5	5.31	80	17	3.29	63	" "
6	5.07	75	9	2.82	43	" "
7	6.29	96	12	3.62	66	Died after convulsion
8	5.70	93	17	2.81	48	Died, pneumonia



TEXT-FIG. 4



TEXT-FIG. 5

*Experiment 5. Text-Fig. 5*

As a supplement to the studies which have been described, this experiment tested the effect of administering 0.5 gm. of amidopyrine daily to 2 dogs over long periods of the feeding of both good and bad diets.

The results are presented in Text-fig. 5. The administration of the drug during a period of normal diet feeding was without marked effect. When the black tongue diet was fed and the drug administration was continued, there was a pronounced decline of the blood values to low levels. When vegex, a commercial yeast preparation, was administered in amounts which were known to be effective in the prevention of black tongue, a remission of the anemia was effected. The blood was maintained at levels approaching the normal as long as the vegex supplement was continued, but fell when it was omitted. This was true in spite of the fact that the amidopyrine administration was continued throughout the course of the study.

## DISCUSSION

In the experiments reported, anemia was brought about in dogs fed the black tongue diet by the administration of amounts of amidopyrine which were without effect when given to dogs fed a normal diet. When sufficient amounts of the drug were administered to dogs fed the normal diet, an anemia did result. It was not as severe as that caused by administering amidopyrine to animals on the inadequate diet and was not associated with stomatitis. Ulceration of the oral and pharyngeal mucous membrane was a striking feature when amidopyrine administration was combined with the inadequate diet. That the anemia and stomatitis were not due to the diet alone is clear from the fact that they occurred before they could be expected if only the special diet was fed. Moreover, they were more severe than are the changes of black tongue and were of a somewhat different character.

The absence of leukopenia was remarkable, particularly since it would have been expected, at least in a mild degree, from the histological appearance of the bone marrow. From this fact it would appear that the action of amidopyrine is neither exactly that of benzol nor of the black tongue diet alone. On the other hand, the toxic effect

on the bone marrow of the administration of an aromatic compound is so dependent upon such factors as dosage, diet, route of administration, and host susceptibility that it is difficult to prognosticate what the effect will be in a given instance.

The histological alterations of the bone marrow were of particular interest. They appeared to be similar to those described in a case of benzol poisoning by Andersen (15) and also to those seen in several cases of aplastic anemia studied in this laboratory in which no history of exposure to benzol was available.

One aspect of the experiments deserves special consideration; the fact that the combination of amidopyrine administration and the diet feeding gave a pathological result not wholly dissimilar from that following a sufficiently long period of the diet feeding alone. The stomatitis was similar to that of some cases of acute black tongue, though different in minor details from most. Anemia of mild degree has been reported to occur irregularly in recurrent, chronic black tongue by Rhoads and Miller (16) and Spies (17). A suppression of maturation of the hematopoietic elements of the bone marrow has been described in both acute and chronic black tongue by the same authors. The suggestion may be advanced that the factor in the diet which makes it productive of black tongue is an aromatic compound or that the diet renders the body incapable of detoxifying some aromatic compound, either present in the diet or a product of intrinsic metabolic formation. Further experiments dealing with this subject are in progress.

#### SUMMARY AND CONCLUSION

1. By feeding dogs a black tongue diet and at the same time administering amidopyrine, acute stomatitis and anemia may be produced.
2. Both stomatitis and anemia occur some time before they could be expected to appear as result of the diet feeding alone.
3. The anemia is associated with suppression of maturation of the hematopoietic bone marrow elements.

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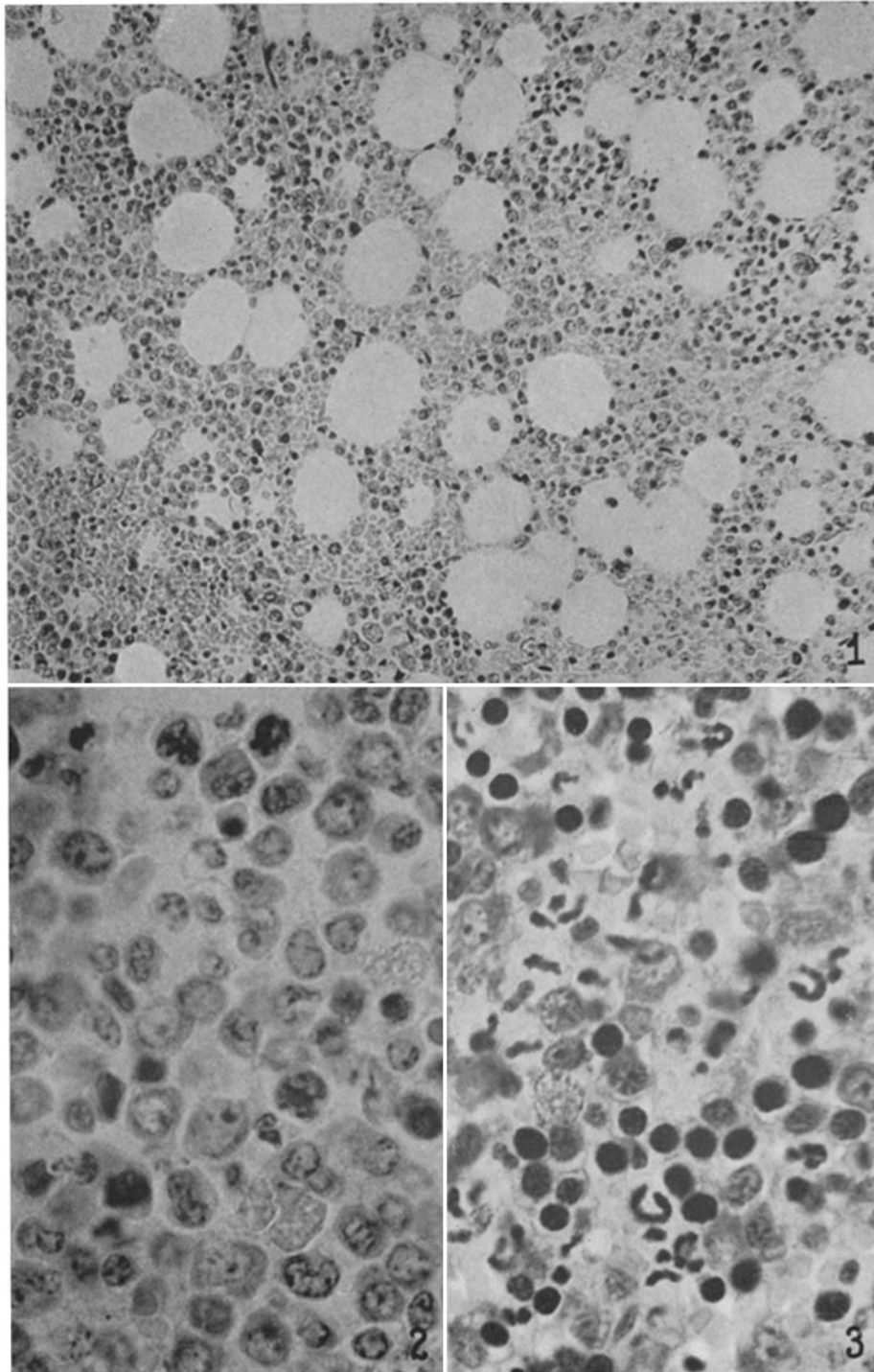
EXPLANATION OF PLATE 10

FIG. 1. Photomicrograph of the bone marrow of dog 2. The marrow is less cellular than normal and the degree of cell differentiation is reduced. Mallory's eosin and methylene blue.  $\times 250$ .

FIG. 2. Photomicrograph of the bone marrow of dog 2. Mallory's eosin and methylene blue.  $\times 1000$ .

FIG. 3. Photomicrograph of the normal canine femoral bone marrow. Mallory's eosin and methylene blue.  $\times 1000$ .





Photographed by Joseph B. Haulenbeck

(Miller and Rhoads: Damage by amidopyrine to hematopoietic function)